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PREFACE

When the first meeting of the Society for Neuroscience took place in 1971, the combined program-abstract booklet contained 350 reports. For 1975, the abstracts volume for the Fifth Annual Meeting contains 1251 reports, many of which are double-length "expanded abstracts."

With this growth in the size and scope of the Annual Meeting of the Society, there has been a corresponding growth of international interest in the proceedings of the Meeting, and it is in response to this interest that the Society has initiated an archival publication, NEUROSCIENCE ABSTRACTS.

Special attention is called to the 42 abstracts which provide summaries of "tape-slide packages," with an audio part (up to 30 minutes) on a magnetic tape cassette and with an accompanying set of 2 × 2″ slides. Many of these "tape-slide packages" are available for loan from the Society for Neuroscience. A list of them is provided following the Table of Contents.

The reports in NEUROSCIENCE ABSTRACTS are arranged according to topic categories which served as a basis for programming the scientific sessions for the 1975 Meeting. Several of the abstracts in the volume were submitted "for publication only." These abstracts have been grouped with appropriate subject categories of abstracts presented at the regular sessions of the Meeting.

EDWARD V. EVARTS, M.D.
President,
Society for Neuroscience

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ABSTRACTS

Chemical Senses

1 TIME COURSE OF GUSTATORY NEURAL ADAPTATION IN THE RAT. David V. Smith, John W. Steadman*, and C. Norman Rhodine*. Depts. Psychol. and Electr. Engr., Univ. Wyoming, Laramie, Wyo. 82071.

Integrated responses to NaCl recorded from the rat chorda tympani nerve typically show an initial phasic portion followed by a slowly declining tonic component that is proportional to stimulus concentration. Although several lines of evidence implicate the transient portion of the chorda tympani response in the coding of sensory information, the mechanisms underlying the production of this phasic burst of activity and the subsequent decline termed adaptation are not well understood. In many sensory systems, e.g., the crayfish muscle receptor organ, adaptation may be the result of several factors. Neural responses were recorded from the rat chorda tympani nerve following stimulation of the tongue with several concentrations of NaCl. These responses were integrated using a fast time constant (47 msec) and the time course of the decline in neural discharge from the peak of the transient response was computer analyzed. The time course of the adaptation process was described by a constant term and two exponentially decaying components, which most likely reflect the existence of two separate mechanisms contributing to the adaptation process in taste. The constant term and the amplitude of the second gradual decay were correlated with NaCl concentration, while the amplitude of the initial rapidly declining exponential component was independent of stimulus intensity. A model incorporating the rate of solution flow predicts that the amplitude of this initial component should not be simply proportional to intensity. Further, the effects of varying the rate of stimulus onset suggest that the initial transient response of the chorda tympani nerve is a function of the rate of stimulus adsorption, while the gradual second decline in the neural response may reflect an adaptive mechanism of the taste receptor cell. (Supported by NINDS Grant NS10211).

2 PHYSICAL CHEMICAL STIMULUS DETERMINANTS OF CAT GENICULATE GANGLION CHEMORESPONSIVE GROUP I UNITS. James C. Boudreau and Thomas E. Nelson. Sensory Sciences Center, University of Texas, Graduate School of Biomedical Sciences, Houston, Texas 77025.

Single unit recordings were taken from geniculate ganglion cells of anesthetized cats. Group I units innervate fungiform papillae primarilly on the back part of the tongue with fibers of large diameter. These units are discharged in general by compounds that form low pH solutions in distilled water. The most active chemical groups are carboxyl groups, phosphate groups and certain amino and imino groups. The most active compounds in the pH region from 5.0 to 7.0 are certain nitrogen heterocycles with pKa values between 5.0 and 7.0. A prominent variable for all group I stimuli is pH in that molecules seem excitatory only in the acidic form; the corresponding basic form is inactive and may be inhibitory. Thus discharge is proportional to the relative concentration of undissociated or protonated molecules present. There are similarities between group I stimuli and stimuli eliciting the sour sensation in the human. This research was supported in part by an NSF Research Grant.

Asterisk (*) indicates nonmember of the Society for Neuroscience.

3 SINGLE UNIT RECORDING FROM CHEMORESPONSIVE NEURONS OF THE GENICULATE GANGLION OF THE DOG. Thomas D. White and James C. Boudreau. (Sponsor: R. Benolken. Sensory Sciences Center, University of Texas, Graduate School of Biomedical Sciences, Houston, Texas 77025.

Single units of the geniculate ganglion were examined for responsiveness to various chemicals applied to the tongues of anesthetized dogs. The cells which were sensitive to this type of stimulation, chemoresponsive neurons, could be classified into three identifiable groups on the basis of response (excitation or inhibition) to a select group of chemical solutions at 50 mM concentration. Group I units are excited by malic acid, HCl, histidine, and distilled water, and are generally inhibited by NaCl. Group II neurons are excited by NaCl, certain amino acids, nucleotides, and nitrogen heterocycles while being inhibited by certain other amino acids and nitrogen heterocycles. Group III neurons consisted of a nonhomogenous set of units unresponsive to amino acids and responsive to a disparate group of compounds (nucleotides, phytic acid, etc.). Spontaneous activity rates were variable within each cell group, although Group II rates tended to be greater than those of the other unit groups. Variability in response was often seen to repeated presentations of a chemical if other stimuli were interposed between presentations. This effect was especially noticable with group II units when Quinine and HCl were interposed between presentations. The classification of chemoresponsive units in the dog is highly similar to that of the cat, indicating a common neural organization of chemoresponsive cells in the geniculate ganglion of Carnivora fissipedia.

4 INTRACELLULAR RECORDINGS FROM SALAMANDER OLFACTORY EPITHELIUM Thomas V. Getchell. Physiol. Dept., Yale Univ. Med. Schl., New Haven, Ct. 06510.

The olfactory epithelium of the salamander, Ambystoma tigrinum and A. maculatum is an advantageous preparation for an intracellular analysis of receptor function. Large cell sizes facilitate intracellular recordings. Receptor cells are bipolar neurons terminating distally in several cilia. Their somatic dimensions are approximately 18 x 10 μm and lie from 50 to 180 μm from the epithelial surface. Sustentacular nuclear dimensions are approximately 26 x 8 μm and typically lie from 12 to 20 μm from the epithelial surface. Two categories of membrane potential transients were recorded intracellularly in response to odor stimulation. The first category of responses, presumably recorded from receptor cell bodies were monophasic positive spikes approximately 10 msec in duration and 10 to 50 mV in amplitude. They were superimposed on a depolarizing slow (generator?) potential which ranged from 4 to 8 mV in amplitude. The first spike occurred from 65 to 700 msec after its onset. The onset of the slow membrane potential change ranged from nearly simultaneous with that of the extracellularly recorder slow negative potential, Veog (-), to approximately 580 msec latency. Graded and differential responses were also recorded in response to odor stimulation. The second category of responses were depolarizing and hyperpolarizing slow potential changes recorded from cells which had membrane potentials ranging from -20 to -70 mV. The responses were graded with stimulus strength but spiking was not observed in response to odor stimulation. The depolarizing responses typically followed Veog(-) by about 500 msec lag. Procion dye marking gave evidence in several instances that spike responses were recorded from receptor cells and certain of the other responses were recorded from sustentacular cells. Supported by NIH research grant #NS 11667-01.

5 REINNERVATION OF GERBIL FUNGIFORM TASTE PAPILLAE BY THE CHORDA TYMPANI NERVE. MaryLou Cheal and Bruce Oakley. Dept. Zool., Univ. Mich., Ann Arbor, 48104.

The time courses and characteristics of reinnervation of the tongue, reformation of taste buds, and recovery of electrophysiological responses to chemical stimulation of the tongue were examined in 89 Mongolian gerbils (*Meriones unguiculatus*) subsequent to cutting or crushing of the chorda tympani nerve. Normally, chorda tympani axons terminate predominately in the fungiform papillae. Regenerating axons, stained by Winkelman's silver procedure, followed the original lingual nerve trunks en route to the epithelium where they reinnervated fungiform papillae. During the second week of recovery there was a progressive increase, first, in the proportion of fungiform papillae containing regenerated axons, second, in taste bud reformation, and third, in physiological recovery. On the basis of the appearance of the first axons in fungiform papillae (8 days) and the first taste responses from the whole chorda tympani nerve (11 days) we estimate that it requires a minimum of 3 days for axons to reestablish a functional taste bud. The time course of reinnervation of fungiform papillae indicates that many fibers regrow at rates in excess of 2 mm per day. Taste buds, defined as pear-shaped clusters of aligned fusiform cells, were seen in fungiform papillae in all gerbils with electrophysiological taste responses. Recently regenerated taste buds mediated responses to a variety of chemicals. Taste responses within a class of chemicals (*e.g.*, salts, acids, sugars) were often highly specific. Changes following cutting or crushing of the chorda tympani are the same except that cutting the nerve delays recovery of taste responses by an average of 1-2 days and produces variable and delayed reappearance of taste buds. Supported in part by USPH Grant NS-07072.

6 REMOVAL OF ODORANTS FROM THE OLFACTORY SAC. David E. Hornung* and Maxwell M. Mozell., Dept. Physiology, Upstate Medical Center, Syracuse,N.Y. 13210.

A radiographic technique was to quantify the rate at which three possible mechanisms (desorption in air, mucus flow, and uptake by the circulatory system) are each able to remove odorants from the olfactory sac.

Our previous isotope studies (Nature 254:617, 1975) showed that tritiated butanol, drawn into the olfactory sac by a negative pressure applied to a cannula inserted in the internal naris, established a steep concentration gradient along the olfactory mucosa. To now study the desorption of butanol molecules from the mucosa, the negative pressure was continued after stimulation in order to draw a stream of nonradioactive room air through the olfactory sac. Butanol molecules that were desorbed into this air stream were collected in a toluene trap located between the internal naris cannula and the negative pressure source. The radioactivity recovered from the trap was readily available for liquid scintillation counting, but to determine the radioactivity left in the animal, the mucosa was frozen, cut, and solubilized prior to liquid scintillation counting. After 480 cc of room air which was delivered during a 30-minute period, only 22% of the total recovered molecules were found in the trap whereas 78% were found in the mucosa. The total recovered molecules were, however, slightly less than the total molecules presented (see below).

Since for this air desorption study, a cannula was sealed into the internal naris, the normal flow of mucus from the olfactory sac into the buccal cavity was prevented. To quantify the rate at which the flow of mucus into the buccal cavity could itself remove odorant molecules from the olfactory mucosa, some animals were prepared without an internal naris cannula and the odorants were puffed into the sac through the external naris. This stimulus was not followed by a stream of nonradioactive room air. In these animals mucus flow was shown by liquid scintillation counting to remove some butanol molecules, but after 30 minutes, 90% of the total recovered stimulus was still found in the olfactory mucosa. The recovered molecules were again slightly less than the total molecules presented.

The uptake of odorant molecules from the olfactory mucosa into the circulatory system was determined from the radioactivity found in arterial blood samples. After 30 minutes 7% of the initial stimulus was recovered from the total blood compartment in those animals which had unobstructed mucus flow. Less than 3% of the stimulus was recovered from the circulatory system in animals with obstructed mucus flow. For both the animals with and the animals without mucus flow, after including the molecules recovered from the circulatory system with those found in the mucosa and toluene trap, the entire presented stimulus could be accounted for.

Unexpectedly, a large percentage of molecules of a sniff of butanol remain in the mucosa after 30 minutes. One wonders how this can be reconciled with electrophysiological investigations which indicated that if there is any receptor adaptation at all, its effect does not last for 30 minutes. Perhaps these data could be reconciled if after initial exposure to the olfactory receptors, the butanol were sequestered in the mucosa such that it would be unavailable to further stimulate the receptors.

To determine if the butanol molecules might be concentrated vertically within the mucosa, 30 minutes after tritiated butanol was presented, the animals were frozen and a section of the roof of the olfactory sac was removed. Using a cryostat, 5 micron sections were cut parallel to the epithelial surface and the radioactivity per section determined by liquid scintillation counting. Almost 90% of the recovered radioactivity was found below the 45 micron level. After 30 minutes most of the sorbed butanol molecules lie below the mucus layer which, according to other investigators, is only 25-40 microns thick. If the cilia are indeed the site for olfactory transduction, the large majority of butanol molecules would appear to be unavailable to further stimulate the receptors. NIH Grant No. NS0394.

7 OLFACTORY PATHWAYS IN THE TREE SHREW (Tupaia glis). L. C. Skeen, Dept. of Anatomy, Duke University, Durham, North Carolina 27710.

Previous experiments (Skeen, 1974) have demonstrated that an architectonically distinct subdivision of Tupaia's medial dorsal thalamic nucleus (MDb) receives projections from a wide variety of paleocortical areas. In order to determine the relationships between these paleocortical areas and the olfactory bulb, bulbofugal and bulbopetal projections were analyzed with anterograde degeneration (Fink-Heimer), anterograde axonal transport (autoradiographic) and retrograde axonal transport (horseradish peroxidase) methods.

Results obtained so far indicate that the ipsilateral bulbofugal projections originate primarily from the mitral cells and terminate among the apical dendrites in the plexiform layer throughout the entire paleocortex except for two small areas: an extreme tip of paleocortex posterodorsally and the angular cortex and subiculum posteromedially. Within the olfactory recipient paleocortex the projections from the main (MOB) and accessory (AOB) olfactory bulb are segregated: the AOB projections terminate throughout the plexiform layer of the medial and posteromedial cortical amygdaloid nuclei, while the MOB projections terminate primarily in the outer plexiform sublamina Ia of the remaining olfactory paleocortex. These MOB recipient paleocortical areas differ both in terms of their cytoarchitecture and their connections. For example, the zone of MOB terminations in sublamina Ia of the anterior pyriform cortex is very thick and uniformly dense while the zones of MOB terminations in sublamina Ia of the other paleocortical targets are thinner and exhibit variations in density. Furthermore, the pyriform cortex, but not the olfactory tubercle, projects to layer Ib, III, and to a lesser extent layer II of all ipsilateral MOB targets. The laminar distribution of these projections compliments that of the MOB projections. Finally, all ipsilateral targets of the MOB, in turn, send reciprocal projections back to the MOB, but the laminar origin of these projections is not the same for all paleocortical areas. For example, the bulbopetal projections of the anterior pyriform cortex originate primarily from the cells in layer II while those of the posterior pyriform cortex originate from scattered cells in layer III, and those of the olfactory tubercle from large cells just deep to the Isles of Calleja. Other ipsilateral bulbopetal projections originate from the nucleus basalis portion of the horizontal limb of the diagonal band.

The paleocortical areas that are intimately associated with the MOB are the same paleocortical areas that project to Tupaia's MDb. These projections arise from the polymorph cell layer III. The thalamocortical projections of MDb, in turn (Skeen, 1974), terminate within a subdivision of the prefrontal neocortex that is heavily myelinated and exhibits a densely granular fourth layer--architectonic features that also characterize Tupaia's primary visual, auditory and somatic sensory cortex. (Supported by the NSF Grant 75-04230 to L. C. Skeen and W. C. Hall.)

8 TASTE OF DIPEPTIDES. Susan S. Schiffman. Department of Psychiatry and Psychology. Duke University, Durham, N. C. 27706.

A strict relationship between qualitative physico-chemical properties and quantitative measures of gustatory quality has not been achieved. Recently, Schiffman and Dackis (Perception and Psychophysics, 1975, 17, 140-146) and Schiffman, Moroch, and Dunbar (Chemical Senses and Flavor, in press) have attempted to relate the chemical structures of amino acids as well as their acetylated counterparts to their gustatory qualities with the application of multidimensional scaling techniques. This paper is an extension of the methodology to the taste of dipeptides. Seventy-eight commercially available dipeptides were rated on a series of fifty-three semantic differential scales relating to gustatory, temperature, tactile, and pain aspects of the stimuli; they were next scaled in a multidimensional space. Most of the dipeptides were described as bitter, sour, or tasteless. However, some were sweet (e.g. glycyl-L-proline) and others had a salty component (e.g. L lysine-L-glutamate H_2O). The order of combinations of the amino acids is important. For example, L alanylglycine and glycyl-L-alanine have different tastes.

9 SEXUAL BEHAVIOR AND ATTRACTION TO VAGINAL ODOR IN MALE HAMSTERS AFTER OLFACTORY AND VOMERONASAL DEAFFERENTATION. Sarah S. Winans and J. Bradley Powers. Dept. Anat. and Neurosciences Lab., Univ. Michigan, Ann Arbor, Mi. 48104.

Bilateral olfactory bulbectomy abolishes mating behavior in male hamsters (Murphy and Schneider, Science, 167:302, 1970), but destruction of the olfactory mucosa (OLF DEAFF) by infusion of the nasal cavities with zinc sulfate (ZS) does not (Powers and Winans, Physiol. and Behav., 10:361, 1973). When the vomeronasal nerves are cut where they pass along the medial surfaces of the main olfactory bulbs (VN DEAFF), severe mating deficits are produced in approximately 40% of the animals. In the remaining 60% of VN DEAFF males, mating behavior is normal, but when zinc sulfate is subsequently applied (VN + OLF DEAFF), none of these males show mounts, intromissions or ejaculations (Powers and Winans, Science, 187:961, 1975). Eighteen of 45 males (40%) showed severe mating deficits after VN DEAFF alone when tested twice weekly for one month. Eight failed to display any mating behavior; 7 mated only once; 3 mated more than once. A subgroup of these 18 males was tested two months postoperatively and in all animals the deficit persisted. In the VN + OLF DEAFF group none of the 22 males mated 2 days after ZS infusion. Sixteen of these 22 animals were also tested on days 8, 15 and 22 after ZS. By day 22 nine males showed behavioral recovery but seven males failed to recover. This variability may be related to differential return of olfactory function following ZS treatment.

Male hamsters are attracted to the odor of female hamster vaginal discharge (FHVD) (Johnston, Behav. Biol., 12:111, 1974). We tested OLF or VN DEAFF hamsters to determine whether changes in their attraction to FHVD might be correlated with changes in their mating behavior. We recorded the total time during a 2 minute test period which males spent licking and sniffing FHVD applied to the wall of a small test arena. OLF DEAFF alone eliminated all sniffing and licking, even though it had no effect on mating behavior. VN DEAFF males which displayed normal mating showed slightly decreased attraction for FHVD when compared to normal males, whereas VN DEAFF animals which had severe mating deficits showed little or no attraction to the discharge. Thus we observed a direct correlation between loss of the attraction for FHVD and loss of mating behavior after VN DEAFF but not after OLF DEAFF. This may reflect differences in the way these chemosensory organs are stimulated, in the kinds of stimuli presented in the two testing situations or in the role these organs play in mediating these behaviors.

10 NEURAL CODING IN THE PONTINE TASTE AREA OF THE RAT. Richard S. Perrotto and Thomas R. Scott. Dept. Psychol., Univ. of Delaware, Newark, Del. 19711.

Patterning theories have proved valuable in describing neural coding mechanisms in the gustatory system. Recordings from first, second, and fourth-order neurons suggest that taste information in largely encoded in patterns of activity across a population of cells. This study provides data on third-order gustatory neurons, and allows for a more coherent account of taste coding through the thalamic level. Single neuron responses, evoked by chemical stimulation of the tongue, were recorded from Pontine Taste Areas (PTA) of acute Nembutalized rats. They reveal that PTA neurons are broadly sensitive to stimuli representing the four putative basic taste qualities. Of 35 neurons, 33 responded to at least three of the four taste qualities. Time course analyses indicate the stimuli which are shown to be similar in behavioral studies exhibit similar response patterns over time. For example KCl, $CaCl_2$, and NH_4Cl (bitter salts) typically evoke the largest transient responses, followed by a gradual decline to a moderate tonic level of activity. Correlations between response patterns indicate that like-tasting chemicals elicit similar profiles of activity across the neural population. Thus the patterns evoked by NaCl and LiCl (salty salts) over a three-second period correlate at the 0.94 level while the activity representing NaCl and $CaCl_2$ (salty vs bitter salts) correlate only 0.68. A multidimensional analysis indicates that three underlying physico-chemical dimensions (as yet undefined) bear upon the neural responses. In certain of their response properties, PTA neurons are like those of the (second-order) solitary nucleus (relative responsiveness to various stimuli, exclusivity of response to chemical stimulation); in other respects they auger the characteristics seen in (fourth-order) thalamic neurons (low absolute response rate, temporal variability, small phasic discharges). It is concluded that PTA is functionally as well as anatomically intermediate between the solitary nucleus and thalamus in the taste processing chain.

11 TASTE RESPONSES IN FETAL SHEEP MEDULLA. C.M. Mistretta and R.M. Bradley. Dept. Oral Biol., Sch. Dent., Univ. Michigan, Ann Arbor, 48104.

To study the development of the sense of taste, recordings were made in the medulla of 16 fetal sheep aged 76-147 days (term) while stimulating the anterior tongue with chemicals. Fetal lambs were delivered by Caesarean section onto a heated table with placental circulation intact. The cerebellum was aspirated to reveal the floor of the fourth ventricle. Using the obex as zero reference, tungsten microelectrodes were driven into the medulla at coordinates derived from previous histology. Chemical stimuli (salts, acids, sugars, and urea) were flowed over the anterior third of the tongue for 30-60 sec, followed by distilled water rinses. Recordings were made from 24 single or few unit preparations that responded to chemical stimulation of the tongue. In eight preparations, electrolytic lesions were made at the recording site. On subsequent histological examination, lesions were found in the region of the tractus solitarius or its nucleus. The most caudal lesion was at the level of the IXth nerve rootlets, the most rostral at the level of incoming VIIth nerve afferents. As in fetal chorda tympani preparations, salts and acids elicited a higher frequency neural response than sugars or bitter stimuli. Five units responded to chemical stimuli only after a latency of 100-500 msec; the significance of this is not yet understood. In the youngest fetus studied, 76 days, responses to salts were rapidly adapting compared to slowly adapting responses in fetuses older than 91 days. Of 18 units tested with tactile as well as chemical stimuli, 16 responded to both. These preliminary results indicate that it will be possible to use fetal tractus solitarius recordings to study the development of sensory function over the entire time course of maturation of the taste system. (Supported by NIH Grant HD-07483.)

12 BEHAVIORAL RESPONSE OF CHANNEL CATFISH, ICTALURUS PUNCTATUS, TO AMINO ACIDS. Edward E. Little* (SPON: L.M.Beidler). Dept. Biol. Sciences, Florida State Univ., Tallahassee, Fl., 32306.

The catfish has been found to have an exquisite sensitivity to simple amino acids in electrophysiological studies of olfactory (Suzuki,N. & Tucker,D., Comp. Biochem. Physiol., 40A:399,1971) and gustatory receptors (Caprio,J., Olfaction & Taste V, 1975). Catfish were trained to associate food rewards with glutamic acid stimulation. The lowest concentration responded to was 10^{-6}M. In another method shock was paired with the addition of an amino acid solution, and catfish readily learned to escape a chamber to avoid shock after solutions of L-serine, L-cysteine, L-proline, or DL-glutamic acid were applied. They failed to do so when plain well water was used as a control. The lowest concentrations of amino acid solutions that evoked a response was within the range of 10^{-7}-10^{-8}M. Fish trained with one type of amino acid solution generalized their response when solutions of novel amino acids were used, but at lower concentrations catfish responded more readily to the conditioned amino acid than to novel ones. The relative importance of olfaction and taste in this behavioral sensitivity is yet unknown. (Supported by NIH Grant NS-05258.)

13 REGIONAL TASTE RESPONSIVENESS ON THE RAT'S TONGUE. Inglis J. Miller, Jr., Department of Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina 27103.

The distribution of fungiform papillae with their single apical taste buds in the rat is consistent among an experimental population of animals. The rat tongue is about 20 mm in length and half of the fungiform taste buds are located on the most anterior 4 mm of the tip. Gustatory responses were recorded from chorda tympani nerves of rats while their tongues were stimulated with chemical solutions. A rubber diaphragm was fitted over the tongue so that the total number of fungiform taste buds could be divided for stimulation into a tip region and mid-region. Summated whole nerve responses were recorded while the entire tongue, the tongue tip alone, or the mid-region alone were stimulated with a concentration series of NaCl, sucrose, or HCl. The tip gave responses which were 80-90% of the responses to stimulation of the entire tongue, while the mid-region gave responses which were only 40-50% of the entire tongue responses for all three classes of chemical stimuli. While there is clearly a quantitative difference between the responses of the tip and mid-region, the differential responsiveness to the three classes of stimuli appears to be nearly the same in both regions. Though the total number of taste buds is the same in both regions, the average density of taste buds per unit of surface area is 3.4 papillae/mm^2 on the tip and 1.3 papillae/mm^2 on the mid-region. Whether the 50% of papillae on the tip receive a higher density of innervation compared with the mid-region is currently under investigation. These observations suggest that regional differences in responsiveness on the anterior portion of the rat's tongue are primarily quantitative and are consistent with the premise that responsiveness to different classes of stimuli is randomly distributed through the receptor population. (Supported in part by NIH Grant NS 10389)

14 THE MORPHOLOGY AND PERIPHERAL DISTRIBUTION OF THE RAT GENICULATE GANGLION. Maximo M. Gomez* and Inglis J. Miller, Jr. (SPON: J. G. McCormick). Department of Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina 27103.

The geniculate ganglion is a group of sensory somata within the petrous portion of the temporal bone which sends its peripheral processes to the anterior tongue via the chorda tympani nerve, to the palate via the greater superficial petrosal nerve, and to the external auditory meatus via the auricular nerve. A morphological study of the ganglion has been undertaken to quantify the relationship between these ganglionic neurons and the taste buds which are innervated by their dendritic terminations. Two ganglia from different rats have been serially sectioned and photographically reconstructed by light microscopy to obtain a total neuron count and an orientation to the cytoarchitechtonic organization. Somata numbering 1754 and 1926 have been identified in two ganglia and these vary in diameter from about 10μ to 40μ with the smallest somata localized relatively more superficially and the larger cell bodies closer to the mass of the seventh nerve fibers. A subpopulation of somata can be identified in association with the emergence of the greater superficial petrosal nerve. The chorda tympani nerve to the anterior tongue innervates approximately 100 taste buds and contains an average of 959 fibers of which 60% are myelinated and 40% are unmyelinated as described by Beidler. The greater superficial petrosal nerve supplies taste buds in the nasopalatine duct which total about 60 bilaterally and approximately 100 taste buds on the palate as described by Kaplick. Degeneration experiments are in progress to determine by chromatolysis the number and localization of geniculate ganglion somata from which axons to the chorda tympani, greater superficial petrosal, and auricular nerves originate.

(Supported in part by NIH Grant NS 10389)

15 ELECTROPHYSIOLOGY OF THE OLFACTORY PATHWAY IN THE PIGEON. Angelica Macadar*, Lyle J. Rausch, and Bernice M. Wenzel. Dept. Physiol., UCLA Sch. Med., Los Angeles, CA 90024.

Previous work in this laboratory has shown 3 primary ipsilateral projection areas of the olfactory bulb (OB) in the pigeon forebrain, viz., prepiriform cortex (CPP), parolfactory lobe (LPO), and ventral hyperstriatum (HV), as well as several secondary areas. We now report results of stimulating pigeons' olfactory nerves (ON) electrically while recording from OB and forebrain. ON conduction velocity is 0.2-0.4 m/sec with thresholds of 1-6 V using pulse durations of 0.05-0.1 ms. Bulbar evoked response (BER) latency is 20-40 ms ipsilaterally and 2-5 ms longer contralaterally; amplitudes are as large as 4 mV. Ipsilateral BER polarity typically reverses across the mitral cell layer. The contralateral BER is often inverted compared to the ipsilateral and also shows a late response (> 60 ms latency). Single ON shocks elicit burst responses with 20-40 ms latency from ipsilateral mitral cells. Bilateral or unilateral transection of OB at different levels with respect to the anterior olfactory nucleus and CPP had different effects on BERs. Responses in ipsilateral primary projection areas are 2-5 ms slower than the BER. With 3-5 Hz repetitive stimulation for 1 min, amplitude drops to 50%; the BER, however, follows 10 Hz without loss for 1 min. Amplitudes in LPO and CPP are 0.8-2 mV and the pattern is similar to BER. Latencies in ipsilateral secondary areas (neostriatum, dorsal hyperstriatum, paleostriatum primitivum and augmentatum) are 50-80 ms, amplitudes are 0.2-0.4 mV, and patterns vary. Amplitude in hyperstriatum accessorium is 0.2-0.6 mV and the latency is typically shorter than in other secondary areas. With contralateral stimulation, latencies vary in these sites and amplitudes are always reduced. Other forebrain areas have been explored with negative results. (Supported by USPHS grant NS 10353 to B.M. Wenzel.)

16 ODOR DETECTION AND DISCRIMINATION IN RATS AFTER ANTERIOR AMYGDALOID LESIONS. Burton M. Slotnick, Jose Simoes-Fontes* and Anne T. Phillips*, Psychology Dept., The American University, Washington, D.C. 20016.

Severe olfactory deficits occur after rostral section of the lateral olfactory tract (LOT) (Slotnick, Prog., Soc. Neurosci., 1972). This study investigated the effects of destroying amygdala and piriform cortical projections of the LOT. Rats were trained in a wind-tunnel olfactometer (Science, 185:796, 1974) to detect the presence of a 1-sec sample of dilute amyl acetate (circa 10-4). After overtraining, they were operated upon and 2-4 weeks later tested for retention and then acquisition of four new 2-odor discrimination tasks of graded difficulty. Rats with the olfactory bulbs removed (N=2), with small neocortical lesions (N=3) and with partial lesions of the amygdala (N=5) served as controls. Experimental rats (N=4) had lesions that extensively damaged the anterior amygdala and piriform cortex at the level of the nucleus of the LOT and interrupted most or all of the olfactory tract fibers at this level. Bulbectomized rats had no retention of the detection problem and only chance performance in 2500 retraining trials. Cortical and amygdala controls had perfect or near-perfect retention and made fewer than 100 errors (mean errors: 22) in each subsequent 2-odor task. Experimental rats showed no discrimination deficits (mean errors: 25) and performed as well as the cortical or amygdala control groups in each postoperative test. These results demonstrate that olfactory projections to the anterior amygdala do not play a significant role in learned olfactory discriminations, and suggest that LOT projections to the pre-piriform cortex and/or the olfactory tubercle provide the anatomical substrate for the retention of odor detection and learning of 2-odor discriminations.

17 OLFACTORY RECEPTOR POTENTIALS AND SINGLE CELL RESPONSES AS A FUNCTION OF STIMULUS INTENSITY IN THE RAT. Charles D. Sigwart* and Robert C. Gesteland Dept. Biol. Sci., Northwestern Univ., Evanston, IL. 60201.

Electro-olfactograms (EOGs) and extracellular action potentials from single receptor cells were recorded from the isolated olfactory epithelia of 1 to 30 day old rat pups. The preparation was maintained in a humidified 95% oxygen-5% carbon dioxide atmosphere. Unit responses could be recorded with either platinized metal microelectrodes or 30 megohm 0.9% saline-filled micropipettes. Responses of a cell were repeatable for at least 30 minutes as long as intense stimulation was avoided. Stimuli used included amyl acetate, n-butanol, butyric acid, dichloroethane, ethyl butyrate, methyl butyrate, pyridine and triethylamine at concentrations between 0.0006 and 0.06 times saturation delivered as brief vapor aliquots in a continuously flowing moist carbogen stream. EOG amplitudes varied with the location of the recording electrode, being largest where the receptor cell density was highest, and with the age of the animal. Maximum response occured in animals 6 days of age and older. EOG amplitude varied linearly with the log of the stimulus concentration. Slopes of the response amplitude versus log stimulus intensity curves were different for different stimulus substances. Receptor cells showed an increase in number of evoked spikes with increase in stimulus intensity up to an optimum value. Further increase in stimulus intensity resulted in a decrease in number of evoked spikes for most cells and most stimuli. Some substances excited a cell and some inhibited it or had no effect. Most cells responded to more than one stimulus. Different excitatory stimuli had different optimum intensities for the same cell. Different cells had different optimum intensities for the same stimulus. There was no obvious variation in stimulus-response relations with animal age. This work was supported in part by NSF Grant No. BMS75-02339.

18 LOCAL ACTIVE REGIONS IN RAT OLFACTORY BULB DURING OLFACTORY STIMULATION. Gordon M. Shepherd, John S. Kauer, and Frank R. Sharp, Yale Univ. Sch. Med., New Haven, Conn. 06510, and NIH, Bethesda, Md., 20014.

An important question in sensory physiology is the role played by spatial patterns of activity in the processing of sensory information. We have attempted to obtain evidence for this in the olfactory system by analysis of activity-related uptake of 2-deoxy-d-glucose (2DG). Rats were injected with 14C 2DG and exposed to different conditions of olfactory stimulation. After 45 minutes the animals were killed, and autoradiographs were prepared according to Kennedy et al. (Science 187:850, 1975). In animals in which one naris had been sutured closed several days before the experiment, there was less uptake of 2DG in most layers of the olfactory bulb on the operated side, as shown by lighter optical densities in the autoradiographs. In animals resting in their holders, breathing laboratory air, the olfactory bulbs often showed very small dots of increased density, localized in or near the glomerular layer. The smallest densities appeared to correspond in size to one or several glomeruli. They often appeared bilaterally symmetrical. Some animals were exposed to a continuously flowing stream of amyl acetate, camphor, or cheese vapors in a closed environment after 2DG injection. Under these conditions, the olfactory bulbs typically showed bilaterally prominent local spots or bands of increased optical density. These were usually located at the level of the glomeruli, but also spread to the adjacent nerve and external plexiform layers. These results demonstrate the most localized sites of CNS metabolic change seen to date with this technique. Preliminary results suggest that patterns of local activity may be different for different odors, and could therefore play a role in the processing of olfactory information.

19 LAMINAR ANALYSIS OF 2-DEOXY-D-GLUCOSE UPTAKE IN THE RAT OLFACTORY SYSTEM. Frank R. Sharp, John S. Kauer and Gordon M. Shepherd. Lab. Neurophysiol., NIMH, Bethesda, MD 20014 and Yale Univ. Sch. Med., New Haven, CT. 06510.

A new method for studying activity-related changes in metabolism of regions in the central nervous system is based on the uptake of 2-deoxy-d-glucose (2DG) (Sharp, Nsc. Abstr., p. 422, 1974). We have used this method to analyze differences in glucose metabolism between histological laminae of cortical structures, taking the olfactory bulb and olfactory cortex as model systems. Rats were injected with 14C 2DG and sacrificed after 45 min. The frontal telencephalon with attached olfactory bulbs was quickly removed and frozen; frozen sections were cut, dried, and autoradiographed on X-ray film. In all animals, the olfactory bulb showed a superficial layer of relatively light optical density corresponding to the outer layer of olfactory nerves. A broad band of increased density was typically present at an intermediate depth in the bulb; this extended from the glomerular layer superficially into the granular layer in the bulbar depths. The central bulb was of relatively light density. Thinner laminae could sometimes be discerned within the intermediate zone. In the prepyriform cortex, there was a superficial zone of relatively light density, corresponding to the lateral olfactory tract and its collaterals. Deep to this was a thin layer of increased density, corresponding to the molecular layer of the prepyriform cortex. In comparison, frontal neocortex showed a relatively uniform density, except in some sections where a thin band of increased density was present at the level of lamina IV. The results show that 2DG uptake can be used to delineate different histological laminae and that differences in uptake occur between layers rich in synaptic neuropil, neuronal perikarya, and unmyelinated and myelinated axons.

20 RESPONSE PATTERNS OF SALAMANDER OLFACTORY BULB UNITS TO STEP PULSES OF ODOR. John S. Kauer and Gordon M. Shepherd. Department of Physiology. Yale University School of Medicine, New Haven, Conn. 06510.

A major problem in olfactory physiology is to identify the basic types of single cell response to odor stimulation under defined conditions. For approaching this problem a method has been developed for delivering step odor pulses monitored during the experiment(Kauer, J.S & Shepherd, G.M., Brain Res. 85:108, 1975). In the present study we have applied this method to the salamander to examine previously suggested categories of response(Kauer, J.S., J. Physiol. 243:695, 1974). Extracellular unit responses were recorded from neurons in the mitral cell body layer as confirmed by dye-marking. Based on the types of activity first seen after the onset of the stimulus, the responses fell into two main categories: suppression and excitation. Sub-categories of these responses could be characterized in relation to odor pulses of different durations and concentrations. Initially suppressive responses typically outlasted the odor pulse(up to 8 secs. duration). These suppressive responses showed little or no change with different concentrations. A few initially suppressive responses were followed by a period of excitation 2-3 secs. after the stimulus onset. Initially excitatory responses showed maintained excitation for the duration of the pulses only if the concentration were held near threshold. This has been termed concentration tuning. When higher concentrations were presented an initial excitation followed by suppression was seen. A second period of excitation following this suppression was observed with longer lasting pulses. These studies have confirmed and extended the characterization of the previously described response categories and substantiated the concept of concentration tuning.

21 AFFERENT CONNECTIONS OF THE OLFACTORY CORTEX. Lewis B. Haberly* and Joseph L. Price. Dept. Anat., Wash. Univ. Sch. Med., St. Louis, Mo., 63110.

The afferent connections of the olfactory cortex are being studied in the rat with the horseradish peroxidase retrograde transport technique. Electrophysiological methods were used to guide the placement of the injections, which were made through micropipettes by pneumatic pressure.

Within the olfactory bulb, mitral cells are labeled throughout the bulb following injections of the prepiriform cortex, olfactory tubercle and anterior olfactory nucleus. Restricting the size of the injection decreases the intensity and number of mitral cells labeled, but in all cases the labeled cells are found in all parts of the bulb. Tufted cells are labeled following restricted injections into the olfactory tubercle and anterior olfactory nucleus but have been seen after injections of the prepiriform cortex only after multiple injections involving a large area of the cortex.

Within the prepiriform cortex a very high density of labeled cells is found in layers II and III and in the part of the claustrum deep to the prepiriform cortex (endopiriform nucleus) distant to small injections into the prepiriform cortex (which involve all layers of the cortex and the endopiriform nucleus). With increasing distance from the injection site, the high proportion of labeled cells is maintained, but the intensity of label in each cell decreases. Labeled cells are not found in the anterior olfactory nucleus, olfactory tubercle and lateral entorhinal area, although labeled cells are found in the prepiriform cortex directly adjacent to these areas. A few labeled cells are also found in the anterior cortical amygdaloid nucleus. A commissural projection from layers II and III of the prepiriform cortex to the opposite prepiriform cortex is revealed by large HRP injections.

Other cells labeled following injections of the olfactory cortex are situated in and deep to the ventral portion of the tenia tecta, at the junction of the vertical and horizontal limbs of the diagonal band, and in an area of the midbrain extending from the dorsal raphe nucleus to the ventral tegmental area.

These results support previous conclusions from experiments using anterograde axon tracing methods that both the mitral and tufted cells project upon the olfactory cortex, and that this projection is not topographically organized, although the tufted cell axons may be predominantly distributed to the anterior olfactory nucleus and olfactory tubercle. They also suggest that the anterior olfactory nucleus, olfactory tubercle and lateral entorhinal area do not contribute to the intracortical association projection which has been shown previously to arise from the prepiriform cortex.

22 TASTE AND SMELL IN AN AQUATIC VERTEBRATE: THE CHEMORECEPTION OF AMINO ACIDS IN CATFISH. John Caprio* and Don Tucker. Dept. of Biol. Sci., Florida State University, Tallahassee, Florida 32306.

Both gustatory and olfactory systems of the channel catfish (Ictalurus punctatus) are highly sensitive to the α-amino acids. This sensory overlap presents interesting questions concerning the taste-smell distinction in aquatic organisms. This abstract describes three distinguishing features of these chemosensory systems: (1) phasic-tonic response characteristics, (2) amino acid thresholds, and (3) structure-activity relations.

Time-averaged gustatory recordings were obtained from twigs of cranial nerve VII (facial) to the maxillary barbel on a Pt-Ir wire electrode. Metal-filled glass capillary electrodes tip plated with Pt-black (ball diameter 15-25um) were used to record summated responses from the surface of the olfactory epithelium. The electro-olfactogram (EOG) was recorded with calomel electrodes via Ringer-agar filled capillary pipettes.

The summated response of the maxillary barbel taste nerve to an amino acid stimulus reveals a quickly adapting phasic response with thresholds ranging between 10^{-9} and 10^{-12}M for the more effective amino acids (Caprio, Comp. Biochem. Physiol In Press). Both EOG and olfactory neural responses exhibit a phasic component followed by a tonic response which remains approximately constant throughout the stimulus duration. Olfactory thresholds determined by either EOG or neural recordings are estimated to range between 10^{-7} and 10^{-9}M. Power functions describe the relationships between the neural (gustatory or olfactory) or EOG responses and the stimulus concentrations, with exponents between 0.07 and 0.14 for the neural recordings; EOG exponents are somewhat larger.

Chemoreceptive specificity was determined with a number of amino acids and their derivatives. L-α-amino acids are the most effective stimuli, although certain D and β-amino acids are more stimulatory than some L-α-amino acids. Derivatives of the α-amino, α-hydrogen, or carboxyl groups of an amino acid are less stimulatory with one exception: esterification of the carboxyl group does not affect activity, indicating an ionically charged carboxyl group is not required for olfactory or gustatory responses.

A major difference between olfaction and taste in the catfish is the different structure-activity relations involving the side chains of amino acids. In the table are listed the six most effective amino acid stimuli for both chemical systems. All comparisons were made at 0.1mM, and the magnitude of the phasic neural response to each chemical is represented as a percentage of that produced by the standard, 0.1mM L-alanine. Mean, standard deviation (S.D.), and number of fish tested (N) are included.

	TASTE			OLFACTION		
Rank	Chemical	Mean±S.D.	(N)	Chemical	Mean±S.D.	(N)
1	L-Ala	100.0	30	L-Cysh	120.8± 8.0	9
2	L-Arg	86.6±11.0	22	L-Aba	107.2± 5.6	5
3	L-Ser	69.5± 9.1	22	L-Gln	104.6±11.2	6
4	L-Aba	65.7± 8.5	9	L-Met	103.5±10.0	5
5	L-Gln	60.6±10.1	5	L-nVal	101.3± 7.0	6
6	D-Ala	57.2± 5.7	9	L-Ala	100.0	20

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23 PERSISTENCE OF AXONAL AND SYNAPTIC FUNCTION AFTER AXOTOMY IN THE GARFISH OLFACTORY SYSTEM. Jeffrey A. Kugel* and Dexter M. Easton, Department of Biological Science, Florida State University, Tallahassee, Fl 32306 (SPON: R.L. Glendenning)

The status of impulse propagation and synaptic transmission was examined at specified times after section of the olfactory nerve in garfish. Axotomy was accomplished by amputation of the tip of the snout, and removal of the cell bodies of the olfactory axons, or by section of the nerve at various places along its 15-20 cm course to the olfactory bulb. The use of a suction electrode in both the in vivo bulb preparation and in the in vitro nerve preparation permitted synchronous excitation of the total olfactory nerve input to the olfactory bulb. The nerve compound action current declined exponentially with a half-life of about 7 days, in contrast to the bulbar response, which appeared undiminished in amplitude as long as 15 days after axotomy. Comparison of stimulus intensity-response curves for both preparations suggests that considerably less than total activation of the nerve fibers produces a maximal amplitude of response in the olfactory bulb. This apparent excess of olfactory input to the bulb over that necessary to evoke a maximal bulbar response may account for the seemingly longer persistence of synaptic function. (Aided in part by Psychobiology Program, Florida State University.)

24 THE OLFACTORY GLOMERULUS OF RAT. ELECTRON MICROSCOPY OF GOLGI STAINED MATERIAL. E. Ramon-Moliner. Dept. Anat., Sch. Med., Sherbrooke Univ. Sherbrooke, Quebec, Canada.

Following lead chromate substitution, the following ultrastructural observations were made: (1) Contrary to what one could have expected, the olfactory axons do not terminate in the form of freely ramifying branchlets scattered between the dendritic ramifications of the mitral cells, external granule cells, etc. They terminate in the form of complex polipoid formations, where they intermingle with other axons, presumably originating in adjacent regions. Only the surface of these polipoid formations provides synaptic contacts with the other glomerular components. (2) By contrast, the "dendritic" components terminate in the form of diffuse and widely scattered branches to form a neuropil matrix which contains the axonal polipoid aggregations. (3) The dendritic processes, at least those of the mitral cells and the external granular cells, offer considerably greater surface of contact with other dendrites than with the afferent axons. (4) The contacts between dendrites and axons were either tangential or in the form of "tunnels" which individual dendrites create within the mass of the axonal polipoid formations. (5) No individual isolated axons were seen surrounded by polidendritic enveloping formations (in contrast to the patterns described in the cerebellar glomerulus, or the L.G. glomerulus). (6) The ramifications of the mitral cell tufts are more sparse than those of the external granule cells. (7) Neuroglia processes were revealed as interstitial narrow spaces interposed between the other processes.

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25 TASTE PREFERENCE-AVERSION OF THE MONGOLIAN GERBIL TO METHYL MANNOSIDES. W. Jakinovich, Jr., I. J. Goldstein* and B. Oakley. Dept. Zool. and Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48104.

Recently it was reported that crystals of β-D-mannose taste bitter to humans (Stewart et al., Nature, 234, 200 (1971)). This finding confirmed earlier reports that the β-anomer of D-mannose in solution is bitter while the α-anomer has a sweet taste (Steinhardt, et al., Science, 135, 367 (1962); Pangborn and Crisp, Experientia, 22, 612 (1966)). Taste preference experiments cannot be easily carried out with these compounds because D-mannose dissolved in water mutarotes to an equilibrium mixture of pyranose, furanose, and acyclic isomers. To avoid the problem of mutarotation one may use methyl glycosides. Taste preference or aversion of the Mongolian gerbil (Meriones unguiculatus) to the two anomeric methyl glycosides of D-mannopyranose was measured and these responses were compared to those obtained with sucrose and quinine.

Naive gerbils, fed ad libitum, housed individually, were kept on a 12 hr light - 12 hr dark cycle and maintained at a constant temperature (25° ± 1°). Taste solutions were available to the gerbils from two inverted graduated cylinders fitted with glass drinking tubes. During the test one cylinder contained distilled water and the other contained taste solution. The concentration of taste stimulants was changed every 48 hr, and every 24 hr the left-right position of a given taste solution was determined randomly. All tastants were presented in an ascending series of 1/3 log molar concentration steps. Sucrose was tested first, and was followed in order by quinine, methyl α-D-mannopyranoside and methyl β-D-mannopyranoside.

The behavioral data are summarized in Figure 1. The animals clearly rejected quinine and methyl β-D-mannopyranoside and preferred sucrose and methyl α-D-mannopyranoside to water. The threshold of acceptance is lowest for sucrose. Concentrations of methyl α-D-mannopyranoside greater than 0.2 M tended to crystallize on the drinking tube and therefore were not used.

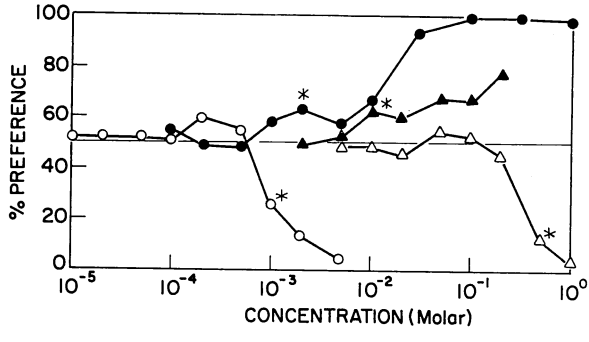

Figure 1. Mean preference of gerbils (N = 6) for quinine (○), sucrose (●), methyl α-D-mannopyranoside (▲), and methyl β-D-mannopyranoside (△).

Percent preference = $\frac{\text{test soln intake}}{\text{total fluid intake}} \times 100\%$

For each curve, * indicates the point of initial significant preference or aversion ($p < 0.05$).

Methyl β-D-mannopyranoside is aversive to the gerbil. These results are similar to findings in humans that the α-anomer of mannose is sucrose-like and the β-anomer is quinine-like. Methyl α-D-mannopyranoside has a sweet quality when tasted by us while methyl β-D-mannopyranoside was extremely bitter.

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26 THE ROLE OF COMPLEX MASKERS IN THE PRODUCTION OF ATTENTIONAL ASYMMETRIES. Kenneth M. Heilman, Dawn Bowers,* and Wiley Rasbury*. Department of Neurology and Clinical Psychology. University of Florida College of Medicine, Gainesville, Florida 32610.

The purpose of this study was to ascertain if there are ear asymmetries in the ability to discriminate tonal sequences (from the Seashore Measure of Musical Talents) in the presence of complex distractors (music and speech).

In the first experiment sixteen college students were presented tonal sequences monaurally (to either the right or left ear) at sensation level (threshold and 40 dB). A music masker (symphonic music) was also presented to the same ear at 30 dB above threshold. The data was analyzed by comparing the following. number of subjects with a right ear effect (REE), number of subjects with a left ear effect (LEE), number of subjects who performed equally well with either ear (NEE). When no masker was used there was no significant difference ($p > .05$); with a music masker the number of subjects with REE was significantly higher than those with LEE or NEE ($p < .001$).

In a second experiment 16 new subjects were used. In this experiment the masker was two people simultaneously reading the same story out of phase and was presented at 80 dB above threshold. Otherwise the experimental paradigm was unchanged. Analysis of data revealed that as in Experiment I with no masker there was no significant difference in the frequency of ear effects ($p > .05$), however, with the voice masker there were significantly more subjects with a LEE than those with a REE or NEE ($p < .05$).

Although in man each ear projects to the ipsilateral as well as contralateral cortex, the contralateral projections appear to predominate. Our data suggests that the left hemisphere which perceives speech stimuli better than the right hemisphere is also more distracted by these stimuli and that the right hemisphere which perceives musical stimuli better than the left hemisphere is also more distracted by these stimuli.

27 RESPONSE TO DELAYED AUDITORY FEEDBACK IN PATIENTS WITH HEMISPHERIC LESIONS

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Studies of delayed auditory feedback (DAF) have suggested that DAF may have considerably different effects on the speech of aphasics than on that of normals. The present study was designed to investigate the response of patients with lateralized cerebral lesions to DAF on verbal and nonverbal tasks at several levels of difficulty. Subjects were three groups of inpatients: 10 with left hemisphere lesions, 9 with right hemisphere lesions, and 9 with no evidence of CNS disease. Each subject was presented five tasks under conditions of simultaneous (SAF) and delayed auditory feedback (DAF). Three tasks were verbal: (A) counting from one to ten, (B) repeating a high probability sentence, and (C) repeating a low probability sentence. The two nonverbal tasks were (D) tapping at a steady rate and (E) tapping repetitions of a simple rhythmic pattern. The subject's response (either speech or tapping pressure on a transducer) was tape recorded and fed back via binaural headphones either simultaneously or with a 360 msec. delay.

The present study presents an analysis of the duration of verbal and nonverbal responses in the form of DAF/SAF ratios, expressed as the percent of increase due to DAF. It was hypothesized that on verbal tasks left hemisphere subjects would show a response to DAF different from that of controls and right hemisphere subjects. Analagously, right hemisphere subjects were expected to show a DAF effect different from that of controls and left hemisphere subjects on the nonverbal tasks. Figure 1 reveals that for normals the extent of disruption due to DAF increased as the difficulty of the task increased, from counting to repeating a sentence on verbal tasks and from tapping to repeating a rhythm on nonverbal tasks. Left hemisphere subjects showed an abnormal pattern on verbal tasks but paralleled control results on nonverbal tasks, while right hemisphere subjects showed an abnormal pattern on nonverbal tasks and paralleled control performance on verbal tasks.

It appears that subjects with lateralized cerebral lesions do show abnormal DAF effects on tasks processed in the impaired hemisphere, although both left and right lesioned subjects showed DAF effects on all tasks. The difficulty level of the task seems to be an important determiner of the extent of DAF effects observed in subjects processing tasks in which they are not using their impaired hemisphere. Considerable variability was noted between individual subjects in the impaired groups, perhaps due to differences in the intrahemispheric locus of lesions, so that one cannot safely speak of uniformly predictable effects of DAF simply as a function of hemispheric localization.

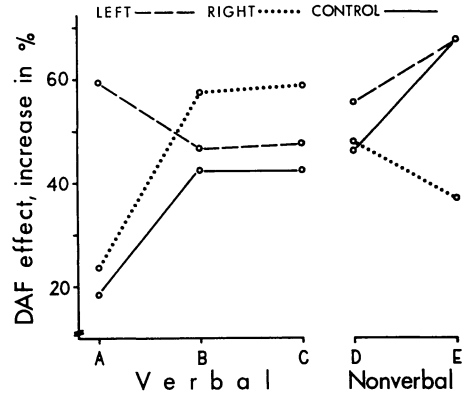


Figure 1. Mean percent duration increase in performance of three verbal and two nonverbal tasks, due to delayed auditory feedback.

28 SPEAKER RECOGNITION BY THE CENTRAL NERVOUS SYSTEM

Manfred Clynes, The Biocybernetic Institute, La Jolla, and UCSD, La Jolla, California 92037

Although differentiation between individual speakers is fairly successfully done by computers and voice prints leading to recognizable patterns for visual or analytic discrimination, little is known about how the nervous system itself performs this auditory function (The problem is quite different from recognizing speech, phonemes and expressive or syntactic inflections).

It was observed earlier in our laboratory that even small steady changes of speed of a tape recording of a speaker's voice interfere with recognition of the speaker's identity, while leaving intact recognition of speech, syntactic, and expressive inflections. This also raised the question whether alteration of speech speed (rate), or of pitch, or both, played a key role in disabling speaker recognition. In order to test this question, an Eltro Rate Changer was used allowing the rate of speech to be changed without changing the pitch, or vice versa. The device operates without significant loss of fidelity as tested by using it twice, the second time to restore the original rate or pitch. Pairs of speakers of the same sex and of comparable voice characteristics were chosen, and 30 second portions of their speech were presented to subjects who could identify the speakers correctly, without error. The same speech segments were then presented, altered in rate without changing pitch, and also in pitch without changing rate, in increasing or decreasing increments chosen at random.

Starting from small 1% increments it was found that when the change reached only 6% (slightly less than a semitone), the change in pitch was sufficient to disable speaker recognition, from errorless to no better than chance, but a 6% rate change still permitted errorless identification. All ten subjects tested in this way showed lack of impairment of recognition when rate was changed only, but not pitch - while showing strong impairment of recognition with changes in pitch, at unaltered rate.

Since when a tape recording is changed in velocity, all frequencies are changed in the same proportion and thus all relative clues remain, the results signify that the nervous system has a surprising capacity, in effect, of remembering absolute pitches for speech, a function which is known to be developed and used only by some for musical sounds. Thus it seems most of us appear to have a stable reference or an aspect of absolute sense of pitch, for patterns, or gestalts, of speech.

29 EFFECTS OF AUDITORY CORTICAL LESIONS ON THE DISCRIMINATION OF SPATIAL LOCATION OF AUDITORY STIMULI. Jack B. Kelly and Steven J. Glazier.* Department of Psychology, Carleton University, Ottawa, Canada, K1S 5B6.

Albino rats were tested following auditory cortical lesions on a discrimination between spatially separated auditory stimuli. The animals were trained to detect a change from a series of left clicks to a series of right clicks presented at a rate of one a second. The behavioral measure of performance was determined by conditioned suppression of an ongoing licking response. The minimum angle of separation between stimuli which could be detected was determined for normal and brain operated animals. Animals with bilateral lesions of auditory cortex could easily discriminate between left and right clicks and minimum discriminable angles were not substantially greater than normal. The results are consistent with the view that auditory cortex is not essential for discriminating differences in sound location.

30 A COMPARISON OF EVOKED POTENTIAL, SINGLE UNIT AND MULTIPLE UNIT RESPONSES IN THE COCHLEAR NUCLEUS DURING REPETITIVE ACOUSTIC STIMULATION. Chi-Ming Huang* and Jennifer S. Buchwald. Dept. Physiology, BRI, Mental Retardation Research Ctr., UCLA Med. Sch., Los Angeles, 90024.

During acoustic habituation procedures, the evoked response has been widely utilized as an index of response plasticity at various levels of the auditory pathway. Such studies have indicated that acoustic evoked responses recorded at cortical and thalamic levels usually show decrements while those from the brainstem acoustic relay nuclei usually do not. In contrast, multiple unit responses from the brainstem nuclei have consistently shown marked decrements during repetitive acoustic stimulation. Such contrasting data preclude a conceptualization of the central nervous processes initiated by exposure to repetitive or non-significant acoustic stimuli, processes of great importance in lieu of the complex acoustic environment out of which normal adaptive behavior must emerge. Thus, we believe that a systematic study of excitability and recovery functions at various levels of acoustic information processing, utilizing both evoked potential and single unit measures, has become a requisite for coherent understanding of acoustic habituation. This report presents evoked potential, single unit and multiple unit data collected from the cochlear nucleus of adult cats during sequences of repetitive acoustic stimulation. In each sequence of 25 trials, stimulus repetition rate (inter-trial interval) or stimulus duration was systematically varied so that a temporal range from 10 msec to 30 sec was parametrically scanned. Data from these experiments have indicated the following: 1) the evoked response and initial 10 ms of single unit discharge showed similar excitability-recovery characteristics, i.e. the amount of response decrement was similar, the time course of the decremental process was similar and the recovery time course of response was similar; 2) the evoked response and initial unit discharge component decremented when stimuli were repeated faster than 5/sec; 3) the decremented evoked response and initial discharge component showed complete recovery within 500 ms after stimulus cessation; 4) a sustained unit response component, elicited by tone durations of 500 ms or more, showed qualitatively different excitability-recovery functions than did the evoked response or initial unit discharge component and these agreed with the excitability-recovery functions of sustained multiple unit responses; 5) the sustained response component decremented when stimuli were repeated faster than 1/10 sec; 6) the sustained response component showed complete recovery within 60 sec after stimulus cessation; 7) the evoked potential and initial discharge component were characteristically different than the sustained discharge component in these decremental and recovery processes irrespective of the unit's initial response latency or ongoing level of firing frequency. These data indicate two temporally disparate excitability-recovery processes in the cochlear nucleus and explain some of the contrasting results of evoked potential and multiple unit studies previously reported. (Supported by USPHS Grants NS-05437, HD-04612, and HD-05958).

31 DIFFERENTIAL CHANGES IN FREQUENCY FOLLOWING AND EVOKED RESPONSES IN SOME PRIMARY AUDITORY AND RETICULAR STRUCTURES. C.L. Faingold and D.M. Caspary, Dept. Medical Sciences, Southern Illinois Univ. Med. Sch., Springfield, Ill. 62708.

Responses of primary auditory structures to tone burst stimuli include the auditory evoked potential (EP) and the frequency following response (FFR). Although EP's are observed in both primary and non-primary structures, the FFR has not been previously observed in non-primary structures (Marsh and Worden, 1968). Averaged potentials were recorded with bipolar electrodes from a non-primary reticular structure (central tegmental field or CTF), the inferior colliculus (IC), and the medical geniculate body (MGB) in locally anesthetized, paralyzed cats. The FFR was observed in IC and cortex but not MGB. However, in contrast to previous findings, an FFR was recorded consistently from the non-primary site.

Differential modifications of EP and FFR were observed depending on anatomical location, masking level and drug treatment. Progressive vertical placements in IC showed consistently different evoked response waveforms and some FFR frequency specificity. When the masking intensity was held constant and the intensity of the tone burst was progressively decreased, the EP in IC and cortex was reduced, while the FFR was relatively unaffected until total masking occured. The relative latencies of FFR and EP differed with stimulus intensity and vertical depth in IC. Pentobarbital administration greatly reduced the amplitude of the FFR and EP in CTF at doses which affected the IC responses to a much lesser degree. Within IC the FFR was reduced in amplitude by pentobarbital at doses which left the EP relatively unchanged. The differential changes in FFR and EP may indicate that separate mechanisms are involved in the generation of these responses. These findings may be indicative of processes subserving the human FFR responses which have been proposed as a method of evaluating brain stem auditory function (Moushegian et al., 1973).

32 AUDITORY CORTICAL AREAS OF MAN. Gastone G. Celesia. Dept. Neurology, University of Wisconsin Center for Health Sciences, Madison, Wisc. 53706.

Average responses to clicks were recorded from the exposed human cortex of 19 adult patients during surgery for the treatment of intracranial diseases. 323 cortical points were examined with no more than 45 points being explored in a single patient. Auditory evoked responses were obtained from 2 areas. Short latency potentials were recorded on the superior surface of the temporal lobe corresponding to the transverse temporal gyri (see figure 1). These responses consisted of two positive waves P_1 and P_2 separated by a negative wave N_1. P_1 had a mean latency of 14.7 ± 1.5 msec, N_1 a mean latency of 19.1 ± 2.6 msec and P_2 a mean latency of 32.2 ± 4.1 msec. Responses to stimulation of the contralateral ear were of higher amplitude than responses to stimulation of the ipsilateral ear. Responses of smaller amplitude and longer latency were obtained from the superior temporal gyrus and the upper lip of the sylvian fissure. These responses had a mean peak latency for P_1 of 40.2 ± 2.6 msec, for N_1 of 62.5 ± 12.5 msec and for P_2 of 97.7 ± 17.2 msec. It is concluded that the cortical auditory region of man may be subdivided in 2 major areas: an area on the supratemporal plane representing the primary auditory area or A_1 and a region surrounding A_1 which perhaps comprises 2 areas, one on the superior temporal gyrus and one on the upper bank of the sylvian fissure including frontal and parietal operculum. The area on the superior temporal gyrus yielded responses with shorter latencies than the one from the area above the sylvian fissure (cross hatching in figure 1).

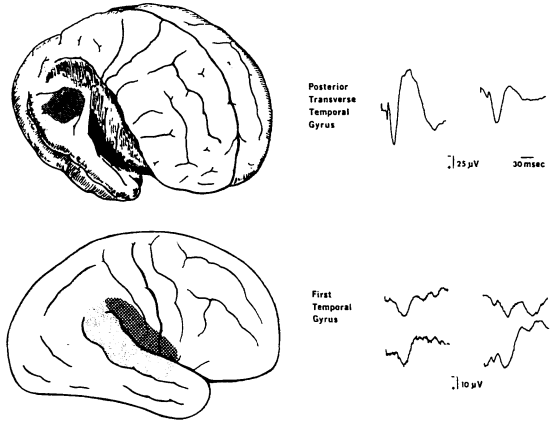


Fig. 1. Summary diagram of auditory cortical areas of man. In hatching primary auditory area. Cross hatching and stippling represents the extent of lateral cortical surface activated by clicks. The responses from the primary auditory area of two patients are shown on the right side. On the lower part of the figure are the responses from the superior temporal gyrus in the same two subjects.

33 ENVIRONMENTAL INFLUENCES ON THE DEVELOPMENT OF AUDITORY NEURONAL RESPONSES IN AVIAN EMBRYOS. Nigel K. Woolf*and Robert R. Capranica. Section of Neurobiology and Behavior, Cornell Univ., Ithaca, N.Y. 14853.

Neurons in the cochlear nuclei (nucleus magnocellularis and nucleus angularis) of Peking duck embryos (Anas platyrhynchos) become responsive to tonal stimuli in an orderly spatial and temporal sequence [Konishi, PNAS 70, 1795 (1973)]. We have confirmed that, under standard artificial incubation conditions, increasing developmental age is accompanied by a gradual lowering of auditory unit thresholds and an increase in their overall range of frequency sensitivity. The development of gross behavioral responses of embryos to auditory stimulation [Gottlieb, Development of Species Identification in Birds, U. Chicago Press, 95-121 (1971)] is closely correlated with this concomitant maturation of sensitivity in the developing auditory system.

Premature exposure of embryos during incubation to auditory stimuli advances the time course of gross behavioral responsiveness, while incubation in acoustic isolation retards the onset of the behavioral responsiveness. Embryos were incubated under conditions of early auditory stimulation or acoustic isolation to explore the limits to which plasticity in the developing auditory system conforms to that of the gross behavioral responsiveness. Single unit recordings were conducted on embryos from day 19 of incubation through two days post-hatching. The results will be presented and the implications of these data on the extent of plasticity of the avian auditory system during early development will be discussed.

(Supported by NIH 1F22NS02675-01 and NS-09244)

34 CHANGES IN THE POSITIVE ENDOCOCHLEAR POTENTIAL (EP) OF THE CAT PRODUCED BY INTRAVENOUS BUMETANIDE. R. Don Brown. L.S.U. School of Medicine in Shreveport, P. O. Box 3932, Shreveport, La. 71130.

Bumetanide is a new "loop" diuretic that is presently under clinical investigation in this country; it is marketed abroad under the trade name Burinex (Leo Laboratories; England). Brown (Neuropharmacol., in press) has demonstrated that it is ototoxic in cats (produces N_1 depression when administered acutely); having a relative ototoxic liability less than that of furosemide and much less than that of ethacrynic acid (Brown, Toxicol. Appl. Pharmacol. 31: 270-282, 1975). Since the ototoxicity of ethacrynic acid could, among other possibilities, be due to its causing a reduction of EP (see review by Brown, Medikon, in press), the effect of iv bumetanide on the EP of cats was investigated. Young, healthy cats were anesthetized with intraperitoneal Dial-Urethane (0.65 ml/kg), one of the tympanic bullae exposed and the cochlear round window membrane removed. Glass micropipettes (5-8 μ tip diam.) were filled with 0.15 M KCl; the tip of one being placed through the basilar membrane into the scala media, the other into a neck muscle. Intravenous bolus injections of bumetanide were found to produce a reduction of EP. This ranged from a 5-10 mV change at 2.0 mg/kg iv bumetanide to an extremely large alteration when 10.0 mg/kg was given. At the larger dose, EP was 15-20 mV negative at the peak effect. In addition, the time course and extent of change in EP produced by the different doses of iv bumetanide appeared to correlate with the changes seen in N_1 in the previously mentioned bumetanide study.

(This investigation was supported by NIH research grant number 1-R01 GM 22-97-01 from the Institute of General Medical Sciences.)

35 RESPONSE CHARACTERISTICS OF CAT AUDITORY NEURONS LOCATED AROUND THE LATERAL SUPERIOR OLIVE. C. Tsuchitani. Sensory Sciences Center, Graduate School of Biomedical Sciences, University of Texas Health Sciences Center at Houston, 77025.

Single unit discharges were recorded extracellularly with stainless steel microelectrodes from auditory units located lateral to the medial superior olive (MSO). Stimuli consisted of monaurally or binaurally presented tone bursts. The response measures obtained were: effective ear, nature of effect, stimulus frequency representation, maximum output, latency of response, and temporal pattern of discharges. The location of the unit studied or the end of the electrode tract and the MSO cell layer were marked with electrolytic deposits of iron from the recording electrode. Following an experiment the location of units studied were determined histologically. All auditory units loacted lateral to the MSO were excited by stimulation of the ipsilateral ear. Units of the dorsal and dorsolateral periolivary cell groups were not effected by stimulation of the contralateral ear, were narrowly tuned, had long latencies and low maximum output. Units of the lateral nucleus of the trapezoid body and the ventralateral periolivary groups were either uneffected or inhibited by stimulation of the contralateral ear. Most units located along the posterior edge of the LSO were not affected by stimulation of the contralateral ear and had longer latencies than LSO units. The cell groups surrounding the LSO appear to be tonotopically organized; with low frequency sensitive units located laterally and progressively higher frequency sensitive units located more medially. Supported by NINDS research grant.

36 EFFECT OF AUDITORY NEOCORTEX ABLATION ON DISCRIMINATION OF CLICK RATES IN THE CAT. Jerry Cranford, Makoto Igarashi, James Stramler, * Dept. Otorhinolaryngology & Communicative Sciences Baylor College of Medicine, Houston, Texas, 77025

Substantial experimental evidence exists which indicates that ablation of auditory cortex severely disrupts the discrimination of changes in the temporal aspects of auditory inputs (e. g., 1, 2, 3). The present report describes the unexpected results of a recent investigation which revealed that auditory decorticate cats exhibit a remarkable acuity in temporal discriminations involving differences in the rate of presentation of clicks. Six adult cats were trained with a shock avoidance procedure to discriminate increases from decreases in the rate of presentation of a continuous train of clicks. Four cats were trained to cross to the opposite compartment of a shuttle box when an on-going train of 5/sec. clicks changed to a rate of 2/sec. (positive trials) and to inhibit crosses when the 5/sec. train changed to one of 10/sec. (negative trials). The remaining two cats were trained to cross to 10/sec. changes and withhold crosses to the 2/sec. change. Cats received 12 trials each day (6 positive and 6 negative) with intertrial intervals (ITIs) ranging between 30 and 90 sec. The voltages to the speakers were varied on special "mock trials" to insure that the cats were not responding to loudness changes. After learning this task three cats received bilateral ablation of auditory cortical subdivisions AI, AII, Ep, SII, and I-T while the remaining three cats had these auditory regions plus the "association" cortex of the anterior lateral gyrus and the anterior and middle portions of the suprasylvian gyrus bilaterally ablated.

Postoperatively, all cats, while initially exhibiting amnesia for the original discrimination, attained preoperative performance levels in approximately the same number of trials as required before surgery. All cats were then given a series of special post hoc tests to further investigate their capacities for discriminating click rates. The first test involved presenting 2 and 10/sec. click trains against a silent background (i. e., without 5/sec. neutral clicks). In four sessions (each containing 6 positive and 6 negative trials) the six cats averaged 92. 6% correct on positive and 97. 3% correct on negative trials. The second test involved reducing the differences between positive and negative click rates in an attempt to determine the cats' thresholds for perceiving rate changes. With this procedure it was found that the cats could correctly discriminate rates of 4 and 6/sec. With the ITIs filled with 5/sec. clicks the cats averaged 77. 5% correct on positive and 98. 7% correct on negative trials (t-test, $p < .01$). In four additional sessions with silent ITIs the corresponding scores were 79. 5% and 88. 5% correct, respectively ($p < .01$). No performance differences were seen with the two lesion groups. The three cats with the largest lesions received a further test to determine whether they could discriminate rates of 4. 5 and 5. 5/sec. In two separate sessions, which contained silent ITIs, the cats averaged 63. 9% correct on positive and 72. 2% correct on negative trials ($p < .10$). These results indicate the need for renewed investigation of the role of neocortex in auditory temporal discrimination.

[1] Allen, W. F. Amer. J. Physiol. 18 (1945) 415-428
[2] Diamond, I. T., Neff, W. D. J. Neurophysiol. 20 (1957) 300-315
[3] Scharlock, D. P., et al. J. Neurophysiol. 28 (1965) 673-681

Supported by a grant from The Deafness Research Foundation

37 LOUDNESS FUNCTION AS REFLECTED IN THE STAPEDIUS-MUSCLE REFLEX.
J. J. Zwislocki. Institute for Sensory Research, Syracuse University, Syracuse, N.Y. 13210

Since S. S. Stevens concluded that sensation magnitudes follow power rather than logarithmic functions of stimulus intensities, several attempts have been made at demonstrating similar functions in the neurophysiological domain. The attempts paid off for cutaneous touch receptors and gustatory receptors. In the auditory system the intensity characteristics of primary neurons differ considerably from the loudness function which has a smaller slope and an intensity range that exceeds that of the primary neurons by at least 10 log units. The measured whole-nerve action potentials follow the loudness function in their magnitude, but saturate at about 10 log units above the auditory threshold, whereas the loudness continues to increase at a constant rate. The saturation may be due to an experimental artifact, but this has not been proven. Recent measurements of the stapedius reflex in the middle ear have demonstrated that the reflex magnitude continues to grow parallel to the loudness function well above the measured saturation level of the acoustic nerve. Since the reflex depends on the output of the nerve, it is likely that the saturation effect arises from an artifact and that the whole-nerve response does provide an adequate code for loudness. The muscle-reflex function was measured by means of acoustic-impedance changes at the eardrum.

38 RECEPTOR SYNAPSES WITHOUT SYNAPTIC RIBBONS IN THE COCHLEA OF THE CAT. Rosemary A. Dunn* (SPON: D. K. Morest). Dept. Anat., Harvard, Boston 02115.

This report describes recepto-neural junctions thought to be chemical synapses without synaptic ribbons or vesicle aggregates. Such synapses occur between the outer hair cells of the cochlea and auditory nerve fibers in adult cats anesthetized with sodium pentobarbital (30 mg/kg) and perfused with an aldehyde fixative through the vascular system and the cochlea simultaneously. These synapses are formed by flat or indented junctions having symmetric membrane complexes associated with smooth endoplasmic reticulum and coated vesicles in the outer hair cell cytoplasm. Inner hair cells have recepto-neural junctions with asymmetric membrane complexes; synaptic ribbons and vesicles gather at one type of junction, while the other type associates with endoplasmic reticulum. Gap junctions are not seen at cochlear synapses but do link supporting cells. To rule out some possible sources of artifact, one cat was anesthetized with ketamine (35 mg/kg) and, following unilateral stapedectomy, white noise at an overall intensity of 70 $\pm$ 10 db SPL was delivered to the unoperated ear at the pinna, so as to minimize contralateral spread, for a period of five minutes before and during the perfusion. The results in both cochleas are comparable to those in unstimulated cats. The findings suggest that inner and outer hair cell synapses have different properties and raise questions about the role of synaptic vesicles and endoplasmic reticulum in the storage and release of transmitters.

(Supported by The Deafness Research Foundation and, USPHS grants 5 T01 GM00406 & 5 R01 NS06115)

39 FREEZE-FRACTURED SYNAPSES IN THE OUTER HAIR CELL REGION OF THE ORGAN OF CORTI. R. L. Gulley and T. S. Reese. LNO & LNNS, NIH, Bethesda, Md.20014

Synapses in the outer hair cell region of the chinchilla organ of Corti were studied with the freeze-fracture technique. Afferent terminals were clustered at the base of hair cells, indenting their plasma membranes. Each afferent terminal in contact with a hair cell was crowned by a field of uniformly large particles on the inner half of its plasmalemma. The adjacent hair cell membrane was marked by patches of smaller particles on its cytoplasmic half and by numerous plasmalemmal deformations of the type found where cytoplasmic vesicles or other internal membrane sacs are fused with the plasmalemma. These plasmalemmal deformations, as well as the asymmetry of the membrane specializations, suggest that the hair cell makes a chemical synapse with the afferents. Efferent terminals were larger than afferents and were indented by hair cells and afferents. Patches on the inner half of the plasmalemma of efferents had clusters of large particles with intervening clear spaces and plasmalemmal deformations of various sizes which, in these respects, were typical of the presynaptic membrane specializations found at chemical synapses. No special distribution of particles was found in the plasmalemma of afferents opposite the efferent presynaptic specialization but the hair cell membrane in contact with the efferent terminals had large clusters of medium-sized particles on its cytoplasmic half. While it is not yet clear whether these particles are part of the subsurface cisterns which lie under the hair cell membrane opposite efferents, or whether they are a postsynaptic specialization, the efferents appear to form chemical synapses with afferents and hair cells. However, the differences in structure of the postsynaptic membrane at these two types of synapses suggests that they may differ from each other in their mode of action. None of the outer hair cell synapses are likely to be electrotonic because no gap junctions were found.

40 THE NEURONAL ORGANIZATION AND EFFERENT CONNECTIONS OF THE INFERIOR COLLICULUS OF THE RAT. D. K. Ryugo* and H. P. Killackey (SPON: N. M. Weinberger). Dept. of Psychobiology, Univ. of Calif., Irvine 92664.

The inferior colliculus (IC) can be subdivided into a number of constituent cell groups. We have examined the neuronal organization of this structure in Golgi, Nissl, and fiber stained material, and have subdivided it into five nuclei on the basis of dendritic morphology and orientation: 1) the central nucleus(CN) constitutes the major core of the IC and is composed primarily of bitufted neurons whose perikarya and dendrites are aligned in rows producing a pattern of laminar-like organization; 2) the intercollicular nucleus(IN) contains medium-sized neurons with disc-shaped dendritic fields oriented perpendicularly to the long axis of the fibers of the IC commissure; 3) the pericentral nucleus(PC) is a sheet of small neurons which forms a dense interdigitating dendritic nest which envelops all of the IC; 4) the dorsomedial nucleus(DM) contains medium-large neurons with radiate-type dendrites with no particular orientation; 5) the external nucleus(XN) contains large radiate neurons, remniscent of isodendritic types observed in other multiple-input regions, and is situated between the CN and PC along the lateral edge of the IC.

The ascending connections of the IC were determined with the Fink-Heimer method. Discrete electrolytic lesions were placed in various subdivisions of the IC and the animals sacrificed 6 days postlesion. The confoundment of fibers of passage made it impossible to trace the connections of IN, PC, and DM. The XN projects only to the ipsilateral medial division of the medial geniculate body (MGm). The CN projects ipsilaterally to the ventral division of the medial geniculate body(MGv) in a point-to-point fashion suggesting a lamina-to-lamina connection from CN to MGv, and to XN. Only the dorsal aspect of the CN has a contralateral projection: it projects to the homotopic point in the contralateral CN, to the XN, and very diffusely to the MGv.

The neuronal organization of the rat IC is remarkably similar to what has been reported in other species (Geniec and Morest, 1971; Rockel and Jones, 1973). Furthermore, these results suggest that the differential telencephalic projections of MGm and MGv (Ryugo and Killackey, 1974) are maintained at the midbrain level by XN and CN respectively.

Supported by NSF Grant #GB 41294.

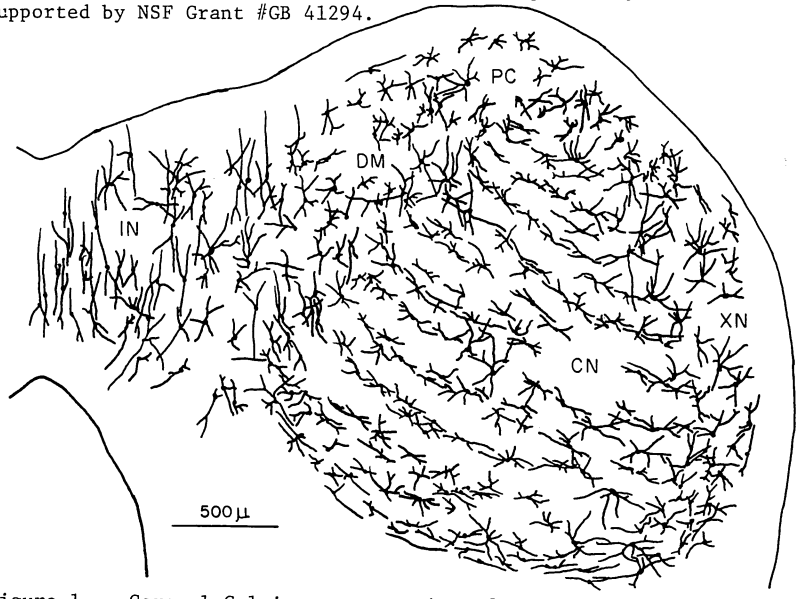


Figure 1. Coronal Golgi reconstruction of the inferior colliculus

41 THE RELATIONSHIP OF THE FELINE TEMPORAL CORTEX TO THE MEDIAL GENICULATE BODY. S.Horenstein and T. Yamamoto.* St.Louis Univ.,St.Louis, Mo.63104.

The reciprocal relationships of the ectosylvian temporal cortex of the cat were studied by means of retrograde axoplasmic flow and nerve fiber degeneration. Lesions were made in cortical areas A_I, A_{II}, EP, SF, and IT, the insular auditory cortex. Small lesions were also made in the dorsal portion of EP. The entire ectosylvian region was shown to receive or send projections to the medial geniculate body. The terminals and cells were all found ipsilateral to the site of the lesion or injection. Most terminals were found in the ventral portion of the medial geniculate's principal (GMp) portion though some were found in the pars dorsalis. Others were located in the magnocellular portion. The A_I and SF cortex bore a precise reciprocal relationship to the rostral ventral portion of GMp. A few fibers were found in the rostral dorsal (PD) and magnocellular (GMm) portions of the nucleus. Both small and large lesions disclosed spatially bound reciprocal pathways. The A_{II} cortex on the other hand bore its principal relationship to the PD and GMm portion. Lesser projections were found in the PV of the nucleus. HRP injections disclosed the cell bodies of fibers afferent to the A_{II} cortex to be mainly in the GMm and posterior portions of GMp. Degenerating fibers, however, were found rather more diffusely in the nucleus and were not bound spatially to the region of the cells labeled by HRP. The EP cortex was regarded as two areas, one ventral (EPv) which is commonly regarded as auditory, and the other more dorsal (EPd) of uncertain significance. Fibers from EPv were found both in the GM and the posterior nuclei of the thalamus. Those in GM were dispersed in the caudal PD. These fibers projected to regions which contained no cell bodies stained by HRP injection of cortex and hence were not spatially bound to the cellular field which projected to the cortex. HRP containing cells were found in rostral PV of GMp. EPd, however, received fibers from the posterior group of the thalamus and also from caudal PD. Terminal degeneration was found in PD. The IT cortex was unique as some afferent cells were mesencephalic being found posterior to GM in the so-called parabrachial nucleus (PBN). Other cells containing HRP granules were found in GMm and caudal PD. This study enlarges upon knowledge of the structural relationships of the temporal cortex and the medial geniculate body. All areas somehow relate to the principal portion of GM. Their unique patterns of projection, however, suggest that each is functionally different. A_I, a region which is tonotopically organized, responds to acoustical stimulation at shortest latency and hence appears in direct line from the cochlear nucleus via the exclusively auditory PV. Its spatial bound recurrent projection to PV may serve a tuning function. A_{II},which generates auditory potentials at longer latency, receives afferents from polysensory GMm as well as PD. Its projections are not bound to their cellular field. Thus, it would appear that the function subserved by this system may be polysensory and committed at a diencephalic level. EPv also gives rise to long latency auditory evoked potentials. Its afferents are auditory as they originate in GMp but its efferents reflect widely in GM and the posterior thalamus where they may interact with other modalities. EPd was clearly shown by this study to receive auditory afferents which are sparse and highly focal and probably are only a small part of EPD's afferents. IT is unique as some of its afferents originate in the brainstem and its projections to GMm may indicate its involvement in reflex reactions such as auditory startle.

42 AUDITORY THALAMO-CORTICAL PROJECTIONS IN THE CAT: A STUDY USING RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE. D. Raczkowski* and J. Winer* (SPON: I.T. Diamond). Dept. of Psychol., Duke Univ., Durham, N. C. 27706.

This study was undertaken to clarify conflicting results of studies of thalamo-cortical projections using anterograde and retrograde degeneration methods. According to the experiments using retrograde degeneration the rostral half of the principal division of the medial geniculate body (GM), which largely corresponds to the ventral division of Morest (GMv) (J.Anat., 98: 611-630, 1964) projects to AI; the caudal extremity of the medial geniculate body (GMc) projects to the insular-temporal area; and the magnocellular division (GMmc) and the anterior extremity of the posterior group (anterior Po) project in a sustaining fashion to several cortical divisions including AI and SII (Rose, J.E. and C.N. Woolsey. In: Biological and biochemical bases of behavior, H.F. Harlow and C.N. Woolsey, eds. U. of Wisc. Press, Madison, 1958; Diamond et al., J. Comp. Neurol., 109: 349-362, 1958). Results of anterograde degeneration experiments support the conclusion that GMv projects to AI but suggest that other divisions of GM and the posterior group (Po m, Po i and anterior Po) may have equally specific cortical targets. For example, Heath and Jones (J. Comp. Neurol., 141: 397-426, 1971) conclude that GMmc and Po m project to insular cortex and the suprasylvian fringe, while the dorsal division of Morest (GMd) projects to the posterior ectosylvian gyrus. In the present study, 0.2-0.4 ul of 10-50% concentration of horseradish peroxidase, Type VI (HRP) was injected in various subdivisions of the auditory cortex in 26 experiments.

The most significant result is the marked contrast between the effects of injections in AI and injections in other subdivisions of auditory cortex. Injections in AI led to a dense band of labelled cells in GMv; labelled cells were also found outside of GMv, but these cells were diffusely distributed in GMd, GMmc and Po. The more extensive the diffusion of HRP within AI the greater the number of cells which contain label but the pattern of the distribution remained the same. In contrast to the effects of injecting AI, small injections in AII, or the insular cortex, led to a diffuse distribution of labelled cells in GMd, GMmc and Po; GMv and the caudal extremity of GM were spared. Again, the more extensive the spread in AII the greater the number of labelled cells while the pattern of the distribution remains the same. Small injections in the posterior ectosylvian gyrus led to a similar picture of widely scattered cells throughout several divisions of the auditory thalamus except that GMv also contained a few labelled cells. The pattern of distribution of labelled cells after injecting the temporal cortex was different from either of the patterns just described. In such cases a concentration of labelled cells was found in the caudal extremity of the medial geniculate.

These results suggest that GMv projects to AI in a highly topographic fashion and that GMd, GMmc and Po project diffusely to several cortical subdivisions. Finally, it seems that the caudal extremity of GM projects to the temporal cortex below AII. These are essentially the conclusions derived from studies of retrograde degeneration. These findings even appear to be compatible with the concept of sustaining and essential projections. For example, the scattered cells in GMmc and Po containing label after AI injection may send sustaining collaterals to AI, and to other subdivisions of the auditory cortex. In this case these cells would be preserved after lesions of AI, but would be labelled after injection of HRP in AI. On the other hand, scattered cell loss could have gone undetected, and therefore we cannot say more than that the present findings are consistent with the predictions which follow from the conception of sustaining and essential projections. We conclude that the principal division of the medial geniculate, which includes GMv and the caudal extremity, differs in its organization from GMd, GMmc and Po, all of which can be regarded as subdivisions of the posterior group. (Supported by NIMH Grant MH-4849 to I.T. Diamond. J. Winer is a postdoctoral fellow supported by NIH-EY01800-01.)

43 ANATOMICAL ORGANIZATION OF THE CAT'S NEOCORTICAL AUDITORY COMMISSURAL SYSTEM. T. Yamamoto,* S. Horenstein, P.A. Young, and J.J. Taylor, Saint Louis University, Saint Louis, Missouri 63104.

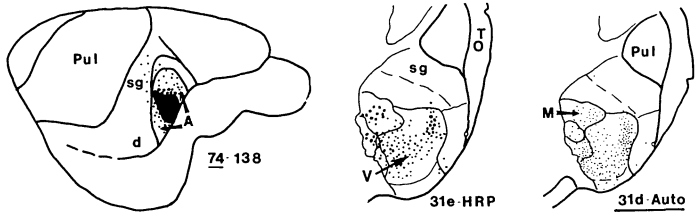
The purpose of this investigation is to describe the anatomic organization of the callosal commissural system of the ectosylvian cortex of the cat. Subpial ablations were made in the subdivisions of the auditory cortex of a series of adult cats. After fixation with buffered formalin, sections of the forebrain of some were impregnated according to the Fink-Heimer method. In others, 0.3 to 2.5 µl of 50% horseradish peroxidase (HRP) were injected into the auditory subdivisions of the ectosylvian cortex. These animals were killed after 48 hours by perfusion of glutaraldehyde-paraformaldehyde mixture and the tissue prepared by the modified Graham and Karnovsky technique for demonstrating peroxidase activity. In a third group of cats, extensive ablation of the entire ectosylvian gyrus was carried out and the animals killed after 2 to 14 days. The tissue from the contralateral posterior ectosylvian cortex was examined by electron microscope. Fink-Heimer study showed that each of the subdivisions of the auditory cortex was connected with its homologue by callosal fibers without exception. In addition, heterotopic axons from A_I projected to contralateral A_{II}, whereas A_{II} gives rise only to fibers projecting to the contralateral A_{II} cortex. In animals allowed to survive 5 days, silver impregnated degenerating axons were observed in the deeper layers of the contralateral cortex. Only a few fibers, however, could be followed beyond layer 3. Instead, numerous fine grains of silver were deposited throughout the first three layers. Electron microscopic observation disclosed that nearly all degenerating synapses were within the superficial three layers of the cortex, particularly layer 1. The degenerating boutons were located on the dendritic spines and no axosomatic or axodendritic stem synapses were found. There were relatively few degenerating synapses in comparison with the total synaptic population. Those observed seemed to be Colonnier's AR type synapses. Most of these terminals appeared dark and shrunken although some were clear and swollen. The dark shrunken and clear-swollen types of degenerating boutons may indicate either heterogeneity of the neurons contributing to the system or different stages of degeneration of the same neuron. HRP study disclosed labeled neurons in the contralateral homologous cortex. The HRP granules were located in small to medium sized pyramidal cells, all but a few of which were located in the third cortical layer. The cells were widely and singly though unevenly distributed though occasionally occurred in clusters. The small numbers of cells with granules may reflect uneven ingestion of the labeling substance, extensive axon branching, or technical failure in staining.

The organization of the ectosylvian commissural system appears therefore to be based upon a small to medium sized pyramidal cell which lies in the third cortical layer. Its axons cross in the corpus callosum and terminate mainly on dendritic spines in the superficial three layers of the homologous contralateral cortex. It appears, therefore, that transcallosal information converges directly upon those pyramidal cells in multiple cortical layers on which specific and non-specific thalamic afferents, association fibers, and recurrent collaterals terminate.

44 CONNECTIONS OF THE MEDIAL GENICULATE BODY WITH NEOCORTEX IN THE TREE SHREW (Tupaia glis). D. L. Oliver and R. J. Nelson* Depts. of Anatomy and Psychology, Duke University, Durham, North Carolina 27710.

The medial geniculate body of the tree shrew contains numerous subdivisions which can be distinguished on the basis of their cytoarchitecture and their connections with the midbrain (Oliver and Hall, 1975). In this study, we examined both the thalamocortical and corticothalamic connections of these subdivisions and used horseradish peroxidase (HRP) for tracing thalamocortical connections, tritiated leucine for corticofugal projections, and anterograde degeneration in both directions. The cytoarchitecture of the medial geniculate body and the auditory cortex as seen in Nissl-stained and myelin-stained material was analyzed in each animal used for tracing connections.

The results of these experiments support the notion that each subdivision of the medial geniculate body is connected with a cytoarchitectonically distinct area of the neocortex. Furthermore, there appear to be corticothalamic projections which correspond to the thalamocortical projections with a remarkable degree of fidelity and suggest that there is a reciprocal projection for each thalamic efferent connection. The central nucleus (V) of the ventral division projects to the primary auditory koniocortex (A) while the remaining marginal portion of the ventral division projects to the region of cortex buried within the rhinal sulcus just beneath the primary cortex. The dorsal division projects to a band of cortex which extends from the rhinal fissure caudal to the primary cortex, dorsally between the target of the pulvinar nucleus (Pul) and the primary cortex, and then rostrally until it abuts the somatic sensory cortex. The dorsal nucleus (d) of the dorsal division projects to the most ventral limb of this band while the suprageniculate nucleus (sg) projects to a zone just rostral to the cortical target of the pulvinar. The "deep" portions of the dorsal division project to a band between the target of the suprageniculate and primary cortex, and the most rostral dorsal division projects to the most rostral limb of the cortical band. The rostral part of the medial division also projects to its own cortical target, an area that caps the dorsal and rostral borders of the primary cortex. In contrast, the caudal medial division (M), as previously suggested for the cat and rat (Colwell and Merzenich, 1975; Ryugo and Killackey, 1975), is unlike the other subdivisions in that it appears to project to both the primary cortex and to parts of the association cortex as well. The insert below illustrates adjacent sections processed to reveal HRP precipitate and radioactivity from a case in which both leucine and HRP were injected into the cortex. Cells labeled with HRP are in register with the autoradiographic signal. Finally, the corticothalamic studies, in particular, suggest that the ventral division of the tree shrew medial geniculate body contains laminae since restricted primary cortex injections often produce a discrete band of label in the ventral division. (Supported by NIH Grant NS-09623 to W.C.Hall.)



45 CYTOARCHITECTURE OF THE TORUS SEMICIRCULARIS IN TUPINAMBIS NIGROPUNTATUS. R. Browner and K. Rubinson. N.Y. Med. Coll., Valhalla, N.Y. and N.Y.U. Med. School., N.Y., N.Y.

The cytoarchitecture of the torus semicircularis (TS) was analyzed in 12 animals. Brains were stained with cresyl-violet or Klüver-Barrera. Golgi Kopsch or Golgi-Cox impregnations were embedded in Araldite and sectioned between 40-120µm.

The TS extends from the superficial caudal mesencephalon dorsal to the exiting trochlear nerve to a position ventral to the middle part of the optic tectum. It is oblique; the caudal pole abuts the midline while the rostral end lies lateral and slightly ventral. Large bundles of tectal fibers course through the caudal TS forming a distinctive, dorsomedial to ventromedial lamination. In the central region of the caudal TS is an area of large (20µm) ovoid to elongate cells. These cells extend rostrally to form the central nucleus ventral to the tectal ventricles. A typical cell contains evenly-distributed Nissl substance and have 3-5 major dendritic trunks extending in several planes (Isodendritic). Dendritic spines appear on the branches and on the major trunks at a distance from the soma.

Capping the caudal end of the central nucleus, dorsolaterally, are layers of smaller (10µm) ovoid to fusiform cells. Cupping the central nucleus ventromedially is a sparse neuropil containing ovoid to fusiform cells and a similar appearing laminar nucleus covers the medial aspect of its anterior part. The cells in these groups have 2 major rectilinear dendrites (Leptodendritic) paralleling the fiber bundles through them. (GRS Grant 41-120-3)

46 SHORT-TERM ADAPTATION IN SINGLE AUDITORY NERVE FIBERS. Robert L. Smith, Institute for Sensory Research, Syracuse University, Syracuse, N.Y. 13210.

Mammalian auditory-nerve fibers exhibit a characteristic short-term adaptation in response to tones of constant sound intensity. Firing rate is maximum at onset and monotonically decays to a quasi-steady rate within 150 msec. Following stimulation, spontaneous activity is suppressed and gradually recovers to its unadapted level. The present experiments aimed at determining some functional properties of the adaptation process and at relating perstimulatory and poststimulatory effects of an adapting tone.

Based on the perstimulatory results, adaptation appears to be inherently additive or linear in nature and not the result of a multiplicative gain change. For example, when increments in intensity were superimposed on an adapting tone, the changes in firing rate produced were equal before and after adaptation. In addition, the relative amount of adaptation, i.e., the ratio of driven onset response to driven steady-state response, was independent of adapting intensity. However, the adaptation process must be preceded by a static nonlinearity in order to account for the saturation of firing rate and decrease in incremental response that occurs at high adapting intensities.

Poststimulatory effects are in basic agreement with the perstimulatory ones. When a test stimulus was placed in the time interval following the adapting tone, a decrement in test response resulted. The decrement followed a saturating function of adapting intensity and was approximately proportional to the perstimulatory response. When the adapting duration was increased, the decrement in test response increased and was monotonically related to the amount of perstimulatory decay.

47 STRUCTURE OF THE CORTI ORGAN OF SEVERAL INBRED STRAINS OF MICE. Stanislav Reinis. Dept. Psychol., Univ. of Waterloo, Waterloo, Ont., Canada N2L 3G1, and Institute for Aerospace Studies, Univ. of Toronto.

Several strains of laboratory mice have been used for the study of different auditory phenomena, audiogenic seizures, etc. In this presentation we provide the basic information on the structure of cochlea of BALB, DBA, A and C57BL/6 inbred strains of mice. The mice at the age of three months were sacrificed in ether anesthesia, their petrose bones dissected and fixed in formalin. Later, the bones were decalcified and the cochlea dissected with ophthalmological scalpel and scissors. The excised Corti organ was stained with hematoxyline-eosine or observed with phase contrast microscope. The comparison of the structure in different strains revealed that at least in C57BL/6 strain the structure of Corti organ in the basal and apical turus is highly irregular. The study of inner ears of C57BL/6 mice at the age of 1, 2, 3, 6, 12 and 18 months showed progressive deterioration and almost complete disappearance of normal structure of the Corti organ.

48 AXONAL TYPES IN THE ANTEROVENTRAL COCHLEAR NUCLEUS (AVCN) OF THE CAT: A GOLGI STUDY. N. B. Cant and D. K. Morest. Dept. Anat., Harvard Medical School, Boston, MA, 02115.

The AVCN can be subdivided on the basis of differences in size and arrangement of the primary afferent endings of the auditory nerve. For example, the endbulbs of Held in the anterior subdivision of AVCN are much larger than those in the posterior subdivision while the external granular layer covering these divisions receives no endbulbs. The present study shows that these distinctions are also consistent with differences in the distribution of the non-primary afferents. Descending fibers entering the AVCN along its medial edge, mostly from the trapezoid body, include 1) thin and medium-sized axons which project to the anterior subdivision, some with large, lumpy terminals forming pericellular plexuses, and 2) medium-sized axons which enter the posterior subdivision, sometimes sending a branch to the anterior AVCN where they end in small terminals in the neuropil. Fibers entering AVCN from the vicinity of the vestibular nerve root and the olivo-cochlear bundle are thin and varicose with large terminals mostly restricted to the external granular and small cell layers of AVCN. Bundles of these fibers travel caudally to the small cell region in posterior AVCN and probably to the dorsal cochlear nucleus (DCN). Two groups of fibers appear to be intrinsic to the cochlear nuclear complex and connect the following regions: 1) medium-sized axons run from the caudal AVCN to its anterior subdivision and form pericellular nests of small, round terminals; 2) thin, varicose axons arise from "corn" cells in the deep layer of DCN and project into the posterior subdivision of AVCN where they end in extensive pericellular nests of small and medium-sized terminals. Thus, while each subdivision of the AVCN receives primary afferent input, the non-primary projections introduce another level of synaptic organization. (Supported by USPHS grants 5R01NS06115 & 1F22NS01220.)

49 RECEPTOR POTENTIALS FROM THE NOCTUID EAR: EFFECT OF TEMPERATURE ON THE TIME-CONSTANTS OF RECEPTION.
William B. Adams Electrical Engineering, Purdue, West Lafayette, Indiana 47907

Receptor potentials arising from the graded electrical activity of one or both sensory cells are recorded from the surface of the scoloparium in the noctuid ear. The response to an acoustic impulse increases in amplitude and decreases in delay and duration as temperature is raised. Arhennius plots of the inverse of the time to the peak response give activation energies of 5 to 12 kcal/mole.

Sinusoidal amplitude modulation of continuous ultrasonic stimuli is used to determine the modulation frequency response. A slight resonance, which may be attributed to the "inductive" component of potassium conductance (Mauro et al, J. Gen. Physiol. 55: 497, 1970), is found for modulation frequencies between 10 and 100 Hz. The resonance is more pronounced and the resonance frequency is higher at higher temperatures. The amplitude and phase angle of the response decrease rapidly above the resonance frequency, indicating at least a fourth-order system with all cut-off frequencies closely grouped.

50 ASCENDING AND DESCENDING PROJECTIONS TO THE INFERIOR COLLICULUS. Joe C. Adams. Laboratory of Neuro-otolaryngology, NINCDS, NIH, Bethesda, Md. 20014.

Ascending and descending projections to the inferior colliculus were studied using the horseradish peroxidase retrograde cell marking technique. Injections were made under visual control after removal of either cerebral cortex or cerebellum of guinea pigs and cats. Results were similar in the two species. Descending projections originate in intermediate layers of the temporal cortex, the peripeduncular nucleus, the suprapeduncular nucleus, the medial division of the medial geniculate body, and scattered cells lying medial to the brachium of the inferior colliculus. Ascending projections originate in all three divisions of the cochlear nucleus, the lateral superior olive, the medial superior olive, the ventral nucleus of the lateral lemniscus, the dorsal nucleus of the lateral lemniscus, and scattered cells lying medial to the medial superior olive. Projections also originate from the central nucleus and extracentral regions of the contralateral inferior colliculus. Most projections arise bilaterally, but the density of projections is usually quite asymmetrical. Predominantly ipsilateral projections arise from the cortex, thalamus, and ventral nucleus of the lateral lemniscus, whereas predominately contralateral projections arise from the dorsal nucleus of the lateral lemniscus and the cochlear nucleus. Projections from the medial superior olive arise from only the ipsilateral side. The lateral superior olive shows equally dense projections from both sides. Differences in degree of asymmetry of other sources of projections lie between these extremes. The tonotopic organization of brainstem nuclei is reflected by the locations of labeled cells following regional injections of the colliculus.

51 TONE BURST AND SWEEP TONE RESPONSES OF SINGLE UNITS IN THE INFERIOR COLLICULUS OF THE UNANAESTHETIZED-PARALYZED ALBINO RAT. Richard A. Reale and Edmund M. Glaser. Dept. Physiol., Sch. Med., Univ. of Md., Baltimore, 21201.

Responses of single cells in the curarized rat inferior colliculus (IC) were examined using tone bursts and sweep tones. The sweep parameters were chosen according to the unit's response area (RA) so as to sweep up to the best frequency (BF) from below, down to the BF from above, and symmetrically through the BF. Our findings indicate a division of the IC similar to that seen in the cat (Rose et. al., J. Neurophysiol., 26, 294 (1963)). Single units isolated for the most part in the dorso-lateral aspect of the IC exhibited RAs up to 7 octaves at moderate intensities, with no clearly defined BF. Advancement of the electrode dorso-ventrally through the nucleus resulted in the appearance of single units tonotopically organized with well-defined BFs and RAs. Cells of higher BF were found to occupy successively more ventral locations. Approximately 85% of the units responded in a sustained manner to acoustic stimulation. However, the discharge pattern was often a complex function of frequency and intensity. In the unanesthetized as compared with the anesthetized preparation there is a far greater incidence of spontaneously active units. The RA of a spontaneously active unit generally has a "W" shape characterizing a central excitatory frequency band and two flanking bands in which the stimulus produces suppression of spontaneous activity. Although the discharge characteristics resulting from sweep tones can sometimes be predicted from the classical RA, in many cases they can not. The units appear to respond preferentially to sweeps. That IC units exhibit such preferential responses reinforces the concept that acoustic coding in this nucleus reflects temporal as well as spectral properties of the stimulus.

52 FUNCTIONAL ORGANIZATION OF MONAURAL AND BINAURAL UNIT RESPONSES IN THE PRIMARY FIELD (AI) OF THE CAT'S AUDITORY CORTEX. Thomas J. Imig* and Hugo O. Adrian* (SPON: J. F. Brugge). Dept. of Neurophys. and Waisman Center on Mental Retard. and Human Dev., Univ. of Wis. Med. Sch., Madison,53706.

The responses of single units or unit clusters to monaural and binaural tone bursts were recorded at short intervals along penetrations into AI of barbiturate anesthetized cats. For each single unit or unit cluster, best frequencies, thresholds and monaural dominance (which is based on the magnitude of unit response) were determined by stimulation of each ear. Binaural interactions at or near best frequency were assessed and classified as summation, inhibition, or complex, e.g. changes in latency, mixed summation and inhibition, or occlusion. Recordings were restricted to units with best frequencies above 4kHz. Following each experiment, the brain was processed histologically for cytoarchitectonic study and reconstruction of electrode tracks.

Units in penetrations oriented nearly parallel to radial cell columns exhibited similar monaural dominance and binaural interactions. In penetrations oriented obliquely with respect to radial cell columns, unit properties remained the same or nearly the same for short distances then changed abruptly. These data suggest a vertical or columnar functional organization of these response characteristics. We refer to zones in which units exhibit similar monaural dominance as monaural dominance columns and to zones in which units exhibit similar binaural interactions as binaural interaction columns. In several experiments, the cortex was mapped in small steps to determine the shape and topographical relationships of these columns. These results suggest that a set of monaural dominance columns form a binaural interaction column and that borders between binaural interaction columns tend to be oriented perpendicular to the frequency gradient of AI. (NS-06225, NS-05326 and 5-P01-HD-03352-07).

53 SUPERIOR OLIVARY INPUTS TO CAT COCHLEAR NUCLEUS.

Eileen S. C. Kane, Dept. Anat., Univ. of Chicago, Chicago, Ill. 60637

Adult cats, subjected to large, unilateral lesions of the superior olivary complex (SOC), survived for 24 hours to seven days. Brains were prepared by modified Nauta methods. Degeneration (both "of passage" and preterminal) was most abundant after four days, but was consistent with patterns at all other survival times. Heaviest preterminal degeneration in both cochlear nuclei occurred in anteroventral regions (AVCN) and in deep dorsal cochlear nucleus (DCN). By contrast, only sparse degeneration occurred in posteroventral regions (PVCN), particularly in the octopus cell area (OCA). Fine to medium-caliber perisomatic and peridendritic fragments occurred in AVCN, in association with larger neurons and granule cells. Preterminal degeneration of medium and fine fragments in DCN was associated with giant cell bodies and proximal dendrites, small cell bodies, and deep dendrites of fusiform cells. OCA preterminal degeneration was almost totally peridendritic; fine and medium fragments occurred along primary shafts, but mostly distally in neuropil. Medium-caliber degenerating fibers of passage occurred throughout PVCN and in deep DCN. Our findings of superior olive projections to cochlear nucleus, via crossed and uncrossed olivocochlear bundles (OCB) are inconsistent with reported distribution of acetylcholinesterase in the lower nucleus. Also, the sparse, peridendritic degeneration in OCA strongly suggests that OCB fibers cannot be responsible for rapid inhibition, characteristic of OCA unit recordings. (Supported by Block Fund, Univ. of Chicago, Deafness Res. Fdn. and PHS Grant NS12071 and Research Career Development Award NS 00008).

54 INTERNEURON NETWORK IN THE DORSAL LATERAL GENICULATE NUCLEUS (LGNd) OF MONKEYS. Pedro Pasik, Tauba Pasik and Jozsef Hámori*. Dept. of Neurol., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029, and 1st Dept. of Anat., Semmelweis Univ. Med. Sch., 1450 Budapest, Hungary.

Interneuron (I-cell) dendrites are known to participate in triadic units as both postsynaptic and presynaptic elements to retinal terminals and principal cell dendrites respectively. In addition, electron microscopic examination of the LGNd of Macaca mulatta reveals the occurrence of synapses between I-cell profiles. Almost all combinations of I-cell to I-cell contacts are observed, i.e. axodendritic, dendrodendritic, dendrosomatic and somatodendritic, the most frequent being the dendrodendritic synapses between the presynaptic dendrites characteristic of I-cells. Quantitation of 5 samples, each consisting of 200 μm^2 of net LGNd neuropil shows that the presynaptic membrane specializations present in I-cell axonic and dendritic elements amount to a mean of 3.73 μm or 8.16% of the surface of such profiles. Only 61% of this extent is in contact with principal cells, and an unexpectedly high 39% engages other I-cell elements. The contribution of I-cell to I-cell contacts is even greater (over 45%) when it is considered in terms of the proportion of profile surface taken by presynaptic sites. Approximately 18% of the neuropil is occupied by I-cell elements, and the following mean differences between the two ending types are significant: dendritic terminals are more numerous and larger; axonic profiles have more of their surface occupied by synaptic sites and each contact is longer. Findings suggest the existence of a network of interconnected I-cells. Since the nature of these neurons is presumably inhibitory, such arrangement can provide a certain measure of anisotropic disinhibition which may be responsible for transformations occurring in the LGNd depending upon the size and velocity of the stimulus as well as of the degree of synchronicity of temporal patterns. Aided in part by U.S.P.H.S. Grant # MH-02261.

55 STRUCTURE OF NEURONS IN THE dLGN OF ADULT RABBITS. Loraine Goodwin Miller* (SPON: K. L. Chow). Dept. Neurology, Sch. Med., Stanford Univ., Stanford, CA 94305.

Neurons in the adult rabbit dorsal lateral geniculate nucleus in thionine stained sections were measured and plotted as a function of their frequency and cross-sectional area. Sixty percent of the cells observed were found to lie between 40 and $90\mu^2$. We believe these cells correspond to Type III cells found by Guillery (J.Comp.Neur.,128:21-49, 1966) in the cat and Kriebel (J.Comp.Neur.,159:45-67, 1975) in the albino rat. Another 30% of the cells fell between 90 and $150\mu^2$. These may correspond to Type I and Type II cells described by Guillery and Kriebel.

Using the Golgi-Kopsch technique (Romeis, 1948; Colonnier, 1974) we found the same distribution of cells with 60% between 70 and $190\mu^2$ and 30% between 190 and $333\mu^2$. Camera lucida drawings of neurons throughout the dLGN showed quite clearly the existence of at least two classes of cells which were easily identifiable on the basis of cell size and dendritic branching.

Results from electrical stimulation of the optic tract while recording from single cells in the geniculate, for the determination of detectable physiological differences which may exist between the two cell types, will be reported at the meeting.

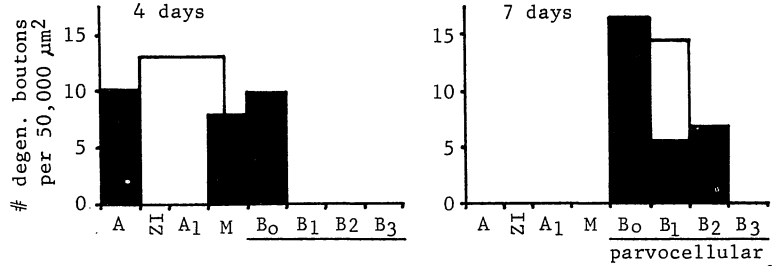
(Supported by the Porter Development Fund Fellowship of the American Physiological Society).

56 ANOTHER LOOK AT LATERAL GENICULATE LAMINATION IN THE CAT. E.V. Famiglietti, Section on Neurophysiology, National Eye Institute, NIH, Bethesda, Md.20014

Laminae A & A_1 of the dorsal lateral geniculate nucleus (LGN) are distinctly separated, but similar cytologically and electrophysiologically. The laminae ventral to these are relatively indistinct and puzzling in their heterogeneity. Guillery[1] advocated renaming the ventral laminae: C,C_1, etc., on the basis of light microscopic studies of retinal afferents. Unfortunately, this simplified nomenclature implies a symmetry between dorsal and ventral laminae which is inconsistent with experimental fact.

The laminar schema advocated here is essentially that proposed elsewhere[2,3] and follows Rioch's tetralaminar division[4]. Thuma's more familiar terminology is retained for layers 1,2&4 (A,A_1,B)[5], while 3 is called magnocellular (M) after Rioch, hence: A,A_1,M,B. The numbering of the B sublaminae has been altered (cf. refs. 2,3) to facilitate comparison with Guillery's innovations, hence: A,A_1M,B_o,B_1,B_2,B_3. This schema is based upon light microscopic observations on cell size and lamination, and upon electron microscopic study of laminar synaptic neuropil and degenerating retino-geniculate afferents.

Three days after optic enucleation nearly all of the optic axon terminals are degenerating in A&A_1 (Fig.). There is no overlap between crossed and uncrossed projections, but the latter overlaps the interlaminar zone (ZI). At 4 days degenerating, crossed,"glomerular" synaptic terminals are seen in M and B_o, while in the upper 1/2 of M a few uncrossed, nonglomerular terminals are present. Manifest at 7 days is a crossed projection of nonglomerular terminals to all of B and an uncrossed projection to the middle of B. (A few binocularly excited cells have been noted in these two zones of overlap [6,7,8]). Parvocellular B_o, presumably included in Guillery's "C", is unique in having at least two distinct, crossed retinal projections.



In most of B, there is good correspondence between the locations of glomerular neuropil & B_o on one hand, and of islands of synaptic "nests"[3] and B_1 on the other. In medial LGN, representing the segment of contralateral visual hemifield nearest the zero vertical meridian, ipsilateral terminals rise to occupy glomerular neuropil and a tongue of primarily contralaterally-innervated small cells (with a few large cells) extends medially beyond A&A_1. The small cells continue down from B_o, occupying the most medial aspect of B.

Six of the 7 heterogeneous "W" retinal ganglion cell types project to B[9,10], and some of the color cells are found above B_1, in B_o, not in M (Guillery's "C")[10]. Certain types of W-cell and a few "Y" (large) cells in temporal retina project contralaterally[11,12,13] to the LGN[9]. The geniculate cells receiving this extension of the visual field across the midline may well constitute the medial tongue of M and the descending tongue of B_o. Thus the revised laminar schema, based upon electron microscopic study, appears to receive support at several points from new physiological findings.

1.Guillery,R., J.Comp.Neur.,138('70); 2.Famiglietti,E., Anat.Rec.,166('70); 3.Famiglietti,E., Ph.D. Dissert.,(©'72); 4.Rioch,D., J.Comp.Neur.,49('29); 5.Thuma,B., J.Comp.Neur.,46('28); 6.Sanderson,K. et al., Vision Res.,9('69); 7.Sanderson,K., Exp.Brain Res.,13('71); 8.Sanderson,K. et al., ibid.; 9.Cleland,B. et al., Abs.Mtgs.ARVO('75); 10.Daw,N., Pearlman,A., J.Physiol., 211('70); 11.Stone,J., J.Comp.Neur.,126('66); 12.Stone,J., Fukuda,Y., J.Comp.Neur.,155('74); 13.Fukuda,Y., Stone,J., J.Neurophysiol.,37('74).

57 INTRINSIC ORGANISATION OF THE RAT DORSAL LATERAL GENICULATE NUCLEUS AS SHOWN BY DIFFERENTIAL DEGENERATION RATES IN THE YOUNG ANIMAL. Jennifer S. Lund, Franque L. Remington* and Raymond D. Lund. Dept. of Ophthalmology, Biological Structure and Neurosurgery, School of Medicine, University of Washington, Seattle, Washington 98195.

The distribution of Fink-stained degeneration was mapped in albino and pigmented rats after enucleation between postnatal days 10 and 25 with survival times of 6hrs to 3 days. In the dorsal lateral geniculate nucleus (dLGN) contralateral to the eye removal the earliest degeneration appears in a lamina occupying the medioventral extent of the nucleus and is rapidly removed; later degeneration fills a central lamina of the nucleus with a patch of degeneration extending to the outer surface of the nucleus at the mediodorsal margin. The latest occurring degeneration fills the outermost lamina and is still obvious when degeneration is largely dispersed from the inner and central laminae; this lamina shows an early filamentous degenerative reaction in the adult. The uncrossed optic pathway occupies a portion of the central lamina of the nucleus and in albino animals it shows a rapid degeneration and dispersion similar to the innermost lamina on the crossed side; in the pigmented animals the uncrossed projection degeneration starts as early as that of the albino but persists as long as the degeneration of the central lamina on the crossed side. The degeneration time of the sprouted uncrossed pathway resulting from unilateral enucleation at birth is the same in albino and pigmented rats and resembles in timing the normal uncrossed pathway of pigmented rats. These results suggest that there are at least three different fiber populations in the rat optic nerve with different distribution in the dLGN. The uncrossed optic pathway of albino and pigmented rats appears to differ in fiber composition; this may relate to aberrations in mapping of uncrossed projections in the albino. Supported by NIH grants EY 1086, 0595.

58 THE AVERAGE DIAMETER OF LABELED AND UNLABELED NEURONS IN THE LATERAL GENICULATE NUCLEUS (LGN) IN PRIMATES FOLLOWING INJECTION OF HORSERADISH PEROXIDASE INTO STRIATE CORTEX.
J. J. Norden. Dept. Psychology, Duke U., Durham, N.C. 27706.

Following injection of horseradish peroxidase into striate cortex in owl monkeys (Aotus trivirgatus), rhesus monkeys (Macaca mulatta), and bushbabies (Galago senegalensis), 94.1-98.0% of all neurons within columns extending through all relay layers in the LGN are labeled indicating an almost complete lack of interneurons (Norden, 1974). Frequency histograms of the distribution of cell sizes of the labeled and unlabeled neurons within these densely labeled columns reveal that the cell size ranges of these neurons overlap suggesting that cell size alone is not an adequate criterion for distinguishing between relay cells and interneurons in the primate LGN. In all three species, however, the mean size of unlabeled neurons was significantly smaller than the mean size of labeled cells ($p < .001$).

The average diameter measurements of cells in bushbaby were also used to determine whether the LGN of this species could be separated into two cell layer types (magnocellular and parvocellular) or three types (small, medium, large) on the basis of cell size. Pairwise comparisons of the mean diameters of cells from layers 1+2, 3+6, and 4+5 were made using Dunn's multiple comparison procedure for orthogonal comparisons among means(Kirk, 1968). All three comparisons were significant ($p < .01$) demonstrating that the LGN of Galago is composed of two large-celled layers (1+2), two layers of medium sized cells (3+6), and two layers of small cells (4+5).

59 THE LAMINAR ORGANIZATION OF LAYER IV STELLATE CELLS IN AREA 17 OF THE TREE SHREW. E. E. Geisert* and R. W. Guillery. Neurosciences Training Program and Dept. of Anatomy, Univ. of Wisconsin, Madison, 53706

Stellate cells in layer IV of the striate cortex were studied with the Golgi method to define their relation to the geniculate input. In the tree shrew, the geniculate laminae are sequentially represented in layer IV. Within the binocular portion of area 17 the outer strip of layer IV is occupied by terminals from geniculate lamina 1. The remaining geniculate laminae are systematically represented, with lamina 6 terminating in the deepest strip of layer IV (Harting et al., J. Comp. Neur.150:393-440,'73). For the purpose of this study, it is assumed that layer IV can be divided into six equal horizontal zones, so that each zone represents the terminal field of a single geniculate lamina. Only stellate cell bodies are seen within layer IV. The dendrites of these neurons are confined within the boundaries of layer IV and 88% of the cells are oriented. An oriented cell is defined as one having a dendritic field with the greater axis at least 1.5 times the length of the axis perpendicular to this. Of the oriented cells 88% are horizontal. Over half of the horizontally oriented neurons extend through one-sixth or less of the vertical thickness of layer IV. Therefore, 40% of the neurons within layer IV are likely to be contacted by afferents from a single geniculate lamina, indicating that in the initial stages of cortical integration, the information relayed by individual geniculate laminae remains segregated. Geniculate laminae 2,3, and 4 receive input from the contralateral eye and terminate adjacent to each other in one-half of layer IV. Within this part of layer IV, dendritic fields retain restrictions to one-sixth of layer IV, suggesting that the stellate cells sample geniculate laminae, not merely right eye/left eye differences. (Supported by Grant NS 06662)

60 CORTICO-CORTICAL CONNECTIONS OF STRIATE AND PRESTRIATE CORTEX IN THE TREE SHREW (TUPAIA GLIS). Lois Lazarus* and Carolyn B. Ware. Dept. Bio. Psych. and Dept. Anat., Downstate Med. Ctr., Brooklyn, N.Y. 11203.

Removal of neocortex lateral to the striate area in tree shrews produces deficits in visually guided behavior. The anatomical relationships of the striate cortex with prestriate and temporal regions are poorly defined. In this study lesions were placed either in striate (area 17) or prestriate (areas 18 and 19) cortex by applying a heated (70°) metal tube directly to the intact dura. Depth of tissue damage was controlled by varying the duration of heat. Four seconds typically resulted in a lesion confined to supragranular layers whereas six seconds produced damage extending to the fourth layer. In all cases the underlying white matter was spared. After survival times of three to six days brains were removed and tissue processed according to Fink-Heimer procedures. Alternate sections were stained with cresyl violet for analysis of cytoarchitecture.

Following lesions of area 17 two discrete foci of terminal generation appeared lateral to the striate region in areas 18 and 19 ipsilateral to the lesion. Callosal projections terminated in area 18, but not in area 17. Lesions of prestriate cortex produced degeneration ipsilaterally in area 17 adjacent to the lesion, in area 19, and in a narrow hand of temporal cortex between area 19 and the rhinal sulcus. Two additional areas of degeneration were seen ipsilaterally in cortex along the medial boundaries of the hemisphere. One spray of degenerating fibers appeared beyond the margin of area 17 along the dorsal lip of the cortex while the second recipient area was located medial to the ventricle in the ventral portion of the temporal region. Contralateral foci of degeneration were seen in areas 18 and 19 only. The pattern of cortico-cortical connections observed supports the concept of multiple regions in temporal cortex involved in processing visual input.

61 SOME EFFERENT AND AFFERENT CONNECTIONS OF A MEDIAL DIVISION OF THE INFERIOR PULVINAR NUCLEUS IN THE OWL MONKEY (AOTUS TRIVIRGATUS). C. S. Lin and J. H. Kaas. Depts. of Anatomy and Psychology, Vanderbilt Univ., Nashville, TN. 37240.

Connections of visual association cortex with the pulvinar complex have been investigated in the owl monkey using autoradiographic methods, silver stains for degenerating axons and terminals, and the retrograde transport of horseradish peroxidase. The connections of the inferior pulvinar suggest that the medial portion of this nucleus is a distinct subdivision of the visual system. Previous experiments using the retrograde transport of horseradish peroxidase indicate that the medial part of the inferior pulvinar nucleus projects to the middle temporal visual area of the temporal lobe (Wagor, Lin, & Kaas, '74). This projection has been confirmed by injections of tritiated proline into the medial inferior pulvinar. The medial inferior pulvinar in turn receives reciprocal projections from the middle temporal visual area. In addition, input to the medial inferior pulvinar nucleus was found from several other divisions of visual association cortex including area 18 and the dorsomedial visual area. Other regions of visually responsive cortex such as the posterior parietal area do not appear to connect with the medial inferior pulvinar. Finally, subcortical input from the superior colliculus was demonstrated by an injection of tritiated proline into the tectum. Thus, the medial portion of the inferior pulvinar nucleus of the owl monkey has a distinct pattern of connections with other visual structures. (Supported by NSF grant GB-36779).

62 FUNCTIONAL CONNECTIONS BETWEEN LGB AND PUL PROJECTION SYSTEMS IN THE CAT AS INVESTIGATED BY CORTICAL HYPOTHERMIA. Richard S. Babb* and William S. Battersby. Queens College of CUNY, Flushing, N.Y. 11367.

The effects of cooling V1 or V2 cortex on the response in both of these areas, to either a single LGB shock or a train of three shocks to PUL, were investigated at both gross and unit levels in 35 locally anesthesized, analgesic cats. A specially fabricated cryogenic unit, with two on-line thermistor readouts, enabled either V1 or V2 cortex to be cooled, while maintaining the adjacent V2 or V1 cortex (respectively) at normal temperature. Results showed: (1) Cooling V1 or V2 cortex produced an enhancement in the amplitude of the gross response to LGB shock in both the area being cooled and the immediately adjacent V2 or V1 cortex, with a maximum at 25-29°C. The summated unit responses elicited by LGB shock also showed an enhancement, primarily in the secondary discharge, with a maximum again at 25-29°C. (2) Cooling V1 cortex produced no systematic change in the gross cortical responses elicited by 3 PUL shocks, either locally in V1 cortex, or in the adjacent V2 cortex. Cooling V2 cortex on the other hand, produced a consistent decline in the relative amplitude of the gross cortical responses elicited by 3 PUL shocks, not only locally in V2 but also in the adjacent V1 cortex. Preliminary data indicate that the unit responses to 3 PUL shocks exhibit definite augmentation, primarily in their secondary discharges, and in part parallel the findings obtained with gross recordings. The findings obtained upon cooling adjacent cortex provided functional evidence for the existence of reciprocal connections between V1 and V2 cortex, associated with both the LGB and PUL projection system, thus extending prior anatomical reports.
(Supported in part by USPHS Training Grant No. MH 10395-08.)

63 ON THE CONNECTIONS OF THE PRETECTUM IN THE TREE SHREW (TUPAIA GLIS). Joseph T. Weber and John K. Harting*, Department of Anatomy, University of Wisconsin, Madison, WI 53706.

We have been utilizing autoradiographic and anterograde degeneration tracing methods to analyze the connectivity of the well developed pretectal complex of the tree shrew.

Based upon myelo- and cytoarchitecture, the pretectal complex of Tupaia can be divided into four distinct subdivisions; the nucleus of the optic tract (NTO), the olivary nucleus (ON), and the posterior (PN) and anterior (AN) pretectal nuclei.

Following intraocular injections of ^{3}H proline, we observed transported label contralaterally within all four subdivisions, with NTO and ON being the principal targets. AN and PN contain only sparse label, but in certain regions islands or patches of label are apparent. Ipsilateral retinal fibers terminate densely within ON and sparsely within AN and PN. NTO does not receive an ipsilateral retinal projection.

In contrast to the organization of retinal input, the striate cortex projects to only two major pretectal subdivisions, AN and PN. However, our data indicate that NTO and ON do receive input from the extrastriate visual cortex.

Following large injections of ^{3}H proline into the pretectum, transported label can be seen ipsilaterally within the 1) dorsal lateral pontine gray, 2) a region immediately dorsal and lateral to the red nucleus and 3) within several regions of the diencephalon. In particular, we observed heavy label within the medial one-half of the ventral lateral geniculate nucleus, the reticular nucleus, regions of the subthalamus and hypothalamus and within a thalamic zone located medial to pulvinar which has been previously termed the lateral nucleus.

Following small injections which were restricted to PN in one case and NTO in another, transported label was observed only within the dorsal lateral pontine gray, the ventral lateral geniculate, the reticular nucleus of the thalamus and within the lateral nucleus.

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64 ORGANIZATION OF THE TECTOFUGAL VISUAL PATHWAY IN PIGEON. Larry I. Benowitz and Harvey J. Karten. Dept. Psychology, MIT, Cambridge, MA 02139 and Dept. Psychiatry, Coll. Med., SUNY, Stony Brook, NY 11790.

In birds, a massive retinal projection terminates in superficial layers of the optic tectum (Cowan et al., J. Anat. 95: 543, 1961); the tectum in turn projects upon the nucleus rotundus thalami (Karten and Revzin, Brain Res. 2: 268, 1966), which then sends its efferents to the ectostriatal core in the center of the telencephalon (Karten and Hodos, J. Comp. Neur. 140: 35, 1970). To study the detailed organization of this major visual pathway, patterns of retrograde transport were examined following small injections of horseradish peroxidase (HRP) into various loci throughout pigeon's ectostriatum (E) or nucleus rotundus (Rt). Ectostriatal injections resulted in discontinuous labeling patterns in Rt, suggesting that discrete subdivisions of Rt project upon corresponding subdivisions of E. The regions of Rt delineated by the HRP studies coincide with cytoarchitectonically distinct divisions within the nucleus. Nucleus triangularis (T), a dorsomedial extension of Rt, was also labeled after each E injection. HRP placements into Rt in turn indicated that each subdivision of that nucleus receives a distinct pattern of afferentation. Anterior and dorsal Rt receive input from superficialmost cells in the stratum griseum centrale of the tectum (SGC; layer 13 of Cajal), medial Rt from the intermediate SGC, and caudal Rt and T from the deepest SGC neurons. A ventral subdivision of Rt receives projections from tectum and from two prectectal nuclei as well. Nucleus reticularis thalami was found to provide additional input into all parts of Rt.

Thus, the ascending tectofugal visual pathway appears to consist of several discrete channels. These originate at different depths in the tectum or in pretectum, are relayed through distinct subdivisions of rotundus, and have characteristic projections upon the ectostriatum.

65 IDENTIFICATION OF TELENCEPHALIC-AFFERENT THALAMIC NUCLEI ASSOCIATED WITH THE VISUAL SYSTEM OF THE FROG. Frank Scalia and David R.Colman*, Dept.Anat. Downstate Medical Center, Brooklyn, N.Y. 11203.

Reports on evoked electrical activity in the medial cortex (primordium hippocampi) and ventrolateral sector (striatum) of the telencephalon following visual or electrical stimulation of the primary optic pathway (Karamian et al.,JCN,1966;Vesselkin et al.,BBE,1971;Gruberg and Ambros,Exp N,1974)suggested the existance of thalamo-telencephalic circuits in the frog's visual system.Silver-staining for anterograde degeneration following thalamic lesions supported thalamo-telencephalic projections,but the specific nuclei responsible for the projections were not identified in these studies.Preliminary,unpublished results (Scalia and Gregory),showing terminal degeneration in the ipsilateral striatum of the bullfrog following lesions of the p-c,p-l complex at mid-thalamic levels,and,in some cases,degeneration of fibers bilaterally in the medial cortex, were also suggestive.To eliminate the possibility of contamination artifact by axons of passage,the p-c,p-l complex was injected with ^{3}H-proline in R.pipiens, and similar results were obtained by autoradiography.However,spread of injection confounded the specific identification of the projection-nuclei. Instillation of HRP into the striatum in R.pipiens now labels cell-bodies, ipsilaterally,throughout that part of the p-l nucleus which is postsynaptic to the tecto-thalamic projection(Rubinson,BBE,1968),and instillation into the medial cortex labels cells,bilaterally,in the anterior part of the p-c nucleus. Cells in this region appear to be postsynaptic to n. Bellonci (Scalia and Gregory,BBE,1970). HRP label in fibers demonstrates that both projections follow Herrick's thalamo-frontal tract; the first involves the lateral forebrain bundle; the second follows the medial forebrain bundle. (Supported by USPHS grant EY-00909).

66 THE ORGANIZATION OF RETINOTECTAL PATHWAYS IN CATS AND PRIMATES. J.K. Harting*, V.A. Casagrande, J.H. Kaas, R.W. Guillery. Dept. of Anatomy, Univ. of Wisconsin, Madison and Dept. of Psychology, Vanderbilt Univ., Nashville, Tenn.

Comparison of the pattern of retinal termination within the superior colliculus of the domestic cat and of three primate species shows both consistencies and differences.

For this study we utilized autoradiographic tracing methods following intraocular injections of ^{3}H proline in the cat, in the lesser bushbaby Galago senegalensis (a prosimian), in the owl monkey Aotus trivirgatus (a new world primate) and in the rhesus monkey Macaca mulatta (an old world primate). Our data permit four general conclusions.

First, the distribution of transported label is in agreement with electrophysiological results which have shown that in the cat the rostral pole of the tectum receives from the contralateral temporal retina, that is, from the ipsilateral hemifield; in primates, in contrast, only the contralateral hemifield is represented. Thus, our autoradiographs reveal that in the cat the rostral pole of the ipsilateral colliculus is free of label, whereas in all three primates the label extends to the rostral tip.

Second, in all 4 species the input from each eye is restricted to the superficial three layers. However, in Galago the ipsilateral input does not extend into the outer zone whereas in the other 3 species there are varying degrees of overlap.

Third, in all species except Macaca the contralateral input forms a continuous terminal zone throughout the colliculus. In Macaca the contralateral input is discontinuous and forms discrete irregular patches. Similar patches were also apparent ipsilaterally in the cat, in Macaca and Aotus, but not in Galago.

Finally in Macaca, differences in density of label were apparent between the anterior and posterior parts of the colliculus, with the rostral pole having the lightest label both ipsilaterally and contralaterally. This density difference, which indicates that the central retina has a lighter projection than the peripheral retina, was not apparent in the other animals.

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67 RETINOFUGAL PATHWAYS IN THE AFRICAN LUNGFISH, PROTOPTERUS ANNECTENS (OWEN). R. Glenn Northcutt. Department of Zoology, University of Michigan, Ann Arbor, Michigan, 48104

Six Protopterus underwent unilateral retinal aspiration. After survival times of 7-47 days the animals were sacrificed by transcardial perfusion and the brains fixed in 10% formalin, embedded in gelatin, and sectioned frozen at 25μ. Sections were then processed by silver impregnation methods. One animal was injected intraocularly with [^{3}H] proline (20μCi), and the brain fixed by perfusion 72 hours later. Mounted paraffin sections were coated with NTB-2, stored for 20 days, developed and stained with cresyl violet. The fibers of the optic nerve decussate completely, and can be divided into anterior and posterior components within the chiasm. The anterior component crosses and terminates rostrally in the ventral half of the neuropil of the periventricular preoptic nucleus. Fibers of the anterior component then continue dorsally to form the thalamic optic tract. Three distinct optic terminal fields can be recognized within the thalamus, from which the anterior optic component continues caudally to the pretectal level where a fifth terminal field occurs within the superficial neuropil. Finally the anterior optic component projects to the entire optic tectum. The posterior optic component decussates in the caudal preoptic zone and appears to primarily terminate in this zone as well as give rise to a basal optic tract which terminates on a few scattered cells forming a basal optic nucleus of the tegmentum. Fibers of the anterior and posterior optic components appear to project mostly to separate targets. (Supported by NIH Grant 11006 and NSF Grant 40134).

68 RETINAL PROJECTIONS IN TRANSFORMING SEA LAMPREYS, PETROMYZON MARINUS. Kalman Rubinson and Michael C. Kennedy. Dept. Cell Biol., NYU Medical Center, New York, 10016.

We have reported previously the retinal projections in larval sea lampreys. As a continuation of these studies, unilateral enucleations were performed on transforming sea lampreys, at various metamorphic stages (five stages have been described by Manion and Stauffer, 1970). After survival times of 7 days, animals were sacrificed, perfused with 4% paraformaldehyde in 0.1M sym-collidine buffer (pH 7.4), and the brains were removed for frozen sectioning and staining with a modification of the Fink-Heimer silver methods for degeneration. In Stage 5 (macrophthalmic) transformers, degeneration was observed in the optic chiasm, contralateral optic tract, basal optic root, lateral geniculate nucleus, pretectum, and optic tectum. Degeneration was also seen in the ipsilateral optic tract and dorsal thalamus; in the larval lamprey, no ipsilateral degeneration was seen.

Cytoarchitectonic studies of the optic tectum of Araldite-embedded brains of Stage 5 transformers showed a more highly developed and organized structure than was seen in the larvae, including the presence of a superficial fiber layer (stratum opticum) and an optic ventricle, both of which were absent in the larvae. The cytoarchitectonic changes that have occurred in the optic tectum of these late transformers may reflect the neuroanatomical modifications which must accompany the emergence and growth of the eye. (Supported by NIH Grant NS 10906-01 and Fellowship 1 F22 EY02259-01 to M.C.K.)

69 RETINO-TECTAL PROJECTION OF THE WINTER FLOUNDER. L. Luckenbill-Edds* and S. C. Sharma (Spon: M. V. Edds, Jr.). Dept. Biol. Sci., Smith Coll., Northampton, Mass. & Dept. Ophthal. N.Y. Med. Coll., N.Y.C.

During metamorphosis of the flatfish larva, one eye migrates to the opposite side of the head; concomitantly, the eyeless side of the body turns to become functionally ventral. The net result: the true dorso-ventral axis of the body is rotated 90° with respect to the animal's visual space. Since visuomotor behavior is not misdirected, some compensatory change presumably must occur in the developing nervous system. As a basis for developmental analysis, we have determined the retino-tectal projection in the adult winter flounder (Pseudopleuronectes americanus) using both anatomical (Roth's modification of Nauta's stain) and electrophysiological mapping techniques. Multiunit responses were recorded from the tectum with the flounder lying in its natural position, eyeless side down. Assessed by either technique, the retino-tectal projections of right and left eyes are symmetrical. Their pattern and extent correspond to those of other teleosts. Despite a 25° binocular overlap in the visual field, no ipsilateral projection could be detected. A sulcus divides the rostral half tectum into dorso-medial and ventro-lateral lobes, each supplied by the corresponding branch of the optic tract. Visually evoked responses could not be elicited in the sulcus. Following enucleation, degeneration was found in the stratum opticum, stratum fibrosum et griseum superficiale and stratum album centrale throughout the contralateral tectum, and in diencephalic nuclei. Since the retino-tectal projection of the adult winter flounder seems typically teleostean, we conclude that neural compensation for the axial rotation must be either intra- or post-tectal. S.C.S. supported by NSF grant GB 43506.

70 THE AFFERENT CONNECTIONS AND SYNAPTIC ORGANIZATION OF THE DORSAL LATERAL GENICULATE NUCLEUS IN THE GREY SQUIRREL (Sciurus carolinensis) J.A.Robson and W.C.Hall. Depts. of Anatomy and Psychology, Duke University, Durham, North Carolina 27710.

In light microscopic studies, we have used anterograde degeneration and anterograde and retrograde axonal transport to demonstrate that the lateral geniculate nucleus in the grey squirrel receives projections from the retina, the small cells in the outer half of stratum griseum superficiale of the superior colliculus, and layer VI of striate cortex. With the electron microscope three main classes of vesicle-containing terminals can be recognized in the lateral geniculate n., two of which can be associated with these afferent pathways. The largest terminals (1.4-4.5μ) contain round synaptic vesicles and mitochondria with broadly spaced cisternae and they form short asymmetrical synaptic contacts. These terminals are usually found in synaptic complexes which contain dendrites, small dendritic knobs, and other terminals which have pleomorphic vesicle populations. They degenerate following eye enucleations. The smallest terminals (.2-.9μ) also contain round synaptic vesicles but they often lack mitochondria and form broad asymmetrical synaptic contacts. Lesions in either the superior colliculus or striate cortex will cause terminals in this class to degenerate. Terminals in the third class are intermediate in size (.7-1.5μ), contain pleomorphic vesicle populations and form symmetrical synaptic contacts. These terminals appear to remain intact following interruption of these three afferent pathways to the lateral geniculate nucleus. (Supported by NIH Grant NS-09623.)

71 RETINAL AND CORTICAL PROJECTIONS TO THE SUPERIOR COLLICULUS OF THE RABBIT. Lawrence H. Mathers, Jr. Depts. of Anatomy and Neurobiology, School of Medicine, Stanford University, Stanford, CA 94305.

The upper layers of the rabbit superior colliculus have been examined in normal animals and following contralateral eye removal with 5-7 days survival, or ipsilateral striate cortex removal and 2-5 days survival. Three upper layers may be distinguished with relative ease. The stratum zonale extends 60-100 micra below the surface and is relatively cell-poor. The stratum griseum superficiale lies between about 100 micra and 800 micra of the surface. Its upper half is nearly free of large myelinated axons, while its lower half contains such axons. Neurons 10-24 micra in diameter are found throughout. Below the 800-900 micron level is the stratum opticum, containing large myelinated axons running predominantly in the mediolateral axis. Retinal axon terminals are distributed almost exclusively in an area 100-500 micra below the surface. Striate cortex lesions produce degenerating terminals in an area between 400-1200 micra of the surface. These two visual inputs are thus partially segregated in their distribution to the superficial laters of the superior colliculus.

72 CONTRASTING EFFECTS OF INFERIOR AND MEDIAL-LATERAL PULVINAR LESIONS ON VISUAL PATTERN DISCRIMINATION LEARNING IN MONKEYS (MACACA NEMESTRINA). Leo M. Chalupa, Richard Coyle* and Donald B. Lindsley. Dept. of Psych., Physiol., and Psychiat. and the Brain Research Inst., UCLA, Los Angeles, 90024.

Three control monkeys without lesions, four with lesions of the inferior pulvinar, three with lesions of the medial and lateral pulvinar and one with combined lesions of medial, lateral and inferior pulvinar were given training on a simultaneous pattern discrimination task. During an experimental session an animal was seated in a dark, sound-attenuating chamber. With the onset of a 1 Khz tone, the monkey pressed and released a set-up lever. 500 msecs after set-up lever release, the discriminative stimuli consisting of an N and Z were presented for 10 msecs on two stimulus-response panels situated at eye-level in front of the animal. Pressing the panel with the Z was automatically reinforced (banana pellet). Monkeys with lesions of the medial and lateral pulvinar did not differ from controls in the acquisition of the pattern discrimination. Monkeys with lesions of the inferior pulvinar were markedly impaired, in that three failed to learn the discrimination (one also had medial and lateral pulvinar lesions) and the other two learned only after more than twice as many trials as controls. In addition, the performance of the monkeys with inferior pulvinar lesions was greatly disrupted by the addition of extraneous visual stimuli (grids or circles) to the discriminative stimuli. Control and lesion monkeys which attained criterion performance showed good retention following a six week interval without training. These results indicate that lesions of the monkey inferior pulvinar prevent or interfere with the acquisition of visual pattern discriminations. (Supported by USPHS grant NS 8552 and MH 25938 to D. B. Lindsley)

73 RETINOCORTICAL PROJECTIONS IN THE TREE SHREW, TUPAIA GLIS. David H. Hubel, Dept. Neurobiol., Harvard Med. Sch., Boston, 02115.

In the macaque monkey, the inputs to area 17 from the 2 eyes project to 2 distinct sets of bands which lie at the same depth in layer IV. This can easily be demonstrated by the method of transneuronal transport[1]. In the tree shrew, Harting et al.[2] found that a small geniculate lesion produced a continuous band of Fink-Heimer degeneration confined to one sublamina of layer IV and concluded that layer IV is subdivided into 6 leaflets corresponding to the 6 geniculate layers. To examine this further I injected one eye of a tree shrew with 1 mCi of a tritiated proline-fucose mixture and examined the brain autoradiographically[1]. Survival time was 14 days. As expected, the dorsal lateral geniculate body contained heavy label in layers 2,3,4 and 6 contralaterally, and 1 and 5 ipsilaterally. The contralateral superior colliculus was also densely labeled throughout the upper grey layer; ipsilaterally there were regularly-spaced tiny clusters of label, 6-8/section, deep in the upper grey. The cortex on the contralateral side showed 2 tiers of label throughout area 17: a thin one in layer IIIB, closely resembling the upper tier of input in macaque, and a thicker one, occupying the full thickness of IV (layer terminology of Harting et al.[2]). Ipsilaterally the label was confined to the binocular region of 17 and occupied all of layer IV except for a narrow, centrally-placed grain-free zone corresponding precisely to the cell-sparse cleft seen in Nissl sections; on this side no upper tier was seen. These results confirm those of Harting et al.[2] in showing a laminar difference in distribution of input from the 2 eyes to layer IV; they differ, however, in indicating that all of IV receives binocular input except for the cleft, which receives contralateral input only. (USPHS Grant EY00605; Rowland Fnd.grant)

[1] Wiesel, T.N., Hubel, D.H. & Lam, D.M.K., Brain Res. 79 (1974) 273.
[2] Harting, J.K., Diamond, I.T. & Hall, W.C., J. Comp. Neur. 150 (1973) 393.

74 THE RETINOTOPIC ORGANIZATION OF THE VISUAL CORTEX IN THE CAT.

R. J. Tusa*, L. A. Palmer and A. C. Rosenquist. (SPON: C. N. Liu).
Dept. Anat., Sch. Med., Univ. of Penn., Phila., Pa. 19174.

In order to precisely determine the number, location and topographic organization of visual areas in the cat cortex, we have extensively mapped the visual cortex of awake, paralyzed cats using single and multiple unit recording techniques. We have found at least 13 separate representations of the contralateral visual hemifield, many of which correspond to cortical fields previously defined by cytoarchitectonics or hodology. Cytoarchitectonic areas 17, 18 and 19 each contain a single representation of the visual field, while area 20 of Heath and Jones contains two representations. The lateral suprasylvian area (LSA) contains six separate representations of the visual field: two located rostral and caudal on the medial bank of the middle suprasylvian sulcus and two mirror images of these areas located on the lateral bank. Two further representations of the visual field within LSA lie on the dorsal and ventral banks of the posterior suprasylvian sulcus and are mirror images of each other. Area 21 of Heath and Jones appears to contain two representations of the visual field but the precise topology is uncertain. Those areas on the crown of the middle suprasylvian gyrus and in the splenial sulcus medial to area 17, which have been reported by others to be visual, could not be visually driven using our techniques.

The visual fields represented within these areas differ strikingly from one another in two ways. First, the portion of the visual field represented in these areas differ. Area 17 has the most complete representation of the visual field; area 18 has only the binocular portion of the visual field represented; area 19 has a limited representation of the vertical meridian, but a complete representation of the horizontal meridian; the four caudal areas within LSA all have an even more limited representation of the vertical meridian and an extensive representation of the whole horizontal meridian. The two areas in 20 and the two rostral areas in LSA all have fairly complete representations of the upper visual field, but very limited representations of the lower visual field. Second, the topographical organization of the visual field represented in these areas differ. Area 17 and the four caudal areas in LSA all have a single order transformation of the visual field in which all adjacent points of the visual field are represented as adjacent points in the cortex. The remaining areas all contain a second order transformation of the visual field in which some of the adjacent points of the visual field are not represented as adjacent points in the cortex. In agreement with J. Allman and J. Kaas (1974), we believe that areas with second order transformations may serve as functional adjuncts to areas containing first order transformations.

Autoradiographic studies of thalamic afferents to the visual cortex generally support these conclusions about the retinotopic organization of the cat visual cortex (see A. C. Rosenquist et al., this volume).

Allman, J. M. & Kaas, J. H. The organization of the second visual area (VII) in the owl monkey: a second order transformation of the visual hemifield. Brain Research 76:247-265 (1974)

(Supported by NSF grant BMS75 02453).

75 THALAMIC EFFERENTS TO VISUAL CORTICAL AREAS IN THE CAT.

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The autoradiographic tracing method was employed to detail the projections of the dorsal lateral geniculate nucleus (LGNd) and extrageniculate thalamic nuclei to the visual cortex. In most cases the thalamocortical organization serves to support our parcellation of cortex based upon electrophysiological mapping methods (see Tusa et al., this volume). For example, injections of tritiated leucine into the lateral posterior nucleus (LP) resulted in heavy labeling of several cortical areas including all 6 representations of the visual field within the lateral suprasylvian area(LSA). In contrast, injections into either the medial interlaminar nucleus (MIN) or the posterior nucleus (PN) resulted in labeling of only 3 of the LSA field representations. The 3 remaining unlabeled regions are mirror image field representations of the 3 labeled areas. We have recently found evidence that extrageniculate thalamic nuclei project to area 17. The projections from MIN, PN, and LP to area 17 are all much less dense than either the projections from laminar LGNd to area 17 or the projections of these same 3 nuclei to extrastriate cortical areas. The most striking property of extrageniculate projections to striate cortex is the presence of a distinct band of label in layer I. Whereas LP, PN, and MIN project to many of the same cortical areas, such as area 19, the laminar distribution of cortical label differs considerably from these nuclei. For example, LP appears to terminate heavily in layer I of area 19, but PN and MIN do not.

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76 VISUAL RECEPTIVE FIELDS IN lam LGN_d, MIN and PN OF THE CAT.

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In the previous paper we have described corticopetal pathways arising from several thalamic nuclei medial to the laminar portion of the lateral geniculate (lam LGN_d) in the cat. Here we describe visually driven unit activity in two of these nuclei, the medial interlaminar nucleus (MIN) and the posterior nucleus (PN), and compare them with lam LGN_d.

Surgery was performed under halothane anesthesia and the preparation maintained with $N_20:0_2$ and local anesthetics. Paralysis of extraocular muscles was achieved by continuous infusion of flaxedil and curarie and artificial ventilation adjusted to maintain alveolar $C0_2$ at 4.0%. Arterial blood pressure and body temp. were also held at normal values. Tungsten electrodes approached the thalamus at an angle 30 degs from the vertical so that ideal penetrations passed thru lam LGN_d, MIN, and PN in turn. Electrode tracks were reconstructed anatomically.

Units in MIN had receptive fields very similar to those of lam LGN_d. Fields were on-or off-center and all were strictly monocular. Responses to moving stimuli were similar in every respect to those of lam LGN_d. However, all units in MIN were activated at short latencies by electrical stimulation of the optic chiasm as opposed to lam LGN_d where a bimodal distribution of optic chiasm latencies was found. In contrast to lam LGN_d, where both X- and Y-cells were found, all units in MIN were Y-cells based on the range of effective stimulus velocities, field sizes, non-linearity of spatial summation, as well as the optic chiasm latency.

As the electrode entered PN, dramatic changes in unitary and background activity were evident. Units in PN were usually binocular, had much larger, uniform receptive fields, and most were direction selective when tested with small moving spots. No orientation selective units were found in spite of the strong input from area 17. Units in PN resembled very closely units in SGS and SO of the superior colliculus.

In addition, we have studied units in lam LGN_d, MIN, and PN using the PST response plane technique of Stevens and Gerstein. This quantitative field plotting method reveals the spatial and temporal variation in firing probability in response to spot stimuli turned on and off within the field. Stevens and Gerstein have identified two types of LGN units based on their PST response planes, units with homogeneous or heterogeneous planes. We have identified homogeneous as Y-cells and heterogeneous as X-cells based on other properties of these units including their latencies to optic tract stimulation. Thus, X-and Y-cells can be distinguished on the basis of their receptive field structure alone. Using the PST response planes, all units in MIN have homogeneous planes and units in PN yield generally unstructured planes.

We conclude that MIN is functionally as well as anatomically a part of the LGN although it contains only Y-cells and no X-cells. PN appears to be functionally very similar to the superior colliculus. It is clear that the cells of origin of the various corticopetal pathways convey fundamentally different messages to their cortical receiving areas.

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77 SIMULTANEOUS UNIT RECORDING FROM LGNd AND VISUAL CORTEX IN THE CAT. C. V. Noback*, A. C. Rosenquist, and L. A. Palmer. Dept. Anat., Sch. Med., Univ. of Penn., Phila., Pa. 19174 (SPON: P. Sterling).

In order to study directly how receptive field properties of visual cortical cells are generated from thalamic inputs, single units were recorded simultaneously from the cortex (areas 17, 18) and LGN. The cortical electrode was placed in a region from which antidromic responses of the LGN cell could be evoked and where receptive fields generally overlapped. Spike trains of the cortical and LGN cells were cross correlated on-line to check for interactions between cells. This information was used in deciding whether further analysis was desirable. In at least 6 cases correlation peaks .5 to 1 msec. in duration were observed whose latencies from the LGN cell spikes in conjunction with antidromic latencies suggest the monosynaptic innervation of the cortical cell by the LGN cell or by other LGN cells correlated through common inputs with the recorded LGN cell. In one of these cases and in others the presynaptic cortical response of the LGN cell was seen by signal averaging the analog cortical signal against the LGN cell. In at least 1 case the presynaptic response was directly observable. In 5 of the 6 pairs the receptive fields were clearly overlapped. Three of the LGN cells were on-centered and 3 were off-centered. All had antidromic latencies of 0.9 msec. or less and responded well to a rapidly moving bar whose contrast was antagonistic to the center response. These properties suggest that the cells were Y cells. The cortical cells were complex. This evidence implies that some complex cells are monosynaptically driven by rapidly conducting LGN cells probably of the Y category. (Supported by NSF Grant No. BMS 75-02453)

78 SUBUNITS IN COMPLEX CELL RECEPTIVE FIELDS IN CAT STRIATE CORTEX. J. A. Movshon* and D. J. Tolhurst*
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Most complex cells in cat striate cortex have receptive fields which cannot be subdivided, like the receptive fields of retinal ganglion cells, LGN cells and simple cortical cells, into discrete excitatory and inhibitory regions. They respond identically to stimulation anywhere in their receptive fields. Nevertheless, these cells are selective for stimuli smaller than their receptive fields, implying the existence of lateral interactions within an apparently homogeneous region. We have investigated these interactions, using computer-generated rectangular-bar and sine wave-grating stimuli.

A complex cell's response to pairs of simultaneously-flashed bars depends critically on the separation between them. If both bars are of the same contrast polarity, the response to the pair is more than the response to either bar alone when the two are close together, but much less when they are separated by more than one-quarter the width of the receptive field. If the bars are of opposite contrasts, the opposite result is obtained: there is summation between the bars at large separations and inhibition at small ones. The result is independent of position in the field: the lateral interactions depend only on bar separation. A plot of response vs. space for such a two-bar experiment yields a "subunit profile". This profile matches the inverse Fourier transform of the spatial frequency tuning curve of the cell, indicating that the subunit revealed may be the mechanism responsible for the cell's tuning for stimulus size. Other aspects of the cell's behaviour may be satisfactorily accounted for by postulating that the receptive field consists of a number of subunits acting as logical OR elements: the most active subunit at any time will determine the cell's response.

79 OCULAR DOMINANCE COLUMNS IN THE CAT'S VISUAL CORTEX. Carla Shatz*, Sivert Lindstrom*, and Torsten Wiesel. Dept. Neurobiol., Harvard Med. Sch., Boston, Mass. 02115.

In this study, ocular dominance columns have been demonstrated autoradiographically in the cat's visual cortex by means of transneuronal transport, as first reported in the monkey by Hubel, Wiesel, and Lam (1974). Both in normal and visually deprived cats the vitreous of one eye was injected with 500 µCi each of 3H-proline and 3H-fucose in 100 µl saline. Ten days later animals were perfused and autoradiographic techniques were used to visualize the distribution of labelled geniculocortical terminals. In the normal cat, ipsilateral to the injected eye discrete patches of label were visible in layer IV of area 17. In the contralateral hemisphere, although there were clear indications of periodic concentrations, the label was almost continuous throughout layer IV. This pattern of labelling in both hemispheres presumably corresponds to ocular dominance columns as physiologically defined. As expected, there was a strip of heavy labelling within the representation of the temporal cresent in the contralateral cortex, and a corresponding absence of label in this region on the ipsilateral side. The columns were best seen in parasagittal sections, suggesting that they run mediolaterally and, as in the monkey, are perpendicular to the anatomical 17-18 border. The size of a pair of left plus right eye columns was roughly 1 mm wide in cortex representing central and peripheral visual fields. The columns were also present in area 18. In one monocularly deprived kitten, the closed eye was injected. Changes in the columns were most apparent in the contralateral hemisphere, in which, unlike normal cats, distinct patches of label were now visible. These presumbaly reflected an increase in dominance of the nondeprived eye consistent with the reported physiological changes. Thus, as in the monkey, ocular dominance columns can be anatomically demonstrated in the cat, thereby providing a useful technique for studying morphological changes related to visual deprivation.

80 NEURONAL MECHANISMS SUBSERVING BRIGHTNESS CODING IN MONKEY VISUAL CORTEX. R. J. W. Mansfield. Harvard University, Cambridge, Massachusetts 02138.

In primates, diffuse illuminants can be discriminated over a large dynamic range of intensity. An analysis of the receptive field properties of neurons in Areas 17 and 18 in the unanesthetized Rhesus monkey reveals the existence of a class of neurons whose spectral sensitivities, dynamic ranges, and intensity-response functions are appropriate for brightness coding. Previous studies that employed anesthetic agents have not observed systematic correlates of brightness coding at the cortical level. The brightness-sensitive neurons of the present experiments resemble in many qualitative and quantitative terms the W-cells described in the cat visual system. The majority had complex or hypercomplex receptive fields and were driven binocularly. In terms of cytoarchitecture, the majority of striate neurons were located in Layers II and III. The neurons respond to stimulus contrast and for diffuse illumination of intensity I, the response rate, R, can be described by the dimensionless equation

$$R/R_{max} = (I/I_{max})^{0.3}$$

in accord with behavioral observations; the upper limit of information transfer in an individual neuron is less than 3 bits. **(Supported by NSF grant BMS 75-08437.)**

81 QUANTITATIVE ANALYSIS OF MOTION SELECTIVITY IN "SIMPLE" NEURONS OF CAT VISUAL CORTEX USING A STROBOSCOPIC MOTION DISPLAY. Leo Ganz and Ralph C. Felder*. Dept. Psychol., Stanford University, Stanford, CA, 94305.

A model to account for sequence-selectivity in "simple" neurons of primary visual cortex has recently been proposed (Bishop, et al, JP 219: 659, 1971) with the following properties: (1) separate cortical mechanisms are postulated for ON-sequence analysis and for OFF-sequence analysis (2) for motion in the preferred direction, disinhibition is followed by excitation and finally by inhibition which is too late to cancel the initial activation of the cortical neuron, (3) for motion in the null direction an initial inhibition is followed by excitation and finally by disinhibition which is too late to be effective.

We have tested this model by analyzing the response of visual cortex neurons to three types of displays: (a) single stationary pulses of light (thin rectangles, white, optimally oriented); (b) sequences of pairs of stationary pulses of light, as in an "apparent movement" display; (c) white or black rectangles moving continuously across the neuron's receptive field. We have recorded from some 103 neurons, from 30 adult cats, the microelectrode located in or near the projection of the area centralis in area 17.

We have analyzed the response of "simple" receptive field neurons to pairs of stationary pulses of light as follows. If the mechanism of motion-selectivity is based on inhibition of the second stimulus when the sequence is in the null direction, then we should expect the S_1-poststimulus time histogram (PSTH) peak for a rectangle presented as the first stimulus at some retinal location to be larger than the S_2-PSTH for that same rectangle presented at the same retinal location but now the second stimulus of a null direction pair. If the mechanism of motion-selectivity is based on facilitation or disinhibition of the second stimulus when the sequence is in the preferred direction, then we should expect the S_1-PSTH peak to be smaller than the S_2-PSTH peak for that same rectangle presented at the same retinal location but now the second stimulus of a preferred direction pair. Our results consistently support the presence of inhibition in null-direction sequences; we consistently fail to find evidence for facilitation/disinhibition for preferred direction sequences. Thus, the mechanism of motion-selectivity seems to be based simply on an anisotropy of inhibition within the receptive field. We do find many "simple" neurons in which ON-selective regions of the receptive field are separated in space from OFF-selective regions.

Our results also show that the inhibition generated by a sequence in the null direction is often very slow in its decay characteristics, lasting over 400 msec. The decay of null-sequence-generated inhibition is slower in neurons with sustained-type impulse responses than in neurons with impulse responses of the transient-type. Moreover, sustained-type cells are found to respond selectively to slower velocities.

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82 RECEPTIVE FIELD PROPERTIES OF PRESTRIATE NEURONS: EVIDENCE FOR PARALLEL PROCESSING. Joan S. Baizer and Bruce M. Dow. Laboratory of Neurobiology, National Institute of Mental Health, and Laboratory of Vision Research, National Eye Institute, National Institutes of Health, Bethesda, Maryland 20014.

We studied receptive field properties of cells buried in the posterior bank of the lunate sulcus in three awake monkeys trained to fixate. We classified cells according to their responses to stationary and moving stimuli of different shapes, sizes, orientations, and colors, and found that the cells fell into six distinct major groups, with little overlap among groups. 1) Orientation-specific cells were sensitive to stimulus orientation but not to the color or direction of movement of an optimal stimulus. 2) Directional cells responded to stimuli moving in the optimal direction regardless of contour, contrast, or color. 3) Color cells showed little or no orientation specificity and were not directionally selective. 4) Suppressed-by-light cells had high spontaneous activity and were insensitive to stimulus orientation and direction of movement. 5) "Pandirectional-spot" cells responded as well to small spots as to slits of any orientation, and equally well to all directions of stimulus movement. 6) "Border" cells responded best to stimuli that filled an excitatory region without encroaching on a powerful suppressive flank. All groups were affected by stimulus size; cells in groups 1 and 5 preferred stimuli that were small relative to total excitatory field size, while cells in the other groups preferred stimuli filling an excitatory region but not extending into inhibitory surrounding areas. The data suggest that in this prestriate region different visual parameters are processed independently and in parallel by different populations of cells. (Supported in part by an NINDS postdoctoral fellowship to JSB.)

83 STRIATE AND PRESTRIATE RECEPTIVE FIELDS IN RHESUS MONKEY: INDICATIONS OF SERIAL PROCESSING. Bruce M. Dow and Joan S. Baizer, Laboratory of Vision Research, National Eye Institute, National Institutes of Health and Laboratory of Neurobiology, National Institute of Mental Health, Bethesda, Maryland 20014.

In the foveal projection area of rhesus monkey striate cortex it is possible, because of the deeply infolded lunate and inferior occipital sulci, to record in a single penetration from cells in striate and prestriate cortex with the same or overlapping receptive fields. In anesthetized, paralyzed monkeys we have mapped receptive fields on a tangent screen and tested successive cells with the same stimuli and movement velocities, thus permitting direct comparisons of foveal striate and prestriate neurons. Orientation-specific prestriate cells have the same receptive field widths as their striate counterparts, but show narrower tuning and require longer stimuli for optimal driving. Prestriate directional cells give polar plots and velocity tuning curves that closely resemble those of their striate counterparts, but have slightly larger receptive fields. The color-tuning of prestriate color cells is not detectably different from that of striate color cells, and about 2/3 of both striate and prestriate color cells lack orientation specificity. Prestriate "pandirectional" cells give muttering responses to any moving stimulus, and have large receptive fields with multiple subregions, a property they share with Class V (on/off) striate cells. Suppressed-by-light cells have similar properties in striate and prestriate cortex. No striate cells have been found with the properties of prestriate "border" cells, and no prestriate cells have shown the properties of noncolor Class I (nonoriented) or Class II ("simple") striate cells. The data indicate the existence of several parallel channels, each undergoing some degree of serial processing in the striate-prestriate projection pathway. (Supported in part by an NINDS postdoctoral fellowship to JSB).

84 NEURONAL MECHANISMS OF THE PARIETAL LOBE FOR DIRECTED VISUAL ATTENTION, STUDIED IN WAKING MONKEYS. T.C.T. Yin, J. C. Lynch, W. H. Talbot*, and V. B. Mountcastle. Department of Physiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Neurons in the inferior parietal lobule (area 7) of the cerebral cortex were studied in waking, behaving monkeys trained to fixate a small stationary or moving spot of light projected onto a tangent screen. Two classes of neurons were identified that discharge in relation to the direction of visual attention to food or to novel objects or other visual targets of interest to the animal. Visual Fixation (VF) cells show an abrupt and sustained increase in their discharge as the animal fixates a stationary spot. Some of these cells are active during fixations of any target within surrounding visual space; others are active with fixations directed into a more limited area, but one rarely smaller than half of surrounding space (i.e., the area of space that can be explored visually with the head fixed). Neurons of the second class are Visual Tracking (VT) cells; their discharges are correlated with smooth pursuit movements of the eyes. VT neurons exhibit a sustained discharge during smooth pursuits of objects moving in one direction and not the opposite, regardless of the position of gaze. The increment in their discharge often precedes onset of the tracking movement; they are not active during fixations of stationary spots. The discharge of VF and VT cells is suppressed before and during saccadic movements interposed during fixations or trackings. We propose that these sets of neurons are cortical command cells directing visual attention to stationary (VF) and moving (VT) targets of motivational interest.

85 A CORTICAL SOURCE OF COMMAND SIGNALS FOR VISUALLY EVOKED SACCADIC MOVEMENTS OF THE EYES IN THE MONKEY. J. C. Lynch, T. C. T. Yin W. H. Talbot*, and V. B. Mountcastle. Dept. of Physiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, 21205.

Experiments were made in waking monkeys trained in a variable foreperiod paradigm to fixate a small light on a tangent screen, follow its abrupt lateral or vertical displacement, and detect its dimming for fluid reward. Microelectrode penetrations were made into the cerebral cortex via chronically implanted chambers. A class of neurons was identified in area 7 that is active in relation to visually evoked saccadic movements of the eyes. These cells are commonly directionally oriented, and discharge as early as 150 msec before eye movement begins. A smaller number is active with saccades in several directions, but discharge earliest for one direction of movement. None is active during spontaneous saccades. During saccadic movements there is a profound suppression of the activity of other command sets of neurons of the inferior parietal lobule, the visual fixation and tracking neurons. This suppression also begins about 150 msec before and persists throughout eye movement; it is followed by a re-fixation increment in the discharge of these cells.

We propose that area 7 of the parietal lobe is one source of the cortical command signals for visually evoked saccadic movements of the eyes, in the monkey.

86 INFEROTEMPORAL AND VISUAL CORTEX ELECTROPHYSIOLOGY DURING VISUAL DISCRIMINATION BEHAVIOR IN MONKEYS: CORRELATIONS BETWEEN WAVESHAPES AND ATTENTION. Marc R. Nuwer and Karl H. Pribram. Departments of Psychology and of Psychiatry and Behavioral Sciences, Stanford University, Stanford, California, 94305.

This study examines the brain electrical activity of monkeys in a selective attention procedure. The task, described in detail by Rothblat and Pribram (Brain Res. 34: 427, 1972) is to respond differentially either to the color or to the pattern of a pair of multidimensional cues flashed briefly (10 microsec.) to evoke an abrupt cortical potential change. Electrode placements included a row along the inferior portion of the occipital and temporal cortex and another from frontal pole to parietal cortex. Here only the results from the inferior temporal (IT) cortex recordings will be detailed because of the marked effect lesions in this location have on visual discrimination. Cortical records were made from surface to depth bipolar electrodes; behavioral records were generated by panel press. Both were processed by a small on line computer (PDP-8).

Stimulus evoked potentials were accumulated over 180 consecutive trials (3 days) as were response evoked potentials. The latter were obtained by averaging both backward (250 msec) and forward (250 msec) from the time of panel press.

These response evoked wave forms recorded from the inferotemporal cortex were found to be correlated with the dimension of the stimulus attended (as defined by the behavioral response): in 70% of the electrodes (11 of 16) when the arrangement of color determined the correct response the evoked brain activity correlated with color; when the arrangement of pattern determined the correct response the evoked brain activity correlated with pattern. This correlation improved with learning but not with overtraining.

The stimulus evoked brain waves were also of interest. Correlation with task dimension was also obtained -- 67% (4 out of 6 mid-IT electrodes). With a shift in tasks (e.g. from color to pattern), there was a lag in the shift of correlated electrical activity as if the monkeys were still attending the prior stimulus although responding appropriately to the new one.

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87 RESPONSES OF INFERIOR TEMPORAL NEURONS TO VISUAL DISCRIMINANDA. Charlene D. Jarvis and Mortimer Mishkin. N.I.M.H., Bethesda, Md. 20014.

In the anesthetized rhesus monkey, some inferior temporal (IT) neurons have complex visual trigger features, and nearly all IT neurons have large receptive fields that include the fovea (Gross et al, J. Neurophysiol., 1972). These characteristics suggested that IT neurons in the awake monkey might be driven selectively by stimuli that the monkey was required to discriminate, and hence fixate. To explore this possibility, two rhesus monkeys were trained to press a panel for juice reward when a positive stimulus appeared on it and to press a blank adjacent panel when a negative stimulus appeared. The positive stimulus (a white ring subtending 5^{o} of visual angle) was paired with up to 24 negative stimuli (other 5^{o} white or colored geometric patterns) in separate blocks of trials, which the animals initiated at a rate of about one trial/sec. Following training, extracellular recordings were made from IT cortex while the subjects performed the task. Out of 141 units recorded from both animals, 40% were activated or (rarely) suppressed by one or more stimuli, at latencies of 80 to 150 msec. While a few of the related cells responded to one stimulus exclusively and a few responded to all the stimuli with which they were tested, most related cells responded to more than one, but not all, of the stimuli. Only two cells fired exclusively to the positive stimulus, and no cell fired to all but the positive stimulus. IT neurons thus appeared to code the stimulus properties of the visual discriminanda and not their reward value. Since differentially responsive cells were easily located using this simple behavioral situation, it may be a convenient one with which to investigate the stimulus code, as well as the effects of non-stimulus variables such as attention, memory, and motivation.

88 SINGLE CELL RESPONSES TO AUDITORY AND VISUAL STIMULI IN THE PREOCCIPITAL GYRUS AND SUPERIOR TEMPORAL SULCUS IN THE MACAQUE MONKEY. Barry Davis and L. A. Benevento. College of Medicine, University of Illinois Medical Center, Chicago, Illinois 60680.

The response properties of cells in the banks of the lunate sulcus, the preoccipital gyrus, and in the banks of the superior temporal sulcus were analyzed following moving or flashing light targets and/or tones. The location of the cells were verified histologically. Cells were responsive to input from either the ipsilateral and/or contralateral eye, and their receptive fields were located within and away from the area of central vision. Cells located in all 3 locations were found to be responsive to light and to have response properties characterized by direction and orientation sensitivity. However, cells located in more anterior preoccipital gyrus and the superior temporal sulcus were generally weaker in their responses to moving stimuli than were the familiar complex cells located in the lunate sulcus. Moreover, cells located in the banks and floor of the superior temporal sulcus were clearly responsive to auditory stimuli, as well as to visual stimuli. The response to sound usually consisted of on and/or off inhibition, which was usually followed by an excitatory afterdischarge. Cases of transient off excitation, and excitation to particular tones were also observed. In attempts to interact auditory and visual stimuli, it was observed that the onset of a preferred tone modified the cell's response to visual stimuli. The cell could be inhibited by the tone to such an extent that a stimulus moving through the excitatory zone of the receptive field had a decreased excitatory influence. The results indicate that the superior temporal sulcus is integrating both visual and auditory information as would be surmised from recently demonstrated anatomical projections.

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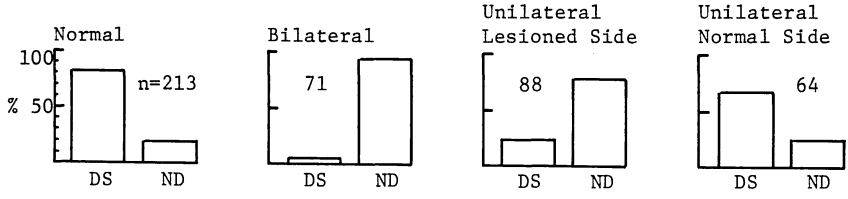
89 EFFECTS OF VISUAL CORTEX REMOVAL ON RECEPTIVE FIELD PROPERTIES OF CELLS IN THE LATERAL SUPRASYLVIAN VISUAL AREA OF THE CAT. Peter D. Spear and Thomas P. Baumann*. Dept. Psychology, Kansas State Univ., Manhattan, Ks. 66506.

Previous anatomical studies show that the lateral suprasylvian visual cortex (LS, or Clare-Bishop area) receives both ipsilateral and contralateral projections from visual cortical areas 17, 18, and 19. The purpose of the present study was to investigate the effects of removing these inputs on the receptive field properties of cells in the LS area. Seventeen cats received bilateral or unilateral visual cortex lesions (areas 17, 18, and 19) 1-5 weeks prior to recording in the LS area, and the receptive field properties were compared to those of LS area cells in normal cats (Spear, 1974, Society for Neuroscience Conv.). In normal cats, 81% of the LS area cells are directionally selective (DS, see fig. I below). The remaining cells have non-directional (ND) receptive fields, including movement sensitive (7.5%), stationary (5%), and diffuse (6.5%) types. In addition, most (65%) of the cells which have receptive fields within 45 deg. of the area centralis are binocularly driven (B, see fig. II). About 34% normally are driven by the contralateral eye only (C) and about 1% are driven exclusively by the ipsilateral eye (I).

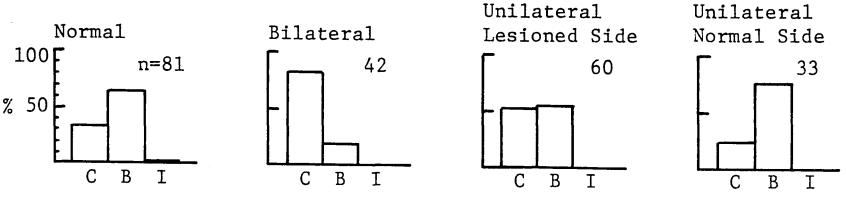
The main effects of visual cortex removals are shown in figs. I and II. Following bilateral visual cortex lesions, there was a marked reduction in directional selectivity among the LS area cells (8%). Over 90% of the cells now fell into one of the three non-directional classes: movement sensitive (47%), stationary (41%), or diffuse (4%). In addition, there was a reduction in the number of binocularly driven cells. Over 80% of the cells with receptive fields within 45 deg. of area centralis were driven only by the contralateral eye following the bilateral lesion. Similar results were obtained recording in the LS area ipsilateral to a unilateral visual cortex lesion, although the effects were somewhat less marked (see figs.). Contralateral to a unilateral visual cortex lesion, the LS area appeared nearly normal.

A variety of receptive field properties of LS area cells were not affected by the visual cortex lesions. For example, most of the cells responded well to small stimuli (½ deg. - 3 deg.) and about 80% showed spatial summation to increases in stimulus size, just as in normal cats. The incidence of surround inhibition (about 35% of the cells) also was normal. Velocity sensitivity of movement sensitive cells and the remaining directionally selective cells also was unaffected by the visual cortex removals.

I. Receptive Field Type



II. Ocular Dominance (Within 45 deg. of Area Centralis).



90 LAMINAR PATTERNS OF GENICULOCORTICAL PROJECTION IN CAT.
Charles D. Gilbert* and Simon LeVay (SPON: Zach W. Hall). Dept. of Neurobiology, Harvard Medical School, Boston, Mass. 02115.

It has previously been shown[1] that the pyramidal cells of layer VI of cat visual cortex project back to the dorsal lateral geniculate nucleus (LGNd). We wished to determine whether this layer received a direct projection from the LGNd. 3H-proline was injected from a recording micropipette into lamina A or lamina C of the LGNd. Subsequent light microscopic autoradiography showed a denser concentration of silver grains over layer VI than over layer V or the white matter, in both areas 17 and 18. Layer VI was much more heavily labeled after the injections in lamina A than after those in lamina C. Electron microscopic autoradiography in this layer showed silver grains over terminals of Gray's type I, which synapsed mainly onto dendritic spines, as in layer IV. Myelinated fibers were also labeled. This result was confirmed by the EM observation of degenerating terminals in layer VI (also making type I synapses on spines) after a lesion in the LGNd. This made it far less likely that the radioactive label in the layer was due to transneuronal transport. The results suggest that the recurrent loop to the LGNd can involve as few as one cortical synapse.

The pattern of projection to other cortical layers was also studied. After injections of 3H-proline into lamina A, heavy labeling filled the entire thickness of layer IV, extending into deep III. Alternating 350 μ wide patches of heavier and lighter labeling were consistent with the columnar organization of geniculate afferents.[2] Injections of lamina C (which contains both left and right eye sublayers) produced a continuous band of label in layer IV. Terminals were also found in the superficial half of layer I after the layer C injections, but not after the lamina A injections. (NIH grant 5T01EY00082-03) 1. Gilbert & Kelly, J. Comp. Neur. in press. 2. Shatz, Lindstrom & Wiesel, this volume.

91 CORTICAL PROJECTIONS OF CORTICORECIPIENT AND TECTORECIPIENT ZONES OF THE PULVINAR IN THE MACAQUE MONKEY.
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Our autoradiographic and Fink-Heimer studies in macaques have shown that the regions of the inferior pulvinar receiving projections from the superior colliculus and cortical areas 17,18,19 are not entirely coextensive. Additional autoradiographic studies revealed that the portion of the inferior pulvinar which receives only tectal projections projects mainly to the cortex within the inferior occipital sulcus and adjacent area TEO of Bonin and Bailey (area 37 of Brodmann or posterior inferotemporal cortex) which have been shown to comprise a functionally distinct region in visual discrimination experiments. In contrast, the "corticorecipient" areas of the inferior as well as the lateral pulvinar project to areas 17,18, and 19 and posterior inferotemporal cortex. The cortical projections of the areas of the pulvinar complex which do not receive projections from the occipital cortex or superior colliculus were also studied. Specifically, the rostral and medial portions of the medial pulvinar project to the floor of the lateral fissure and to prefrontal cortex about the inferior limb of the arcuate sulcus and the orbital cortex. The caudal pole of the pulvinar (medial pulvinar) projects to area 20 between the anterior middle temporal and the occipitotemporal sulci. Thus, it is the regions of the pulvinar which receive projections from the superior colliculus and occipital cortex which project to occipital and posterior temporal cortices concerned with visual function with area TEO being a main target of the superior colliculus-inferior pulvinar pathway.
(Supported by NSF Grant BMS 75-07349)

92 SOME PROJECTIONS OF THE POSTERIOR BANK AND FLOOR OF THE SUPERIOR TEMPORAL SULCUS IN THE MACAQUE MONKEY. Steven C. McLoon*,Rebecca Santos-Anderson*and L. A. Benevento. College of Medicine, University of Illinois Medical Center, Chicago, Illinois 60680.

The posterior bank and floor of the superior temporal sulcus of the macaque monkey has been shown to contain units sensitive to visual stimuli. In addition, this area receives convergent axonal projections from visual cortex (i.e., areas 17,18,19) and certain portions of the pulvinar (Anat. Rec. 181:461, 1975). The lateral pulvinar and the dorsolateral portion of the inferior pulvinar project to areas 18 and 19 and the posterior bank and floor of the superior temporal sulcus. Injections of tritiated leucine and proline into occipital cortex show that this region projects to the lateral pulvinar and dorsal and lateral portions of the inferior pulvinar. In addition, injections into the posterior bank and floor of the superior temporal sulcus show that this area also projects to the same regions of the pulvinar. The superior temporal sulcus also projects back to the preoccipital gyrus (area 19) and the lunate sulcus (area 18). The results indicate that the superior temporal sulcus and occipital cortex are being interrelated by cortico-cortical connections as well as by connections with the pulvinar.

(Supported by NSF Grant BMS 75-07349)

93 TOPOGRAPHIC ORGANIZATION OF PROJECTIONS FROM VISUAL CORTEX TO THE MEDIAL INTERLAMINAR NUCLEUS AND LATERAL POSTERIOR NUCLEAR COMPLEX IN THE CAT. Bruce V. Updyke. Dept. of Anatomy, Univ. of Wisconsin, Madison WI, 53706.

The retinotopic organization of the medial interlaminar nucleus (MIN) and a part of the lateral posterior complex (LPC) was analyzed by autoradiography following injections of areas 17 and 18 with ^{3}H-proline. The retinotopic identities of injection sites were determined by comparing the projections to the laminar part of the lateral geniculate nucleus (LGNd) with Sanderson's (J. Comp. Neurol.,143:1971) map of the nucleus.

Areas 17 and 18 both project in register onto MIN and LPC. Within MIN the projections terminate as thin bands oriented parallel to the dorsoventral axis of the nucleus. Cortex representing the vertical meridian of the visual field projects adjacent to the MIN/LGNd border; that representing lateral visual field out to about 45° projects medially in MIN. Cortex representing upper visual field projects caudally; that representing lower visual field projects rostrally. The projection patterns show MIN to be organized as a compressed mirror image of the representation within LGNd.

Within LPC the cortical projections also terminate as bands which extend through the lateral posterior nucleus (LP) and the posterior nucleus of Rioch (PN). The bands originate rostro-medially in LP and are inclined caudally and ventro-laterally into PN. Cortex representing the vertical meridian projects ventro-medially in LP and PN; that representing the lateral visual field projects dorso-laterally. The lateral most projections share a common border with MIN. The cortex representing the upper visual field projects caudally; that representing the lower visual field projects rostrally. The representation of the visual field defined by these projections resembles an expanded mirror image of the representation found within MIN.

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94 RETINOGENICULATE PROJECTIONS OF B10.D2/nSn MICE. Irwin S. Westenberg*, V.A. Hosp., Phoenix, Az., 85012, and Donnell Creel, V.A. Hosp., Salt Lake City, Ut., 84113.

Reduced retinal pigmentation has been correlated with reduced and/or disorganized retinogeniculate projections (RGP) in mammals. However, it has not been determined if it is the genes for reduced retinal pigmentation that lead to modified RGP. To answer this question it is necessary to compare animals that are genetically identical except for the genes for reduced retinal pigmentation, e.g. albino (B10.D2/nSn-c^{4J}) and normal B10.D2/nSn mice. In order to provide the basis for such a comparison, the RGP of B10.D2/nSn mice were mapped. The right eyes of six 60-day-old male mice were removed, and six days later the mice were killed. Brain sections were stained for degenerating axonal processes. In serial maps large crossed RGP and smaller, more localized uncrossed RGP were observed. The uncrossed RGP were similar to those of other normal rodents. However, the crossed RGP were unusual; they were uniformly distributed throughout the dorsal lateral geniculate nucleus, including the region receiving uncrossed RGP. The size and localization of the uncrossed RGP suggest that the B10.D2/nSn strain is suitable for testing the hypothesis that the genes for albinism lead to reduced or disorganized uncrossed RGP. The unusual crossed RGP in the B10.D2/nSn strain raises new questions. The technical assistance of Ms. C. Kalaha-Brunst is acknowledged.

95 THE EFFECT OF DARK ADAPTATION ON NEURONAL RESPONSES IN THE CAT LATERAL GENICULATE NUCLEUS (LGN). B.B.Lee, V.Virsu+& O.D. Creutzfeldt+. Max Planck Inst. für biophysikalische Chemie, Göttingen, W.Germany.

It has been suggested that both the centre and surround components of the receptive fields of LGN neurones are generated by the centre responses of ganglion cells (Singer & Creutzfeldt,1970). If so, then LGN neurones should retain the same centre-surround structure following dark adaptation despite the loss of ganglion cell surround mechanisms at low illumination levels. The centre-surround organisation of neurones from layers A and A1 of the LGN has been studied with background illuminations between 1000 trolands and almost complete darkness (less than 10^{-5}td.) using two techniques. We have measured by how much the response to a spot flashed on the centre of a receptive field is inhibited by the simultaneous presentation of annuli of various diameters in the surround, and have analysed responses of discs of various diameters (area summation curves). For both ON- and OFF-centre neurones very little change in centre-surround organisation occurred following dark-adaptation. However,definite changes in the structures of the responses to flashed and moving stimuli were found. For example,responses to flashed stimuli often became more sustained in the dark,and subsidiary peaks and troughs in the PSTHs of neuronal responses to moving stimuli tended to disappear. Some of these changes suggest that there is a decrease in recurrent inhibition within the LGN as background illumination decreases.

Singer,W., Creutzfeldt,O.D. Exp.Brain Res.,10311-330,(1970).

96 FREQUENCY ENTRAINMENT IN THE CAT LATERAL GENICULATE NUCLEUS. Conrad Wall III*, Wlodzimierz M. Kozak* and Arthur C. Sanderson*. Carnegie-Mellon Univ., Pittsburgh, Pa. 15213. (SPON: David L. Tomko. Univ. of Pittsburgh Sch. Med., Pittsburgh, Pa. 15213.)

We have investigated the effects of externally applied periodic signals upon spontaneous alpha-like gross potentials that occur in the cat lateral geniculate nucleus (LGN), testing the hypothesis that it is an oscillating system which can be entrained by periodic signals of appropriate frequency and strength. On-line spectral analysis of the response reveals an ongoing natural oscillation under quiescent conditions and a "low pass" type of amplitude response with a suppression of natural oscillations while entrained. There is also a phase lead for periodic stimulation below that of the natural frequency and a phase lag in the response for stimulation above the natural frequency. A mathematical model of an entrainable nonlinear oscillator is proposed. It demonstrates all of the physiological effects described above. In addition, the experimental results show a frequency component which persists at the driven frequency after the end of periodic stimulation. The duration of this persistence effect is estimated to be between 0.1 and 1.0 seconds.

97 ATTENTION UNITS IN THE FROG'S OPTIC TECTUM

David Ingle, McLean Hospital, Belmont, MA 02178

Within the superficial neuropil of the frog optic tectum one records fiber terminals from the retina which contact ascending dendrites from deeper neurons. We have also recorded visually-driven units that appear non-retinal. One class has response properties similar to units recorded in posterior thalamus, and may be axon terminals derived from thalamis neurons. The other class survive a large lesion of thalamus; they appear to be intrinsic axons from tectal neurons of deeper laminae.

Units of the presumably intrinsic class differ from retinal axons in that they continue to discharge after a bug-like stimulus has left the field. Some respond only after the stimulus has disappeared. Both sub-classes of unit reflect a post-stimulus excitability within a local tectal region, which is elicited by prey-like stimuli.

The behavior of these particular tectal units (whatever the circuitry which provides their novel properties) may participate in two behavioral phenomena which also imply post-movement excitability within the prey-response system. (1) both frogs and toads may give a delayed snap to a recently stopped stimulus. (2) frogs have a tendency to snap more frequently at the second of two very brief stimulus movements. Both behavioral and physiological data suggest that self-exciting mechanisms within the frog tectum may contribute to short-term "focal attention" to prey objects.

98 SUPERIOR COLLICULUS VISUAL EVOKED POTENTIALS IN UNANESTHETIZED RATS. Robert S. Dyer and Zoltan Annau. Dept. Environmental Medicine, Johns Hopkins Univ., Baltimore, Md. 21205.

Rats were chronically implanted with bipolar recording electrodes in the superior colliculus and studied with their pupils dilated in a chamber constructed to reflect flashes. The response to flashes (0.5/sec averaged in blocks of 50) was more complex than has previously been reported, consisting of 5 positive and 5 negative peaks within 240 ms of the flash, not including afterdischarges. All components reversed polarity at the level of the stratum griseum superficiale. Amplitudes of P1 and N4 gradually increased over the first 1.5 hr of recording, but subsequently remained stable for the next 4.5 hr. Latency and amplitude recovery cycles were plotted for P1 and N4 using a subtraction procedure, and N4 was found to be easily confused with N3 at short interflash intervals. Amplitudes dropped to 50% of control at 150 ms intervals for both P1 and N4, but P1 could still be identified in some animals at intervals as short as 6 ms. Changes in light intensity produced differential changes in amplitude of the different waves. An 88% reduction in flash intensity reduced the amplitude of P1 to 58% of maximum, but reduced the P3 amplitude to 8% of maximum. The P3 wave was the most sensitive to decreased flash intensity, and was among the first waves to disappear at decreasing interstimulus intervals, appearing as only a notch on the rising slope of N4 at interstimulus intervals less than 250 ms. When lightly anesthetized (30mg/Kg Nembutal), both the P1 and N4 amplitudes increased by more than 45%; P1 latency remained unchanged, but N4 latency increased by 7%.

99 LUXOTONIC UNITS IN STRIATE CORTEX OF ALERT MACAQUES. Yukihiko Kayama*, Ronald R. Riso*, John R. Bartlett and Robert W. Doty, Sr. Center for Brain Research, University of Rochester, Rochester, New York, 14642.

Luxotonic units, i.e., those continuously discharging at least twice as fast in diffuse light as in darkness or vice versa, are common in striate cortex of unanesthetized Saimiri. (J. Neurophysiol. 37: 621, 1974.) To assay their presence in alert, nonparalyzed macaques two animals were prepared for chronic transdural recording with microelectrodes. The monkey's head was painlessly fixed during recording and the limbs gently restrained. Data were obtained from three areas, each 12 mm in diameter, sampling most of the occipital operculum. Pattern vision was eliminated by covering the constricted pupils with opalescent contact lenses and placing the monkey within an illuminating opalescent hemisphere. Under these conditions, of 209 units examined for 10-100 min roughly 40% were luxotonic, 20% displayed transient responses to diffuse steady light, 20% responded only to stroboscopic flashes, and 20% failed to respond to either flashes or steady light. Analysis for 47 electrode penetrations roughly perpendicular to the cortex showed no significant tendency for grouping of luxotonic units. While essentially all were binocularly influenced, only 75% gave equivalent responses from each eye. For many of these the rule 1+1=1 applied, i.e., each eye alone gave a comparable response and there was no binocular summation. In 20% of the luxotonic and nonluxotonic units tested their discharge was modulated by saccadic eye movements in the dark. The general tendency was for suppression of discharge from approximately the onset to 150 msec after the saccade, followed by enhancement at about 200 msec. (Supported by Grant NS 03606 from the National Institute of Neurological Diseases and Stroke.)

100 SINGLE CELL RESPONSES TO AUDITORY AND VISUAL MODALITIES IN THE LATERAL ORBITAL AND ADJACENT CORTEX OF CAT AND MONKEY. James H. Fallon* and L. A. Benevento. College of Medicine, University of Illinois Medical Center, Chicago, Illinois 60680.

Extra- and intracellular recordings were made in the orbito-insular cortex of cats and the lateral orbital cortex of rhesus monkeys. As we reported previously units responded to ON, OFF, and ON-OFF presentations of auditory and visual stimuli as well as to visual targets of various shapes having a specific orientation and direction of movement. 10.4% of the cells responded to visual stimuli only, 14.6% to auditory stimuli only and 46% to both types of stimuli. A dominant feature of cells in anterior insular cortex of the cat was the frequency-dependent suppressive responses to the onset, offset and/or duration of tones. Both active inhibition (IPSPs) and presynaptic blockage (inhibition occuring before the recorded cell) were features of cells exhibiting "best frequencies" for inhibition. In some cells, the afterdischarges and subsequent hyperpolarization that followed initial hyperpolization to the onset of tones delivered to one ear could be enhanced or negated by similar or different afterdischarge patterns evoked by stimulation of the other ear. Auditory and visual stimuli were also presented simultaneously and paired with delays up to 1 sec. In these cases cells which exhibited no clear responses to either visual or auditory stimuli presented alone, demonstrated convergent input by virtue of resultant active inhibition, presynaptic blockage or facilitation evoked by pairing the stimuli at certain delays. Interactions of the afterdischarge patterns were also seen.

(Supported by NSF Grant BMS 75-07349)

101 VISUAL EVOKED POTENTIAL CHANGES IN MONKEY INFEROTEMPORAL CORTEX CORRELATED WITH SELECTIVE ATTENTION. Benjamin M. Dawson and Leo Ganz.
Department of Psychology, Stanford University, Stanford, CA. 94305

Two general mechanisms have been proposed to underlie an organism's ability to selectively attend to relevant features in a complex stimulus while ignoring irrelevant features. In a filter or efferent model, the organism "tunes" selective information filters in primary processing centers (Hernandez-Peon, et al., Sci. 123: 331, 1956; Broadbent, Percept. and Communication, 1958). In a hierarchical or afferent model, successive centers further analyze the information until a decision can be made (Deutsch & Deutsch, Psych. Rev. 70: 80, 1963; Carpenter & Ganz, Percept. & Psychophys. 12: 57, 1972).

Pribram, et al. (JCPP 62: 358, 1966; EEG Clin. Neurophysiol. 29: 146, 1970; Brain Res. 39: 427, 1972) suggest that in the visual system, inferotemporal cortex (IT cortex) works in an efferent fashion to tune lower visual centers. Others (Gross in Handbook of Sensory Physiol., Vol. 7, 1973; Mishkin in Frontiers in Physiol. Psych., 1966) suggest that IT cortex is a further step in a predominantly afferent system. To further investigate these two models and the role of IT cortex, we studied visual evoked potentials (VEP) in macaque monkeys performing a delayed match-to-sample task.

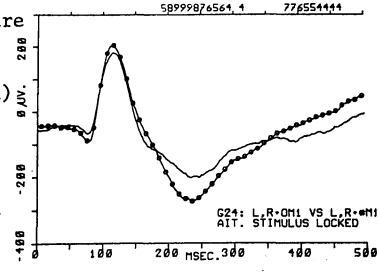
Component stimuli from two classes (circles or stripes) were combined to form compound stimuli. In a typical trial, a compound stimulus was presented tachistoscopically (duration = .6 msec) on the middle panel of three response panels. Half a second later possible matches were presented on the two side panels. In the circle relevant condition a compound stimulus consisting of a circle overlayed with 3 stripes might be presented, and possible matches would be either a single circle or 4 circles (Fig. 1a). In the stripe relevant condition a similar compound stimulus might be presented, but now the possible matches would be 3 horizontal or 3 vertical stripes Fig. 1b). Thus given the same compound stimulus, under different conditions the animal must attend to circles and ignore stripes or vice versa. All aspects except the relevance condition were randomized.

Fig. 1a

Fig. 1b

VEP's (bipolar depth recording, head mounted amplifiers, 500 msec epoch) generated by the same compound stimulus under the two relevance conditions were compared (see Fig. 2). Differences (tested with a modified t-test) must be due to internal shifts in processing as the retinal stimulus is invariant. The results are clear and unequivocal: in all 4 animals tested, VEP changes correlated with shifts in relevance condition (attention) are found strongly in IT cortex. The other 40 electrodes (LGB, striate, FPS, pulvinar, frontal, motor, parietal, superior temporal) showed little or no consistent changes in stimulus locked VEP's.

Fig. 2



In this difficult task (mean trials to criterion = 60,000), selective attention is delayed until IT cortex, supporting a hierarchical (afferent) model. The filter (efferent) model would predict changes at primary (striate) and secondary (prestriate cortex) as well as IT cortex, due to the interposed filter.

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102 ANTIDROMIC ACTIVITY DURING THE EVOKED CORTICAL RESPONSE. Arthur D. Rosen. Division of Neurology, State Univ. of N.Y., Health Science Center, Stony Brook, N.Y. 11790

Previous studies from this laboratory have demonstrated antidromic activity in the visual radiation fibers during penicillin induced focal seizures in the striate cortex. The present study was carried out to determine if antidromic activity could be demonstrated during a more physiological event in the striate cortex, the evoked response. Extracellular recordings were made from 39 post-synaptic geniculate units in 17 adult cats. The refractory period following orthodromic activation of geniculate units was determined for antidromic activation of the same unit via stimuli delivered to the striate cortex. The value thus obtained was compared with the refractory period determined in the same manner from the spontaneous activity of that unit during the later phases of the evoked cortical response. In 15 of the 39 units thus studied, the refractory period for spontaneous activity occurring 5-15 msec. after the onset of the evoked cortical response was significantly less than that determined for the units response to orthodromic activation. These results are interpreted as indicating that antidromic activation of geniculo-cortical neurons may occur during the later phases of the evoked response in the striate cortex. In addition, long latency responses to antidromic stimulation were often seen when the antidromic stimulus was delivered following a spontaneous (and presumably orthodromic) unit discharge occurring 40-80 msec. after the onset of the evoked cortical response. This phenomenon is believed to represent an antidromic response developing during the direct cortical response.

103 **EXACERBATION OF CORTICAL VISUAL DISCRIMINATION DEFICITS BY PULVINAR LESION IN THE MACAQUE.** **Michael C. Trachtenberg and Ellen C. Gower*. Neurology Svc., VAH, Boston, MA 02130.**

Two macaque monkeys with combined bilateral lesions of the lateral striate cortex and pulvinar are significantly impaired in the post-operative acquisition of several distinctly different visual problems: viz., concurrent object discrimination, landmark reversal, and difficult pattern discriminations to which an annulus has been added.

In contrast, these animals experience no difficulty in learning color or object pair discriminations, nor do they show any deficit in post-operative retention of pre-operatively learned concurrent object or difficult pattern discriminations.

The deficits exhibited by these animals are reminiscent of those seen after separate cortical lesions restricted to either inferotemporal cortex (concurrent object discrimination), dorsocaudal posterior parietal cortex (landmark reversal), or foveal prestriate cortex (difficult pattern problems). Together they represent a unique pattern of functional difficulty which cannot be explained by any simple localization hypothesis. In particular, these data indicate that that part of the retino-geniculo-cortical pathway terminating in extrafoveal striate regions is adequate for the performance of visual tasks provided the integrity of parallel retinofugal systems is maintained. In the absence of an intact pulvinar a number of sophisticated visual problems (sensitive to lesion of the three above-mentioned cortical areas which receive input from both pulvinar and visual I) are solved only with greater difficulty. This strongly suggests that the input provided by the pulvinar to these regions contributes meaningfully to cortical function, and in the presence of damage to other cortical visual areas this contribution may be essential. However, the specific character or function of the information supplied by the pulvinar route remains to be clarified.

104 VISUAL ACUITY DEFICITS IN DESTRIATE TREE SHREWS AS A FUNCTION OF STIMULUS AREA AND STRIPE SEPARATION. Jeannette P. Ward, Joy Frank*, and Marilyn Moss*. Dept. of Psychol., Memphis State Univ., Memphis, Tenn. 38152.

The first study of visual capacity in the tree shrew, Tupaia glis, following ablation of striate cortex reported little or no deficit in basic visual function (Snyder & Diamond, Brain, Behav. Evol., 1968, 1, 244-288). Preservation of basic sensory capacity in the absence of primary visual cortex was unexpected in view of the results of similar studies with other mammals. Ware, Casagrande, and Diamond (Brain Behav. Evol., 1972, 5, 18-29_ showed that destriate tree shrews were able to discriminate even a fine pattern of stripes presented on a large stimulus area. Using a small stimulus area, however, Ward and Masterson (Brain, Behav. Evol., 1970, 3, 421-469) found that tree shrews with complete removal of striate cortex were not able to discriminate any striped pattern while tree shrews with lesions that spared a portion of striate cortex could discriminate some stripe widths although deficient in acuity as compared to intact animals. The purpose of this study was to investigate the relationship of stimulus area and stripe width to the acuity of the destriate tree shrew.

Six tree shrews were trained pre- and post-operatively in a two-choice apparatus with food reward. Visual tests were employed in which stripe separation was either 1", 1/2", 1/4", 1/8", or 1/16" and stimulus area was varied. Subjects were first trained on a descending series of stripe widths with area constant at 4" x 4". When each subject had finished the series or failed to discriminate at any level, the descending area series was begun. Using the finest stripe that an animal was able to discriminate, the area was gradually decreased in discrete steps: 3"x 3", 2.5"x 2.5", 2.0"x 2.0", 1.5"xl.5", and 1"x 1". A titration method was used such that if an animal was not able to perform at criterion in 5 sessions on a given stimulus combination, the stripe width was increased. This usually restored the animal's performance to criterion level at which point the area was again decreased. This procedure resulted in an acuity threshold curve for each destriate tree shrew which was a joint function of area and stripe width. Sets of stimulus pairs were selected for each animal based on its threshold curve and each subject was retested using the method of constant stimuli.

Tree shrews were also tested with striped stimuli for which the area was varied in horizontal and vertical rectangular pairs from 3"x 2" to 3" x 0.5" in order to assess the effect of the decrease in stripe length which accompanies a decrease in area by squares. In a final test, epicritic vision was assessed with flecked patterns composed of square bits of black tape of different widths which were discriminated from a gray of matched luminous flux and the total area of both stimuli was varied by square area as above.

Intact tree shrews were able to discriminate all stimuli in the battery. Following ablation of striate cortex all subjects had an acuity deficit which was a function both of stimulus area and stripe width. Thus, the tree shrew like other mammals with extensive loss of primary visual cortex does have a deficit in epicritic visual functions. The necessity for a decrease in stimulus area in order to demonstrate the deficit and the reestablishment of discrimination of a small area stimulus with an increase in stripe width probably reflect characteristics of the remaining tectocortical system. Research with other species is necessary in order to learn whether the tectocortical visual system of the tree shrew is unique in these characteristics.

105 THE RESIDUAL SPATIAL VISION OF CATS WITH LESIONS OF THE VISUAL CORTEX. G. D. Ritchie*, Patricia M. Meyer and Donald R. Meyer. Dept. Psych., OSU Columbus, Ohio 43212.

Cats were trained on three classes of visual discriminations. The first was a large checks vs small checks problem in which the stimulus panels varied both in the total amount of contour and the number of enclosed spatial figures. The second was a contour problem in which the number of objects per panel was constant, but the amount of contour was greater in one panel than in the other. The third was a numerosity problem in which the stimuli contained more objects in one panel than the other, but the amount of contour was the same for both panels. The discriminanda in all three classes were equated for flux, and were designed to eliminate local flux and local contour differences as cues for solving the discrimination.

Normal cats learned all three classes of problems and subsequently sustained removals of the visual neocortex. Postoperatively, the animals could discriminate stimuli that varied in both contour and numerosity (class 1) or that varied in contour and were equal in numerosity (class 2). However, when contour was equated and numerosity was greater in one stimulus than in the other, the cats never exceeded chance performance after nearly a year of retraining. These results indicated that the cats were form-blind despite their abilities to learn some classes of flux-equated tasks.

After completing the learning phase of the experiment, visual placing was studied in these animals. With very few exceptions, placing was absent during periods of recovery of over one year, but could be reinstated for periods up to three weeks after a single injection of d-amphetamine.

106 VISUO-MOTOR FUNCTION IN MONKEYS WITH VISUAL CORTEX ABLATION AND WITH CROSSED CORTICO-TECTAL ABLATIONS. Joseph G. Malpeli and Peter H. Schiller*. Department of Psychology, M.I.T., Cambridge, Mass. 02139.

Sprague (Science 153: 1544-1547, 1966) has shown that cats made hemianopic by a posterior cortical lesion regain some visual function in the cortically blind henifield following ablation of the contralateral superior colliculus. A similar restitution of vision is observed when, instead of ablating the contralateral superior colliculus, the intercollicular commissure is severed. We have investigated the effects of crossed cortico-tectal lesions on visual target acquisition in monkeys. Juvenile rhesus macaques were trained in a task which required them to shift their gaze rapidly from a central fixation point to a peripheral target appearing in an unpredictable location on a tangent screen. Accuracy of the first saccade to target was assessed prior to and following unilateral ablation of the striate cortex with extensive involvement of prestriate visual cortex. With a 1 degree target the accuracy for saccades into the cortically blind hemifield varies greatly with target eccentricity, being quite good at 10 degrees, fair at 20 degrees, and poor at 30 degrees. We have not observed any improvement in this parameter of visuomotor function up to 4 weeks after ablation of the contralateral superior colliculus. Sectioning of the intercollicular commissure rather than destruction of the contralateral superior colliculus is also being investigated. (Supported by N.I.H. grant EY00676 and N.I.H. fellowship 1 F 22 EY02280)

107 A BEHAVIORAL ANALYSIS OF MIDDLE TEMPORAL AND VENTRAL TEMPORAL CORTEX IN THE BUSHBABY (GALAGO SENEGALENSIS). Martha Wilson, I.T.Diamond, Richard J. Ravizza*, and Karen K. Glendenning. Depts. of Psychology, Univ. of Conn., Storrs, 06268, and Duke Univ., Durham, N.C. 22706.

Three experiments were carried out in order to investigate the organization of visual cortex in the bushbaby. One thalamocortical relay can be identified as the geniculo-striate system; another ascending sensory system, the tecto-pulvinar-middle temporal system has recently been defined. A third system,projecting from the rostral portion of the pulvinar nucleus relays to areas 18, 19 and ventral temporal cortex. Afferents to this system, if any, are presently unknown. Our specific aims were (a) to find out the behavioral effects of removing the middle temporal (MT) area, and (b), to clarify the nature of the visual impairment produced by lesions of ventral temporal (VT) cortex (Atencio and Ward, Abstr. Neurosci, Soc., 1974). Three visual tasks were given to eight bushbabies, which were divided into MT and VT groups after preoperative training. The first task evaluated their ability to respond to a visual cue which was separated from the response site and varied in spatial location. Then the groups were tested on tasks involving selective attention to available stimulus information. Finally, postoperative sensory status was compared in terms of size discrimination thresholds.

Results showed that MT but not VT lesions consistently impaired visual spatial abilities while VT but not MT lesions impaired the ability to focus and switch attention. There were no differences in size discrimination thresholds in the two groups. It was concluded that these two areas of extrastriate cortex play different functional roles in vision.

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108 ROLE OF INFERIOR TEMPORAL CORTEX IN PERCEPTUAL EQUIVALENCE OF STIMULI IN THE LEFT AND RIGHT VISUAL FIELDS. Lynne Seacord*, Charles G. Gross, and Mortimer Mishkin. Dept. Psychol., Princeton Univ., Princeton, N. J. 08540, and N.I.M.H., Bethesda, Md. 20014

Most neurons in the inferior temporal (IT) cortex of the rhesus monkey have large receptive fields that extend well across the vertical meridian into both visual half-fields (Gross et al, J. Neurophysiol., 1972). The responsiveness of these IT neurons to stimuli in their ipsilateral half-field depends on the splenium and anterior commissure (Gross et al, Fed. Proc., 1974), the same commissural pathways that support interocular transfer of discrimination habits in chiasm-sectioned monkeys. Since IT neurons have the same trigger features in both half-fields, and are also binocular, they may provide the neural convergence that underlies the interocular transfer. To test this possibility, monkeys were trained to a stringent criterion with one eye and then tested for transfer to the same criterion with the other eye on each of several pattern discrimination problems. Prior to any training, five experimental monkeys received bilateral IT lesions combined with midsaggital section of the optic chiasm, while ten controls received either the bilateral IT lesion alone, the chiasm section alone, or no surgery. Only the experimental monkeys showed impaired interocular transfer, as measured by a) initial errors on the transfer tests, b) total errors on the transfer tests, and c) savings scores. Presumably, IT neurons mediate interocular transfer by providing perceptual equivalence for patterns in the left and right visual fields, and, by implication, perhaps for patterns in different parts of the same field as well.

109 INFERIOR TEMPORAL CORTEX LESIONS DO NOT IMPAIR DISCRIMINATION OF LATERAL MIRROR IMAGES. Charles G. Gross, Melissa Lewis* and David Plaisier*. Dept. Psychol. Princeton Univ., Princeton, N. J. 08540.

Bilateral ablation of inferior temporal (IT) cortex in the macaque produces a severe impairment in visual pattern learning. The degree of impairment has been thought to be a function of the difficulty of the discrimination problem as measured by the performance of normal animals. However, in two previous studies (Cowey and Gross, Exp. Brain Res., 1970; Gross et al, J. Comp. Physiol. Psychol., 1971) monkeys with IT lesions were not impaired in learning to discriminate lateral mirror images, although normal monkeys found these discriminations relatively difficult. These paradoxical results suggest that lateral mirror images may be a special class of discriminanda for animals with IT lesions. To test this possibility, 6 monkeys with bilateral IT lesions and 6 control animals (with lateral striate or no lesions) were trained on a series of discriminations of mirror image and non-mirror image pairs. The animals with IT lesions showed the usual visual discrimination deficit on the non-mirror image tasks, but learned the mirror image tasks as quickly as the normal animals. The control animals, unlike the animals with IT lesions found the mirror image tasks much more difficult than the non-mirror image ones. IT cortex may be involved in the perceptual equivalence of lateral mirror image stimuli and perhaps in other perceptual equivalences.

110 EFFECTS OF TECTAL LESIONS ON PERIPHERAL FIELD VISION IN THE MONKEY. E. Gregory Keating. Veterans Administration Hospital and Depts. of Anatomy and Neurology, SUNY - Upstate, Syracuse, N.Y. 13210.

Current theory assumes the superior colliculus to be important for the control of 'ambient' or peripheral vision. Removing the tectum causes little visual deficit in primates perhaps because the lesioned monkey can shift to more foveal geniculo-striate pathways to solve most visual tests. In one experiment tectal lesions did impair accuracy in locating targets if the visual stimuli flashed too quickly for the monkey to shift all of them into its foveae (Keating, 1974). The present experiment measured the effects of tectal lesions on monkeys entirely prevented from using macular vision.

Five rhesus were trained on several tests including pattern and luminance discriminations and a third test which measured their accuracy in reaching for the dimmer of two lights flashing at various points in the visual field. Two monkeys also learned to distinguish a moving from a stationary shadow. The animals were retested after removal of the superior colliculus alone or in combination with a lesion of the central 6^0 - 8^0 of both retinae. Tectal lesions impaired accurate localization of briefly appearing stimuli but adding the retinal lesions did not greatly enhance the deficit. Combined tectal-retinal lesions (which sometimes included pretectum) did not impair pattern or luminance discriminations. Neither did the combined lesions cause the cortical blindness, visual agnosia, or movement discrimination deficits described by Anderson and Symmes (1969) to result from tectal-foveal striate ablation. Even adding foveal striate removal to the tectal-retinal lesions in two animals failed to enhance the effect of tectal lesion alone. (Supported by NS 10576.)

111 IMPAIRED PERFORMANCE OF VISUAL STIMULUS REVERSALS BY CATS WITH LESIONS OF THE SUPERIOR COLLICULUS-PRETECTUM. J.M.S. Winterkorn. Anatomy Dept. Cornell Univ. Med. Coll., New York, N.Y. 10021

In a prior study, cats with bilateral lesions of the superior colliculus-pretectum (SC-P) were shown to be able to locate and discriminate between visual stimuli widely separated in space. However, as has been shown in hamsters by Schneider, cats with lesions of the SC-P committed more alley-entrance errors than unoperated cats, suggesting that cats with lesions of the SC-P may have a deficit in inhibiting incorrect or unrewarded responses. This hypothesis was tested by training cats in a sequence of 2-choice visual discriminations (light/dark) in which the reinforcement polarity of the 2 stimuli was successively reversed.

Five cats were trained for food reward in either an automated Y-maze or an automated double-ended straight maze, both before and after bilateral ablation of the SC-P. When a cat had achieved a high performance criterion in the initial light(+)/dark(-) discrimination, the polarity of reward was reversed and the cat was retrained to criterion on a dark(+)/light(-) discrimination. In total, each cat was trained in the initial light/dark discrimination followed by ten reversals, both preoperatively and postoperatively.

Cats with lesions of the SC-P relearned the initial light/dark discrimination as rapidly as unoperated cats. However, compared with their preoperative performance, cats with lesions of the SC-P required more trials and committed more errors before achieving criterion on each of the 10 successive reversal tasks. These results suggest that cats with lesions of the SC-P have a deficit in inhibiting unrewarded responses.

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112 EYE MOVEMENTS OF INFEROTEMPORAL, FOVEAL PRESTRIATE AND PULVINAR ABLATED MONKEYS DURING VISUAL DISCRIMINATION BEHAVIOR. Carol Christensen*, Leslie Ungerleider and Karl Drake*. Neuropsychology Laboratories, Dept. Psych., Stanford University, Stanford, California, 94305.

The eyemovements of normal (N), inferotemporal (IT), foveal prestriate (FPS) and pulvinar (P) ablated monkeys were monitored with a corneal reflection technique during visual discrimination training. Training consisted of four 40 trial sessions in which subjects were reinforced for fixating the positive of two simultaneously presented visual stimuli. Video tape records of these fixations for the first and fourth sessions were analyzed. Three of the measures calculated for the two sessions were the percentage of total fixation time within the display boundaries, the percentage of this on display time in which either of the two stimuli were fixated and the percentage of on display time spent fixating the positive stimulus. During the first session IT ablated subjects showed a significantly greater percentage of fixation time on display than did N subjects. In addition both IT and FPS subjects fixated the stimulus portions of the display longer than did N subjects during the first session. These differences disappeared by the fourth session. N subjects, however, showed a greater absolute increase in the time spent fixating the display and stimuli from the first to the fourth session than did the other two groups. Analysis of the time spent in differential fixation of the two stimuli showed that while all groups fixated the stimuli approximately equally during the first session, by the fourth session the N subjects had learned to preferentially observe the reinforced stimulus. IT and FPS ablated subjects were equally impaired in learning the discrimination. Preliminary analysis of data collected from P subjects indicates that they are not different from N subjects in these measures.

113 EFFECT OF POSTNATAL ENUCLEATION OF THE EYE ON CORTICO-CORTICAL CONNECTIONS OF THE RAT'S STRIATE CORTEX. Vicente M. Montero*, Maria A. Carrasco*, and Victor Fernandez*. (SPON: C.N. Woolsey). Dep. Physiol. Biophys., Faculty of Medicine, Univ. Chile, Santiago, Chile.

The rat's striate cortex sends direct connections to several peristriate areas which appear to have different retinotopic organizations (Montero et al., Brain Res., 1973, 53:197-201;202-207). In the present study we have examined the effect on these connections of removal of one eye 14 days after birth, just before eye-opening. Electrolytic lesions were placed in the monocular sector of area 17 in both hemispheres after the rats had become adults. Four days later the ensuing cortical degeneration was examined with the Fink-Heimer technique, with the following results: (a) The number of degenerating fields and their topographical pattern were similar in the enucleated rats and in controls, i.e., degenerating connections to posterior, posterolateral, laterolateral, lateromedial, anterolateral and anteromedial peristriate areas were found in both. (b) On qualitative inspection and quantitative analysis (number of degeneration granules per unit volume of tissue), it was found that the density of granules of the laterolateral field in the contralateral hemisphere to the remaining eye, was markedly greater than in either the ipsilateral hemisphere of the four enucleated animals or in the hemispheres of two normal controls. No significant interhemispheric difference in granule density was found in the other fields of degeneration. It is concluded that the afferent imbalance to the two striate areas resulting from eye enucleation induces a proliferation of preterminal arborization, and probably synapses, in this specific striate-peristriate pathway in the rat. The functional implications of this structural alteration has yet to be determined. (Supported by NIH Grant NS-03640 to the University of Chile).

114 VISUO-MOTOR PROPERTIES OF NEURONS IN A NONSPECIFIC THALAMIC STRUCTURE: N. CENTRALIS LATERALIS OF CAT. Madeleine Schlag-Rey* and John Schlag. Dept. Anat. and BRI, UCLA, Los Angeles, 90024.

Eye movement-related activities of centralis lateralis (CL) neurons have been recently described (J. Neurophysiol. 1974, 37: 982). Such cells change their firing before or during saccades in specific directions, even in complete darkness. The continuation of this study shows that the same cells also respond to photic stimuli in absence of eye movements.

Recordings were made with tungsten microelectrodes in chronic cats. Retinal projections of stimuli were computed from on-line recordings of stimulus and eye positions. We found that eye movement CL units: (1) can respond to bright diffuse flashes in 25-40 ms, (2) are sensitive to very dim, low contrast patterns (e.g. 3° dia. on faint red background), (3) give on, off, on-off, or sustained responses, and responses to moving stimuli, (4) have usually large receptive fields (e.g. 30° dia.) close to center and most often contralateral. The receptive field was always on the side toward which saccades were preceded or accompanied by increased firing. In some instances, saccade-related firing only occurred when the saccades were directed toward a visual target.These results imply that neurons in the dorsolateral region of the cat's nonspecific thalamus participate in visuo-motor processing.

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115 TIME-COURSE OF THE TOPOGRAPHIC REGULATION OF A HALVED OPTIC TECTUM IN ADULT GOLDFISH. Myong G. Yoon. Dept.of Psychology Dalhousie University, Halifax, Nova Scotia, Canada.

The visual system in adult goldfish is capable of re-adjusting to various types of size-disparity, experimentally induced between the retina and the optic tectum. If the caudal part of the tectum is excised and the contralateral optic nerve is sectioned and then allowed to regenerate into the operated tectum, the remaining rostral half-tectum eventually reacquires the whole visual projection from not only the appropriate temporal half of the retina but also from the foreign nasal half of the retina in correct retinotopic order. The patterns of the newly reestablished visual projections, however, depend on the duration of postoperative periods allowed to the halved tectum before it becomes re-innervated by incoming optic fibers. If the regenerating optic fibers are allowed to invade the denervated half-tectum earlier than 33 days following excision of the caudal tectum, the newly restored visual projection is restricted from only the temporal hemiretina onto the reamining rostral half-tectum, without showing any sign of a field compression at this early stage. On the other hand, when the incoming optic fibers are controlled to invade the half-tectum about one and a half months after excision of the caudal tectum, the newly restored visual projection shows an orderly field compression from the whole extent of the retina onto the remaining rostral half-tectum in correct retinotopic order. In a further study, the caudal half of the left tectum was removed in a new group of adult goldfish. The right optic nerve was also sectioned. Three or four months later, the restored visual projections were mapped. All of these operated fish showed an orderly field compression from the entire right retina onto the remaining rostral half of the left tectum. Immediately after the mapping experiment, the caudal half of the right tectum was removed, and both the left optic tract and the right optic tract were also sectioned near their entrances to the left half-tectum and the right half-tectum, respectively, in each experimental fish. These fish were revived after the second surgery for further experiments. Retinotectal projections were re-mapped for both the right half-tectum and the left half-tectum at relatively early postoperative periods between 17 and 22 days after the second surgery. The newly restored visual projection onto the remaining rostral half of the newly operated right tectum did not show any sign of a field compression: only the left temporal hemiretina projected onto the right rostral half-tectum. On the other hand, the newly restored visual projection onto the remaining rostral half of the left tectum (which received the tectal surgery about four months ago) showed a complete field compression from the entire right retina, at the same mapping session. The present results suggest that it takes at least a month for the halved tectum to complete a topographic regulation of itself into a whole. The topographic regulation will enable the halved tectum to accomodate incoming optic fibers from not only the appropriate temporal hemiretina but also from the foreign nasal hemiretina in an orderly compressed topographic pattern.

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116 BEHAVIORAL AND ELECTROPHYSIOLOGICAL ANALYSIS OF SENSORY NEGLECT FOLLOWING DIENCEPHALIC LESIONS IN CATS. C. S. Wier* and Dennis M. Feeney. Depts. Psychol. and Physiol., U. of New Mexico, Albuquerque, 87131.

In different animals the effects of unilateral lesions of the ventromedial hypothalamus (VMH), lateral hypothalamus (LH), anterior cerebral peduncle (ACP) or substantia-nigra/cerebral peduncle (SN-CP) were compared on visual and somatosensory function. Behavioral tasks included approach to food presented at various visual angles, suppression of licking by lights signaling shock at various visual angles, latency of limb withdrawal to immersion in water or paw shock. Dramatic and persistent (60 days) effects were obtained with ACP and SN-CP lesions, less severe, short lasting effects with LH lesions and no effect following VMH lesions. The syndrome was a contralateral deficit and an ipsilateral facilitation of visual and tactile responses compared to prelesion control. Although transient motor impairments were also observed, the demonstration of lack of responsiveness using the visual suppression technique indicates the syndrome is due to a sensory impairment. Despite the presence of profound sensory changes on the behavioral tests, only very transient and inconsistent reductions in the amplitudes of evoked potentials (ipsilateral to lesion) to flash and paw shock were seen in visual and somatosensory cortex, association cortex, caudate nucleus, superior colliculus and mesencephalic reticular formation. Additionally no changes were observed in gross sleep and waking EEG patterns. (Supported by NINDS Grant NS 10469-02)

117 SUBJECTIVE METHOD FOR THE DETERMINATION OF MONOCHROMATIC ABERRATIONS OF THE EYE. Howard C. Howland* and Bradford Howland Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853 and M.I.T. Lincoln Laboratory, Lexington, Mass. 02713.

Using a method similar to the Hartmann test[1] a spectrally pure point source is viewed through a millimeter grid, oriented at 45° to the axes of a ± 5 diopter crossed cylinder lens[2]. The subject draws the grid which he sees. The drawing is then analyzed by a computer program which yields an orthogonal polynomial approximation of the wave aberration disc at the pupil.

The initial results obtained by this method on approximately 35 subjects have shown a variety of symmetrical and asymmetrical third and fourth order aberrations of positive and negative sign. These results are not in accordance with the view that the normal eye contains a fixed amount of positive spherical aberration, and they provide (to our knowledge for the first time) quantitative estimates of third order, coma-like aberrations.

The significance of these measured aberrations at various pupil apertures for the degradation of the modulation transfer function of the eye has been investigated with numerical ray-tracing techniques. This analysis shows that great differences may exist between individuals in the optical performance of otherwise normal eyes at large apertures. In some cases (e.g. severe symmetrical spherical aberration) a part of the resolution of the eye can be restored by corrective lenses, and it is possible to predict the theoretical optimum prescriptions for different pupil sizes and hence light levels.

1. John Strong, Concepts of Classical Optics, Freeman, San Francisco (1958).
2. Bradford Howland, Appl. Opt. 7, 1587 (1968).

118 EFFECT OF CORTICAL EEG FREQUENCY COMPOSITION ON THE QUALITY OF APPARENT MOTION IN MAN. Stephen Coffin* (SPON: D. Young). Dept. of Psychology, UCLA, Los Angeles, Calif., 90024.

Earlier work (in preparation) has shown that both subjects' two-flash discrimination threshold and productions of a subjective five second duration were well correlated with the frequency composition of EEG recorded over visual cortex (Oz). As thresholds lowered, and duration productions shortened, EEG frequency composition within the alpha band (8-12 Hz.) shifted to faster activity.

The present experiment extended these results to an apparent motion task. Subjects were asked to rate the quality of apparent motion in trial-wise presentations of stimuli separated by about 3.5° of visual angle. Interval between offset of first and onset of second stimulus (ISI) was varied between 10 and 90 msec. EEG activity during stimulus presentation was assessed by a frequency spectral analysis technique.

Results with 10 subjects showed that high quality apparent motion was associated with faster EEG activity at short ISI, and lower frequency peaks at longer ISI. Of the five electrode sites chosen (Oz, O1, O2, P3, and P4), only results at Oz were significant ($p < .05$). Changes were again in the 8-12 Hz. frequency band.

Results suggest that a mechanism associated with components of the spontaneous EEG serves to pace information processing within the primary visual cortex, in several spatio-temporal perceptual tasks.

119 **A HEBBIAN SYNAPTIC MODIFICATION EXPLANATION OF THE MCCULLOGH EFFECT.** **F. S. Montalvo* (SPON: A. Trehub). Dept. of Comp. and Info. Sci., U. Mass., Amherst, Ma. 01002.**

A computer simulation of a neural network model involving Hebbian synaptic modification between a retinal layer and a cortical layer is proposed as a possible explanation of the McCullogh effect, without hypothesizing fatigue adaptation. Hebbian modification of initially randomly weighted synapses during random presentation of all color-orientation combinations produces a uniform distribution of color-orientation specific units. With additional presentations of alternate red-vertical(R-V) and green-horizontal(G-H) stimuli during synaptic modification, the network responds to black-and-white-horizontal(W-H) and black-and-white-vertical(W-V) stimuli with the McCullogh effect. This is because the shift in response of the R-V units to the R-V stimulus causes the depletion of the slightly red-sensitive cells from the W-V population response. Likewise the shift in response of the G-H units to the G-H stimulus produces a depletion of the slightly green-sensitive cells from the W-H population response. The result is a slightly green bias in the W-V response and a slightly red bias in the W-H response.

Synaptic modification affords a more plausible explanation of this effect than adaptation, given its very long durability and its lack of decay during sleep.

120 THE EFFECT OF CARBON MONOXIDE EXPOSURE ON THE CONTRAST SENSITIVITY OF SQUIRREL MONKEYS. William H. Merigan*. (SPON: W. Hodos). Department of Psychology, University of Maryland, College Park, Md. 20742.

The spatial contrast sensitivity of three squirrel monkeys (Saimiri sciureus) at a field luminance of 3cd/m^2 was measured in a previous study. Here, two contrast sensitivity determinations were made at each of four carbon monoxide (CO) levels (100, 200, 300, and 400 ppm) at both a medium (3.6 c/deg) and a high (13 or 23 c/deg) spatial frequency. These CO levels produce a COHb level at equilibrium of approximately 11, 19, 27, and 33%. For each determination, the monkeys were brought to equilibrium with the CO atmosphere and then tested in a morning and an afternoon session. Thresholds were measured five days a week, and CO administered on Tuesdays and Fridays. The order of CO exposures was generally increasing.

The contrast threshold of one monkey was elevated at most CO levels at both medium and high spatial frequencies. A second monkey showed a few threshold elevations at the two highest CO levels. The thresholds of the third monkey were unaffected at both medium and high spatial frequencies under all CO levels. These findings support previous results (McFarland and Halperin, J. Gen. Physiol. 24, 69, 1940; Stewart et. al., Arch. Envir. Hlth. 21, 154, 1970), which showed that photopic visual acuity is relatively insensitive to both CO-induced and hypoxic hypoxia. (Supported by NIEH grant # ES 00757-03)

121 APPARENT MOTION IS MEDIATED BY BOTH MOTION DETECTORS AND STIMULUS INTEGRATING PROCESSES. P. J. Burt (SPON: M. A. Arbib). Computer and Information Science Department, Univ. of Mass., Amherst, Ma. 01002.

The mechanisms of apparent motion were studied with a spatially periodic display which could be perceived to move in either of two directions. A row of dots was flashed 25 to 50 times per second on a CRT so that with every presentation the pattern was shifted a distance S along the axis of the row. A dot in one presentation might appear to move to the position of either nearest neighbor in the following presentation. When $S=\frac{1}{2}D$ (D is the dot separation in a row) the stimulus is equally strong for motion in either direction. It was found that motion can be seen only in one direction at a time; the motion stimuli do not cancel to give zero perceived velocity, nor do they combine to give a sense of motion in both directions simultaneously. The appearance of motion must therefore depend on processes which select one of the possible modes of stimulus integration. On the other hand, a preferred direction of apparent motion was found which depends on retinal location, and which can be reversed by fatigue. This is evidence that motion detectors play a role in guiding integration processes. By presenting stimulus patterns alternately to the two eyes it was shown that these motion detectors must be binocular.

A neural model is proposed in which stimulus integration corresponds to the development of a stable pattern of activity in a neural net. Motion of activity in the net reflects image motion (real or apparent) on the retina. Separate processes related to the neural response to a brief stimulus and to motion of the response activity, interact dynamically to produce constraints on stimulus integration which match a wide variety of motion phenomena.

122 LATERALITY EFFECT ON TESTS OF GEOMETRIC DESIGNS. Santosh Kumar, John P. Allen*, Darleen Powars* and L. Julian Haywood*. Division of Pediatric Neurology, University of Southern California School of Medicine, Los Angeles, California 90033.

The right and left hemispheres are specialized in gestalt and analytical functions respectively. The tendency to perceive the stimulus as a whole (gestalt) is responsible for the geometric illusion in the Muller-Lyer Figure in which the slants on the horizontal lines of comparison making arrowheads or feather-ends cause perceptual distortion. The greater the left hemisphere participation (i.e. analytical approach) the less the Muller-Lyer Illusion.

Accuracy in reproducing the geometric designs of the Bender Visual Motor Gestalt Test depends on perceiving them as a whole. The greater the right hemisphere participation the less the error in Bender Test performance.

A negative correlation may be expected between the Muller-Lyer Illusion and the Bender Error Scores even in the absence of brain damage.

Results of testing 25 children with Sickle Cell Anemia (3 having right cerebro-vascular accident -CVA, 8 having left CVA and 14 having no CVA) show a Rho of -.44 and a $p < .05$ between the Muller-Lyer Illusion and the Bender Error Scores. On the average, patients with right CVA had less illusion than those with left CVA ($p < .01$). Patients with left CVA had smaller Bender Error Scores than those with right CVA ($p < .01$). Patients without CVA had smaller Bender Error Scores than either CVA groups, and also had illusion effect in-between the two CVA groups. Thus, these tests which are based on geometric designs reliably reflect laterality differences following localized brain damage.

123 VISUAL ACUITY IN THE NORTHERN BLUE JAY: BEHAVIORAL AND ANATOMICAL CORRELATES. Katherine V. Fite, Robert J. Stone* and Michael Conley*. Dept. Psychol., Univ. Mass., Amherst, 01002.

Despite numernous references to the high visual acuity of birds, direct measurement of spatial resolution has been reported for only a few species, and these have not revealed unusually high capabilities. In the present study, operant psychophysical measures of near-field acuity (minimum-separable) have been obtained in the Northern Blue Jay over a wide range of spatial frequencies and luminance levels using a two-choice response task. Psychometric functions were obtained for 3 subjects which related % correct response to variation in pattern luminance for each of 9 spatial frequencies (square-wave, 50% gratings). Luminance-acuity thresholds (75% correct response) were used to construct a function relating luminance-acuity thresholds to the angular subtense of a single stripe-width across grating patterns.

Visual acuity in this species improves rapidly with increasing luminance above -2.0 log ml., reaching a maximum resolution of 1.6-2.0 min. of arc between 1.0-1.5 log ml., but then decreases with further increases in luminance.

The Blue Jay is a diurnal species with a pronounced, convexiclivate fovea located in the central retina, unlike the domestic pigeon which has a shallow fovea and a second area of high ganglion-cell density in the superior temporal retina. Further comparisons will be made with observations previously reported on pigeons and nocturnal owls with regard to measures of visual acuity and retinal-anatomical data, including special reference to the role of the avian fovea in spatial resolution.

124 CNV CORRELATES OF SEXUAL INTEREST. Victor Milstein, Joyce G. Small, Iver F. Small, and Richard N. French.* Indiana Univ. Sch. of Med. & Larue D. Carter Mem. Hosp., 1315 W. 10th St., Indianapolis, IN 46202 USA

Costell et al (Science, 1972) reported that there are reliable differences in the amplitude of the contingent negative variation (CNV) which are proportional to the presumed degree of interest in sexual and non-sexual visual stimuli of normal male and female adults. Moreover they demonstrated greater CNV amplitude with same-sex stimuli than with neutral material in women, but not in men. In the present study we sought to replicate these findings. We used identical visual stimuli as Costell with an additional series of blank slides of diffuse light. Our experimental design was similar in many respects although we added additional safe-guards to avoid artifact contamination and measurements of baseline reactivity. DC activity was recorded from both midline and homologous areas on both sides of the head to determine whether or not there might be lateralized CNV differences. Bilateral occipital EEG activity was recorded for off-line analysis of mean energy, frequency and auto- and cross-correlation. The subjects were adult paid volunteers who had participated in previous laboratory procedures and who had normal clinical EEGs without significant lateralized differences. Male and female subjects viewed repeated slide slimuli with concurrent recording of six channels of electrophysiological data.

The results of the study indicated that the amplitude of the CNV was greater when subjects were exposed to sexually explicit material than to ambiguous or non-sexual stimuli or to diffuse light. There were some lateralized differences in the CNV which were complexly related to sex of subject, experimental sequence and content of the stimulus. Our findings generally confirm the potential suitability of the CNV and also quantitative analysis of the EEG as objective ways of measuring some aspects of sexual reactivity.

125 VISUAL DISCRIMINATIVE CUES AND PREFRONTAL CORTEX (PFC) UNIT ACTIVITY. Shozo Kojima* and Thomas J. Tobias. Primate Research Institute, Kyoto University, Inuyama, Aichi, Japan.

Two groups of PFC neurons recorded in behaving monkeys respond to presentation of visual cues. Activity from one kind of cell is closely correlated with presentation of a light which signals an opportunity for response and reward (discriminative stimulus, S+.) A second cell type responds to this light both in the S+ paradigm,and in tasks where the identical lamp is an irrelevant cue(S-).The present study sought to examine activity of single neurons during performance of several tasks, for which eye and hand movement were regulated, with successive presentations of S+ and S- cues. For example, while the monkey fixed its gaze upon a center light, an S- peripheral lamp was illuminated. In a subsequent task, this same lamp (now S+) indicated the possibility of responding with eye movement towards the peripheral lamp when the center light extinguished. Then the position or color of this peripheral lamp was altered, or the S+ visual cue was replaced by an S+ tone, and the effects of these substitutions on the same neuron's discharge rate were recorded. Some cells which responded to both S+ and S- cues displayed differential activation according to spatial position of the visual cue; no PFC neurons responded strongly to color change; and for some cells, presentation of an S+ tone resulted in an inhibition relative to activity elicited by the visual cue. Finally, most cells responded differentially to the discriminative stimulus (S+) irrespective of a requirement for hand or eye movement in executing the task, and a few neurons showed correlation to the movement required, but not to presentation of S+or S- cues

126 CORTICAL EFFECTS OF EARLY SELECTIVE EXPOSURE TO DIAGONAL LINES. Audie Gene Leventhal* and Helmut V.B. Hirsch. Dept. Biology, SUNY Albany, Albany, N.Y. 12222

The effects of early visual exposure to diagonal lines and to horizontal or to vertical lines on the response properties of neurons in the striate cortex of the kitten were compared.

Consistent with previous results, exposure to horizontal or to vertical patterns leads primarily to the development of neurons that respond best to horizontal or to vertical lines. By contrast, exposure to diagonal patterns results in the development of many cortical cells that respond best to horizontal or to vertical lines in addition to those responding best to the corresponding diagonals. In addition, cortical neurons with receptive field properties different for the two eyes were found in cats exposed to 45^{o} lines with the left eye, and simultaneously, to 135^{o} lines with the right eye but not in animals exposed to horizontal lines with one eye and to vertical lines with the other eye. These cells responded most strongly to 45^{o} lines when activated through the left eye and to 135^{o} lines when activated through the right eye. The proportions of cells responsive to visual stimulation, activated binocularly, and selective for orientation is reduced by restricted early visual exposure that is discordant for the two eyes. These reductions are smaller following exposure to diagonal lines than following exposure to horizontal or to vertical ones. Normal visual experience subsequent to the selective exposure does not alter the distribution of preferred orientations of cortical neurons but increases the proportions of cells responsive to visual stimulation, activated binocularly, and selective for orientation.

Our results demonstrate that cells which prefer horizontal or vertical contours do not require a specific input for their development; neurons that respond preferentially to diagonal lines do.

127 SELECTIVE ABOLITION OF DIRECTION SPECIFICITY IN CAT VISUAL CORTEX. Max Cynader. Dept. Psychol., Dalhousie University, Halifax, N. S., Canada.

Cats were reared in a visual environment in which the only illumination source was a strobe light. The brief (10 microsecond) flashes occurring at 8 flashes/sec ensured a series of stopped images on the cats retinae. At six months of age, the cats were removed from their rearing enclosure and the response characteristics of single visual cortex neurons were examined.

The results indicate that the occurrance of direction specificity in cortical neurons of these cats is drastically reduced. In normally-reared cats over 80% of the neurons encountered exhibit direction specificity but only 14% of the units found in strobe-reared cats displayed this property. A similar reduction of direction specificity was observed in both areas 17 and 18. Nearly all units in both normal and strobe-reared cats exhibited orientation specificity and tightness of orientation tuning was similar in the two groups. The velocity tuning of units in the two groups was similar. As in normally-reared cats, units in cortical area 18 of the strobe-reared cats prefered much higher stimulus velocities than did units in area 17.

Preliminary evidence suggests that some recovery of direction specificity is possible following prolonged exposure in a normal environment.

128 EFFECTS OF BRIEF MONOCULAR EXPERIENCE ON SINGLE UNITS IN KITTEN VISUAL CORTEX. P.B. Schechter and E.H. Murphy. U. of Chicago Dept. of Behavioral Sciences, Committee on Biopsychology, Chicago, Ill. 60637.

Eleven kittens were dark-reared from 10 days of age. When they were 4 weeks old, 9 of them had 1 eyelid sutured, and were placed in a bright visual environment for either 3 hours (3-hour group; n=5) or 12 hours (12-hour group; n=4). The remaining 2 kittens received neither suture nor visual experience. Following their visual experience, the 3- and 12-hour kittens were returned to the dark. Ocular dominance was examined for single units in the visual cortices of all kittens during their sixth week of life.

Three hours of monocular experience did not produce a cortical ocular dominance bias toward the experienced eye, but it did result in a marked binocular abnormality: About equal numbers of cells were driven by the experienced eye only, by the naive eye only, and by both eyes. The 12-hour group showed a distinct bias toward the experienced eye, but 1 animal differed from this pattern, resembling a 3-hour kitten. (It is possible that individual differences in gestation and/or development, resulting in individual differences in the onset of the "critical period," may account for this difference.) About 90% of the responsive units in the dark-reared kittens' cortices were binocularly driven.

It appears that 2 processes may be involved in producing the effect of monocular experience on cortical binocularity. One, requiring only brief periods of monocular exposure, disrupts binocular connections. The other, requiring somewhat longer monocular experience, results in an ocular dominance bias strongly favoring the experienced eye.

129 RECOVERY FROM DARK REARING: BEHAVIORAL AND ANATOMICAL OBSERVATIONS. R.E. Kalil* (SPON: J. Gibson). Dept. Anat., Univ. Wisconsin, Madison 53706

In the cat, dark rearing retards cell growth in the dorsal lateral geniculate nucleus and leads to a marked atrophy of these neurons (Kalil, Anat. Rec., 181, 1975). In the present experiments behavioral and anatomical methods have been used to determine whether the effects of dark rearing are permanent or if recovery is possible. Cats were reared in complete darkness for 4, 8, 12 and 16 wks., and then brought into the light and allowed normal visual experience for at least 3 months. At the end of this period, the 4 and 8 wk. dark reared (D.R.) cats were sacrificed and geniculate cell areas were measured. Upon removal from the dark, the cats appeared to be blind. They failed to avoid objects, and showed no depth perception, visual avoidance, following or placing. These deficits disappeared quickly in the 4 and 8 wk. D.R. cats, requiring only 1 wk. of normal vision. Recovery took 2.5 wks. for the 12 and 16 wk. D.R. animals. Visual fields, measured with a food perimetry technique, were normal in the 8 and 12 wk. D.R. animals, but the 16 wk. D.R. cat displayed a severe loss of peripheral vision. Geniculate cell areas in the 4 and 8 wk. D.R. cats were of normal size, indicating a complete recovery from the effects of dark rearing. In a similar manner visual behavior also appears to recover completely provided that dark rearing does not exceed 12 wks. If dark rearing is prolonged for 16 wks. a persistent visual field defect results. It remains to be determined if this visual field loss is accompanied by a permanent atrophy of geniculate cells.

130 EFFECTS OF BINOCULAR DEPRIVATION ON CAT STRIATE CORTEX. David W. Watkins* and S. Murray Sherman. Department of Physiology, University of Virginia, Charlottesville, Virginia, 22901.

Single unit recordings were used to study 66 neurons in striate cortex of six binocularly deprived adult cats (deprived by eyelid suture at 4-10 days age). Average response histograms were prepared for 22 of these neurons. We found no more than 4 of the cells had normal receptive field properties. Abnormalities included: a) many (18/66) visually nonresponsive neurons, b) few orientation-selective neurons, c) more neurons with obvious spontaneous activity, d) low rates of stimulus-evoked neuronal discharge, e) rapidly adapting and inconsistent responses, and f) a general lack of inhibitory sidebands in the receptive fields. Some visually inexcitable cells had purely inhibitory or suppressive receptive fields. It is suggested that the striate neurons of binocularly deprived cats lack normal stimulus specificity because they lack the normal configuration of excitatory and inhibitory receptive field components. This defect may, in part, obtain from a dysfunctional intracortical inhibitory pathway. (Supported by NSF Grant BMS 73-06938 and NEI Grant 1 R23 EY 1492-01)

131 EFFECTS OF MONOCULAR DEPRIVATION ON CAT STRIATE CORTEX. James R. Wilson and S. Murray Sherman. Dept. of Physiology, University of Virginia, Charlottesville, Virginia, 22901.

Single unit recordings were made in striate cortex of 13 monocularly deprived cats. Previous studies have shown that most (95%) of the cells with receptive fields in the central visual region are driven exclusively by the non-deprived eye. Binocular competition has been proposed to account for this phenomenon (Wiesel and Hubel, J. Neurophysiol., 28:1029, 1965). In this study we wished to consider both the central and peripheral fields of the deprived eye. In particular, we emphasized recordings from cells located in the dorsal bank of the splenial gyrus contralateral to the deprived eye. This area contains cells having their receptive fields located in the visual field seen only by the deprived eye (monocular segment, 45-90°). Since binocular competition cannot occur in this region, the effects of visual deprivation alone could be studied by comparing the receptive fields of this area to fields in the corresponding area of the opposite hemisphere or to those of a normal cat's monocular segment (cf. Guillery and Stelzner, J. Comp. Neur., 139:413, 1970). Our results are fourfold: 1) the deprived eye does drive some cells with apparently normal peripheral receptive fields; 2) a significant number of monocularly driven cells - driven by either eye - were found to have abnormal receptive fields; 3) there is a pure deprivation effect as shown by a paucity of functional cells in the monocular segment; 4) whereas all cells in the binocular segment of normal cat striate cortex have excitatory and/or inhibitory input from both eyes (Henry et al., Vis. Res., 9: 1289, 1969), cells driven only by the non-deprived eye also lacked inhibitory fields for the deprived eye. The last point indicates that binocular inhibition on cortical cells is normally accomplished by intracortical mechanisms. (Supported by NSF Grant BMS 73-06938)

132 Effects of extraocular muscle surgery and alternating monocular occlusion on receptive fields in cat superior colliculus. Barbara Gordon, Linda Gummow*, and Joelle Presson*, University of Oregon, Eugene, Oregon 97403.

We have cut the right medial and lateral rectus muscles of kittens during their second week of life and studied the resulting ocular dominance histograms in the visual cortex and superior colliculus. We have found dramatic differences between these two structures. The colliculus of adult strabismic cats contained many more binocularly driven cells (ocular dominance groups 3,4,5) than did the cortex. The unoperated (left) eye was, however, far more effective in driving collicular cells than was the strabismic (right) eye. Contralateral to the unoperated eye only 37% of the cells were binocularly driven, and the monocularly driven cells were all dominated by the unoperated eye (groups 1, 2). Contralateral to the strabismic eye 77% of the cells were binocularly driven and about half the monocularly driven cells were dominated by each eye. In contrast only about 17% of the visual cortex cells were binocularly driven. In the cortex the unoperated eye showed only a very slight tendency to dominate more cells than the strabismic eye.

Since collicular cells have large receptive fields, many cells might receive synchronous binocular input even when the two eyes are badly misaligned. This binocular input might be responsible for the large number of binocularly driven collicular cells. Therefore, we raised cats with alternating monocular occlusion during the first 3 months of life. Some were later allowed normal binocular vision and were studied at about 20 months. Others were studied at 3 months and had no binocular visual experience. About 77% of the collicular cells were binocularly driven, in contrast to 17% of the visual cortex cells. In 3 animals we studied both right and left colliculi and found no differences between the two sides. The animals allowed binocular vision after 3 months of age did not differ from those with no binocular experience.

Finally, we wondered if binocular driving in the colliculus might depend on either binocular visual input or normal motor control. Therefore, we raised cats with both alternating occlusion and section of right medial and lateral rectus muscles. This procedure also forced the cats to use the strabismic eye half the time. The colliculus of these animals resembled the colliculus of kittens subject only to alternating monocular occlusion. The two colliculi were identical and did not show the asymmetry that is so prominent in cats raised with artificial strabismus alone. About 70% of the cells were binocularly driven. Most of the monocularly driven cells were dominated by the contralateral eye.

We have also examined directional selectivity in strabismic, alternating occluder, and strabismic-occluder cats. All three groups had a normal number of directionally selective cells (65-75%) but the distribution of preferred directions was decidedly abnormal. In the normal colliculus only about 9% of the cells had preferred directions within 30^{o} of vertical, while in the deprived cats about 26% of the cells had preferred directions within 30^{o} of vertical.

We conclude, first, that binocularly driven cells in the superior colliculus probably do not require input from binocularly driven cortical cells, but can receive input from both group 1 and group 7 cortical cells. Second, if one eye cannot move normally, the ability of that eye to drive collicular cells decreases unless the animal is forced to use that eye. Third, if an animal cannot use binocular vision to control eye movements, the number of collicular cells preferring vertical stimulus movement increases and the number preferring horizontal movement decreases.

133 MODULATION OF SINGLE UNIT LGN RESPONSES BY VISUAL CORTEX FEEDBACK. J. D. Daniels*, Joyce L. Norman* and John D. Pettigrew. Behavioral Biology, California Institute of Technology, Pasadena, CA 91125

Anatomists have identified a projection from the visual cortex back to its primary input, the lateral geniculate nucleus. The visual response properties of LGN cells, however, show no obvious influences of the cortex. We have been trying to find a more subtle influence by presenting LGN cells with visual stimuli which are known to maximally stimulate cortical cells, then looking for biases in the LGN responses which might indicate the nature of the cortical influence. Orientation is one of the parameters we have studied.

Most LGN cells showed no orientation bias but a few had asymmetries in the polar plots obtained with moving bars which were not present when they were tested with moving spots. To see if we could enhance orientation biases in LGN cells we raised a series of kittens whose only visual experience was in vertically or horizontally striped cylinders. Half the animals, in addition, had one eye sutured. Our sample from these animals indicated that, though none of the cells had orientation preferences as narrow as cortical cells, the biases seen were either parallel or orthogonal to the orientation of exposure. A few cells from layers which never saw the stripes (in the monocular animals) also had biases, indicating a possible cortical influence. Compared to our normal sample, there appeared to be only a slight increase in the percentage of asymmetric cells. Our data suggests that the cortex may produce no responses of its own in the LGN, but may selectively modulate inhibitory mechanisms already present in the retina-to-LGN circuitry.

134 ABSENCE OF SURROUND ANTAGONISM IN UNIT RESPONSES OF THE LGN IN YOUNG KITTENS. Joyce L. Norman*, J. D. Daniels* and John D. Pettigrew. Behavioral Biology, California Institute of Technology, Pasadena, CA 91125.

Studies of visual cortex development have shown that cells there become more specified in their responses to oriented stimuli, reaching mature orientation and disparity selectivity at one month of age. While these changes are generally attributed to cortical development, we have considered the possibility that some of the immaturity seen in cortical cells may be a reflection of the developmental state of cells of the lateral geniculate nucleus.

We have recorded single unit responses from the LGN of kittens 6-30 days of age and have found a developmental progression in center-surround organization. Discrete receptive fields with centers sometimes as small as 1-3 degrees are found in kittens 6-10 days of age even though the optics are very poor. Striking, however, is the absence of surround antagonism in cells of young kittens. Units often give no response to annular surround stimulation. Also, cells show no response decrement when tested with targets of increasing size. Cells with surround responses appear prior to those with surround inhibition during the second week, but even by the end of the third week, cells without surrounds are still encountered. Immature LGN cells have very low spontaneous rates, require long interstimulus-intervals, and respond poorly if at all to movement faster than 5 degrees/second. Additionally, there is a high proportion of ON-OFF type cells and cells which are binocularly driven. The proportion of units which can be categorized as W-, X-, or Y-cells based on the presence or absence of linear summation, latency to optic chiasm stimulation, and response to standing contrast increases with development.

135 MODIFIED RETINAL PROJECTIONS IN RATS WITH UNILATERAL CONGENITAL EYE DEFECTS. P.W. Land*, E.H. Polley and M.M. Kernis*, University of Illinois College of Medicine, Chicago, Illinois, 60612.

We have examined the retinal projections of rats born with teratogen induced unilateral eye defects. Previous investigations have shown that trypan blue produces unilateral eye defects in rats at a stage of gestation prior to ganglion cell axonal outgrowth. Pregnant, Long-Evans, black-hooded rats injected on day 8 of gestation with a 1.8% aqueous solution of trypan blue (167mg./kg.) gave birth to a variable number of live young with unilateral eye defects, many surviving to maturity. The normal eye of adult anophthalmic or microphthalmic animals was removed. These animals and similarly enucleated controls were perfused with Karnovsky fixative 5 days after surgery. Serial, frozen sections of the brain were stained by the Fink-Heimer method or a modification of the Nauta method (Lund and Westrum, '66). The contralateral terminal degeneration resulting from removal of the single intact eye of experimental animals resembles the control pattern. The ipsilateral projection expands diffusely throughout the LGNd and SC. Altered patterns of projection to other retinal terminal areas were also observed. Other investigators have reported expanded ipsilateral retinal projections in adult rats which had one eye removed at birth (Lund, et. al., '73). They attributed that expanded projection to either misdirected axons from the remaining eye (a failure to cross in the chiasm) or collateral sprouting of normally decussating fibers; interaction of fibers from both eyes being required for axonal crossing characteristic of the normally developing animal. Our data suggest that interaction of optic nerve fibers from both eyes at the chiasm is not required for crossing to occur. However, bilateral interaction may be necessary to restrict developing retinal projections to their appropriate terminal area in brainstem and thalamic nuclei. (Supported by PHS GRSG #613, University of Illinois).

136 EFFECTS OF INTER-OCULAR COHERENCE AND AUDIO-VISUAL CORRELATIONS ON THE DEVELOPMENT OF VISUAL CORTEX IN KITTENS. F.K. Lenherr and D.N. Spinelli. Ctr. for Systems Neuroscience, Grad. Res. Ctr., Univ. of Mass., Amherst, Mass. 01002

Eight kittens were dark-reared from birth. Their entire visual experience until the time of recording was limited to two patterns, each consisting of three black bars on a white background. In pattern H the bars were horizontal, in V, vertical. One group of kittens viewed H and V on alternate days, always using the same eye; those in a second group also saw H and V alternately, but in different eyes; a control animal viewed H and V simultaneously, one pattern in each eye. In addition, kittens in the first two groups heard one of two different pure tones whenever H or V was illuminated. Tone A (1.0 kHz) was always presented together with one of the patterns, and tone B (4.5 kHz) when the other could be seen. Tone and pattern were therefore always correlated during exposure.

Results showed that frequency-specific auditory responses of units in visual cortex (Area 17) were weakly correlated with visual orientation tuning: the few cells that responded selectively to both modalities were all stimulated best by the particular tone which had been paired with their preferred orientation during development. It was also found that "incoherent" stimulation--the simultaneous presentation of different patterns to each eye (control animal)--was far more effective in destroying cortical binocularity, and in biasing orientation preferences, than either monocular (first group) or alternate monocular (second group) presentation. Theoretical studies of the development of visual cortex, using a computer simulation model, suggest that these experimental findings are consistent with the hypothesis that the genetic programs which govern the maturation of geniculo-cortical synapses on the one hand, and cortico-cortical synapses on the other, do not operate in isolation from one another, but rather interact extensively during the course of development.

137 NEURAL CORRELATES OF VISUAL EXPERIENCE IN SINGLE UNITS OF CAT's VISUAL AND SOMATOSENSORY CORTEX. D. N. Spinelli, J. Metzler, R. W. Phelps. Departments of Computer and Information Science and Psychology, University of Massachusetts, Amherst, MA 01002.

Restricting the visual world of kittens during development to a few, known experiences has proved to be a very powerful method in the study of neural plasticity. In a continuing effort to distinguish between an atrophy from disuse and a memory hypothesis, and to give behavioral significance to the experience, the following experiment was performed. Kittens were reared in a dark room until about 13 weeks of age. Beginning with the 4th week of age, each day the kittens were trained to a simple visual discrimination: vertical bars presented to one eye meant that the kitten had to lift the ipsilateral foreleg; horizontal bars presented to the other eye meant that the kitten had to lift the other foreleg. Failure to perform within 6 seconds was followed by a mild electrical shock to the appropriate forearm. After the kittens had mastered this discrimination, single-cell receptive fields were mapped in visual and somatosensory cortex. Visual receptive fields had the shape of vertical or horizontal bars and could be driven only by the eye that had the corresponding experience. The most dramatic findings were in somatosensory cortex. First, the area containing cells responsive to forearm skin stimulation was enormously larger than in normal kittens. Secondly, a large proportion of these cells could be driven by visual stimuli: vertical or horizontal bars were most effective. Cells responsive to vertical bars could be driven only by the eye that had seen vertical bars and by the foreleg that had been associated with that stimulus; cells responsive to the other foreleg could be driven only by horizontal bars seen through the horizontal eye. These results demonstrate that the number of cells committed to a specific function in the cortex can be altered dramatically by experience and lend support to the memory hypothesis. (Supported by NIMH Postdoctoral Fellowship 1 F02 MH44282-01 to JM and NIMH Grant No. 7 R01 MH25329 to DNS.)

138 POSSIBLE MOLECULAR MECHANISMS ASSOCIATED WITH RETINO-TECTAL SYNAPSE FORMATION IN BUFO MARINUS, REVEALED BY THE USE OF LABELLED SNAKE NEUROTOXINS. John A. Freeman, William A. Lutin*, and R.N. Brady*. Departments of Anatomy and Biochemistry, Vanderbilt University, Nashville, Tenn. 37232.

The retino-tectal system of the toad B. marinus provides a model system for the study of molecular events underlying the spatial ordering of synapses. Using a computer controlled mapping procedure, and 3-dimensional current source-density (CSD) analysis (Freeman & Nicholson, J. Neurophysiol. Vol. 38 #2, 1975) to distinguish pre- from postsynaptic activity, we find that lesioned optic nerves regenerate over a period of 6-10 weeks to reestablish a normal pattern of synapses. Sites for synaptic formation are selected on the basis of affinity between neurotransmitter and postsynaptic receptor protein as well as appropriate spatial location. Previous studies have provided evidence (Gruberg & Freeman, M.I.T. Quart. Prog. Rep., 1975) that ACh is an excitatory amphibian optic nerve neurotransmitter. Other studies (Freeman & Lutin, Neurochem. Soc. Abst. #132, 1975) have shown that the post-synaptic receptor protein (RP) is nicotinic cholinergic and selectively binds snake α-neurotoxins. High resolution maps (50 μm spacing) were obtained before and after chronic localized application of α-toxin to the tectal surface. (Because of its high binding affinity for the RP, localized application of toxin produced a circumscribed region of functionally denervated tectum, as shown by EM autoradiography using ^{3}H-α-toxin and by ACh microiontophoresis.) Toxin-bound RP was stable for a period of several weeks, as judged by iontophoresis, EM autoradiography, and radioassay for labelled toxin-receptor complex in membrane fractions. Localized application of α-toxin to circumscribed regions of tectum during optic nerve regeneration resulted in initial invasion of regenerating fibers into toxin treated regions, followed by subsequent loss of regenerating terminals there, and regrowth into surrounding untreated tectal regions. Chronic application of α-toxin to restricted regions of the tectal surface, with the optic nerves intact, resulted in loss of optic nerve synaptic terminals in the treated zones, and the appearance of new terminals at sites outside the zones, accompanied by displacement and reordering of optic terminals there. These data are consistent with the idea that synapses in both the regenerating and the intact retinotectal system require functional interaction between neurotransmitter and receptor for their stabilization and continued maintenance, and they provide additional evidence for dynamic competition between neighboring optic terminals for synaptic sites. We suggest that alterations of receptor protein of tectal neurons during regeneration and possibly during embryogenesis may play a causal role in determining whether retinal fibers growing past a given location form enduring synapses there. According to this view, spatial retinotectal synaptic ordering could be simply achieved by a temporal and spatial variation in receptor protein properties during innervation. (Supported by USPHS Grants E40117 and NS09916 from the NIH.)

139 DEVELOPMENT OF PYRAMIDAL NEURONS IN MACAQUE MONKEY PRIMARY VISUAL CORTEX. Ronald G. Boothe, Dept. of Ophthalmology, Univ. of Washington School of Medicine, Seattle, Washington 98195.

Primary visual cortex of fetal and infant macaques (127 days gestation to 6 months postnatal) has been prepared by the Golgi rapid method. Pyramidal neurons were examined to determine their dendritic branching patterns and distribution of spines on the cell surface. At 127 days gestation the basal dendrites and the side branches off the apical dendrite shaft are short, thin, and beaded. Dendritic spines typical of the adult (having a thin stalk with an expanded tip) have not been seen at this age although small irregular processes occur occasionally on dendrites. By 155 days gestation apical dendrites of most pyramidal cells show a laminar distribution typical of the adult, although many still become thin and beaded distally. Basal dendrites of cells in the middle laminae (IVB and V) appear well developed with secondary and tertiary branching, while those in more superficial and deepest laminae (II,III,IVA,VI) have a thin beaded appearance. Irregular processes are found frequently on dendrites and occasionally spines typical of the adult are found. As apical dendrites develop a moderate population of spines, a reduction in the density of these processes is seen as the shaft passes through IVC and IVA. At the time of birth (approx. 170 days gestation) both apical and basal dendrites look relatively mature with secondary and tertiary branching. Moderate numbers of typical adult spines are found distributed on the dendrites, and processes also occur on the soma. Somatic processes are rare before 160 days gestation or after 3 months postnatal. By 6 months after birth, the dendritic branching and distribution of spiny processes resemble the adult.

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140 THE INTERACTION OF RETINAL LESIONS AND EYELID CLOSURE IN THE MONKEY DORSAL LATERAL GENICULATE. Anita Hendrickson, Jennifer Lund and Robert Kalina. Dept. of Ophthalmology, Sch. Med., Univ. of Washington, Seattle 98195.

Photocoagulation lesions were made in the left inferior temporal retina of four Macaca fascicularis monkeys during the third postnatal week. The lids of the right eye were sutured in two animals. One open-eye and one closed-eye animal were perfused at four months and the other pair at six months of age; in all animals the lesioned eye was injected with H^3 amino acid the day before sacrifice. The dorsal lateral geniculate nuclei (dLGN) were frozen sectioned, radioautographed and stained with cresyl violet, samples of 100 cells each were drawn from 1) three laminae in the lateral left dLGN overlying the atrophied region due to the retinal lesion, 2) all six laminae of the medial left dLGN, and 3) all six laminae in the lateral right dLGN. Cell sizes were determined by a computer assisted sonic drawing tablet.

Preliminary results indicate that 1) with both eyes open there is no change in cell size in laminae overlying the atrophied region, 2) when one eye is sutured, 'deprived' laminae show less cell shrinkage opposite the lesion zone than when opposite 'open eye' laminae, and 3) when cell sizes are compared between crossed and uncrossed laminae served by the same eye, cells in the 'open eye' crossed laminae are larger than the cells in the uncrossed laminae and a greater degree of shrinkage is found in crossed 'deprived' compared to uncrossed 'deprived' laminae. Supported by NIH grants EY-01208, -39039, and -01086.

141 FRONTAL AND OCCIPITAL VISUALLY EVOKED POTENTIALS IN VISUALLY DEPRIVED HUMANS. J. Glass, J. Kennerdell*, and J. Merikangas*. Univ. of Pittsburgh Sch. of Med. Pittsburgh, Pa. 15261.

Diffuse light rearing from birth produces irreversible alterations in the stimulus specificity of visual cortex neurons in cats. Deprived cats are also incapable of visually guided behaviors, but this deficit abates following a period of normal visual stimulation. The author has previously shown the deprivation to affect the visually evoked potential (VEP) from non-specific cortex (precruciate gyrus) to a greater extent than the VEP from visual cortex (marginal gyrus). Only the VEP from the precruciate gyrus changed as a result of the post-deprivation experience.

We have now studied humans with visual defects allowing them light perception but not pattern vision in one eye from birth or soon after birth. The VEP was recorded from the scalp overlying the occipital pole and the precentral gyrus. In two subjects, age 19 and 27, a comparison of the VEP evoked from the normal eye with the VEP evoked from the deprived eye shows that the deprivation altered the VEP from both recording sites. However, with the deprived eye, the VEP from the frontal site was reduced in amplitude to a much greater extent than was the VEP from the occipital pole. In one subject, a five year old boy, the occipital VEP evoked from the deprived eye was actually larger than the VEP evoked from the normal eye. Even in this case the frontal VEP was reduced when evoked from the deprived eye. Therefore, the changes in the VEP from non-specific cortex are not merely secondary to changes in the primary projection pathways. Our findings indicate that the deprivation alters the non-specific sensory pathways independently of the primary visual pathways.

142 ALTERATION OF DIRECTIONAL SENSITIVITY IN THE CAT CORTEX BY SELECTIVE DEPRIVATION: EVIDENCE ON THE CRITICAL PERIOD. Nigel W. Daw and Harry J. Wyatt*. Physiology Department, Washington University, St. Louis, Missouri 63110.

Two cats were raised in a drum with vertical stripes moving continuously right, for two hours per day from 25 days to 50 days of age. For the rest of the time they were in the dark. Recordings from the visual cortex showed that 90% of the unidirectional cells, with preferred directions more than 15° away from vertical, responded to rightward movement rather than leftward movement. There were also a substantial number of bidirectional cells, some responding to both rightward and leftward movement. Six other cats were raised in the same drum, with the drum moving left for a period of time, then right for a period of time, to define the peak of the critical period for this type of deprivation. The change from leftward movement to rightward movement was made at 3, 3 1/2, 4, 4 1/2, 5 and 7 weeks in different kittens. The peak of the critical period may be earlier than the peak of the critical period for monocular deprivation.

143 DEVELOPMENT OF VISUAL STIMULUS-SEEKING IN KITTENS. Victor F. Emerson*, Brian N. Timney* and P.C. Dodwell* (SPON: M.W. Donald). Dept. of Psychol., Queen's Univ., Kingston, Ont., Canada.

Kittens were given access to patterned light, contingent on a panel press, for an hour a day from postnatal days 20 to 150. Each of 7 dark-reared kittens showed a level of responding equivalent to less than 1 minute of exposure to light per hour of opportunity until about 2 months of age (63.8 $\pm$ 1.72 days), when the self-produced exposure time increased abruptly to between 5 and 25 minutes per hour (14.1 $\pm$ 5.5 minutes). Controls reared on an alternating 12-hour light-dark cycle did not show this increase.

One of the light-deprived kittens had access to a pattern on half the days and to a tone on alternate days; it demonstrated the increase in self-produced exposure to light at 65 days, but showed no change in exposure to tone. Another dark-reared kitten was given visual access to a stationary vertical grating on some days and to the same grating in motion on alternate days; no preference was apparent, the functions for both stimuli increasing sharply at 66 days.

This age-dependent onset of light-seeking behavior occurs a month later than the peak sensitivity of the known feature-detectors of the visual cortex. Thus, the change in the value of light as a reinforcer can apparently not be explained solely by the plasticity of the visual cortex. Rather, it may reflect another stage in the development of the visual abilities of the cat.

144 PERCEPTUAL CAPACITIES OF KITTENS FOLLOWING ROTATION OF ONE EYE. Carol K. Peck and Sheila G. Crewther*, Dept. Psychol., Pomona College, Claremont, Calif. 91711 and Div. Biol., Cal Tech, Pasadena, Calif. 91786.

Eight kittens had one eye intorted at the time of eye opening. Angle of rotation ranged from 10^{o} or less to 70^{o}. Whem tested for simple visuomotor behaviors at the age of 2 months with only the rotated eye open, they showed visual following of both slowly and rapidly moving objects and visual placing when slowly lowered to a broad, uninterrupted surface. In addition, they showed visually-guided reaching of two types: 1. They hit accurately and precisely at balls and other small objects which were dangled at various locations within the visual field; and 2. When placed on a platform in front of an interrupted (pronged) surface, they reached directly and accurately for the prongs, and avoided the gap between prongs, on at least 9 of 10 trials. Moreover, they performed well while running a simple obstacle course and jumped accurately from table tops, grading the force of the jump to the distance between floor and table.

The kittens were then trained on a series of two-choice visual discriminations, using food reward. The rotated eye was able to learn both brightness and form (square/plus, horizontal/vertical, and diagonally-oriented line) discriminations. Although learning through the rotated eye tended to be slower than through the normal eye, both eyes learned at rates within the range of variation found in normally reared cats. Both cats showed substantial amounts of interocular transfer of these discriminations as measured by changes in the initial percentage of correct responses in the first and second eye trained, and on most problems, the second eye trained also showed substantial savings in the number of trials required to reach criterion.

145 TRANSIENT RESTRICTION OF DEVELOPMENTAL PLASTICITY OF THE PRESUMPTIVE PIGMENT RETINA (PPR) DURING THE PERIOD OF SCLERAL CARTILAGE INDUCTION IN THE CHICK EMBRYO. Patricia A. Dimond and D. J. McCallion* Dept. Neurosciences, McMaster Univ. Med. Ctr., Hamilton, Ont. L8S 4J9

The primitive forebrain of the chick gives rise to two anatomically defined layers of the developing eye; the outer pigmented layer, which forms part of the adult choroid, and an inner neural layer which forms the definitive retina. During the early stages of embryogenesis either of these retinal layers has the ability to generate the other retinal layer under appropriate conditions. However there is one circumstance during which the presumptive pigment retina (PPR) appears to become drastically but transiently restricted in this ability. This is the time when it is involved in another activity, i.e. the induction of the scleral cartilage from the periorbital mesenchyme, which occurs during the 3rd and 4th days *in ovo*. To investigate this we grafted the PPR plus the periorbital mesenchyme from donors of 2-5 days *in ovo* to the chorioallantoic membrane of host chicks. The grafts were subsequently examined for neural retina and cartilage. The generation of neural retina fell significantly during the period of cartilage induction; however it rose again when the periorbital mesenchyme was determined, even though the actual formation of cartilage occurred only 4-5 days later. The periorbital mesenchyme that forms the sclera and its cartilage originates in the cranial neural crest and migrates to the developing eye. We suggest that cranial neural crest cells arriving at the eye are responsible for the reduced ability of the PPR to generate neural retina; however when they are determined to form cartilage, they lose this restrictive influence.

146 THE ADULT GOLDFISH RETINA GROWS BY ADDING NEW CELLS. Pamela R. Johns and Stephen S. Easter, Jr., Dept. of Zoology, Univ. of Mich., Ann Arbor, 48104.

The eyes from goldfish (*Carassius auratus*) 4 to 20 cm in body length were sectioned radially using paraffin histology and stained with cresyl violet or gallocyanin and eosin. The retinal area and total number of ganglion cells and cones was computed.

Ganglion cells were identified on the basis of nuclear morphology and constituted less than half of the cells in the ganglion cell layer. Displaced ganglion cells were identified by their chromatolytic response to optic nerve section and accounted for 1% of the total ganglion cells. Cone inner segments were identified by their morphology and position.

The retinal area increased from 7 to 60 mm^2 while the ganglion cell density decreased from 6000 to 2000 cells/mm^2 with increasing body length. The total number of ganglion cells per retina increased from 40,000 to 140,000, and their average nuclear diameter increased from 5 to 9 µm. The total number of cone inner segments also increased, with the ratio of cones to ganglion cells remaining constant at about 3:1. Preliminary growth studies show that hundreds of cells are added to the retina each day.

Previous results (unpublished) indicate that the extent of the visual field in goldfish is invariant (185° ± 5°) during growth. This fact together with the increase in cell numbers implies that a given cell's receptive field size must change either in degrees visual angle subtended or in size measured in µm on the retina as the fish grows. In addition, since new cells are added at the margins, a cell located at the edge of the retina in a small fish will be gradually displaced toward the center as the fish grows, and its receptive field will likewise move centrally in the visual field. Both of the above imply that rewiring of synaptic connections occurs. (PHS grant EY-00168 and MMPP grant 494, and Mich. Chap. Travel Award)

147 EFFECT OF TRANSPLANTATION OF AN ADDITIONAL OPTIC PRIMORDIUM ON DEVELOPING NUCLEAR CENTERS IN THE CHICK. C.H. Narayanan and Y. Narayanan*. Dept. of Anat., L.S.U. Medical Center, New Orleans, Louisiana, 70119.

In a previous study on the effect of peripheral overloading following transplantation of an additional eye in chick embryos, a significant neuronal hyperplasia was observed in the developing ciliary ganglion of the operated side. We have now extended the analysis to the accessory oculomotor nucleus (AN) and the trochlear nucleus (TN) in the experimental animals, with the aim of obtaining information on the nature of cell loss in these nuclear centers. Chick embryos with well differentiated composite eyes (native eye plus graft) were sacrificed on days 9, 11, 13, 15 and 17 of incubation. The number of neurons in the AN ipsilateral to the graft and the contralateral TN were compared with counts of neurons on the unaffected side. A significant neuronal hyperplasia was observed in both these nuclei. While the increase in the number of cells in the TN may be related specifically to the increased musculature, the increase in the AN forms ground for speculation as to the mechanism leading to cell loss in the normal AN during a period when the developing ciliary ganglion is undergoing cell loss. Interestingly enough, the increase in cell number in the AN bears a direct relationship to the increase in the number of cells of the ciliary ganglion on the operated side.

148 DEVELOPING SYNAPSE SETS IN THE DIFFERENTIATING MOUSE RETINA. Leslie J. Fisher. Dept. of Anatomy, U. of Michigan, Ann Arbor, Mich.

The pattern of synaptic connections found within an adult nervous system may develop in several possible ways. For example, a surplus of synapses may be formed with subsequent disappearance of some as the animal grows or, alternatively, a limited number of synapses corresponding to the final number found in the adult may be formed over a specific period. To determine which of many possible modes of synaptic set development is actually followed, a quantitative study of developing synapse sets was performed.

Retinas of mice (C57BL/6) were removed and prepared for electron microscopy at daily intervals from birth until day 16 and at longer intervals until day 40. The inner plexiform layer at a fixed locus within these retinas was scored for density of synaptic profiles within amacrine and bipolar cell processes. Synaptic densities were corrected for sampling parameters and plotted as a function of time after birth. Both amacrine and bipolar synapse densities are characterized by two phases of growth: 1. an initial phase of rapidly increasing density, 2. a plateau phase of nearly constant density. Significantly, the breakpoint between these two phases in both cell types occurs at day 15, the age at which the eyes open in these animals.

Amacrine cell synapses are first seen at day 5, while bipolar synapses begin to appear at day 11. Amacrine synapse density increases at a mean rate of 1.1 synapses/hour/1000μ^3 until day 15. Bipolar synapse density increases at a slower rate:0.67 synapses/hr./1000μ^3 until day 15. Serial synapses remain at a very low density throughout the period studied suggesting few amacrine-amacrine synapses (supported by NSF grant GB 41553).

149 PHYSIOLOGICAL CORRELATES OF EARLY VISUAL EXPERIENCE. Jacqueline Metzler and D. Nico Spinelli. Departments of Computer and Information Science and Psychology, University of Massachusetts, Amherst, MA 01002.

Early selective visual experience has dramatic effects on the functional characteristics of single cells of the visual cortex of kittens. To distinguish between a memory and an attrition hypothesis and to identify those factors that control the maintenance or disruption of binocularity, the following experiment was performed. Two groups of kittens were dark-reared from birth until 3 1/2 weeks of age at which time they viewed an 'L' pattern with one eye and either the horizontal or vertical components of this same pattern with the other eye. The horizontal and vertical bars were positioned so that they stimulated corresponding parts of the retinas of both eyes. One group of kittens saw the horizontal and vertical components of the 'L' with the one eye on alternate days for a period of 8 to 12 weeks, at which time recordings were taken from single units in Area 17. The other group viewed either the horizontal or vertical bar with the one eye for a 4- to 6-week period. Recordings were then taken from these kittens. They were subsequently revived and exposed to the orthogonally oriented bar for an additional 4 to 6 weeks, at which time recordings were again taken. The results of these recordings indicate that the receptive fields of the units sampled mimic what the kittens saw during the first few months of life. Most of the receptive fields were either horizontally or vertically oriented and several had 'L'-like shapes corresponding to the dimensions of the 'L' pattern to which the kittens had been exposed during development. The remaining receptive fields were classified as diffuse. Moreover, nearly all of those units with well-defined receptive fields could be activated by either eye. The finding of cells with receptive fields not present in normal cats lends support for the memory hypothesis. (Supported by NIMH Postdoctoral Fellowship 1 F02 MH44282-01 to JM and NIMH Grant No. 7 R01 MH25329 to DNS.)

150 BEHAVIORAL AND ELECTROMYOGRAPHIC RESPONSES TO LIGHT IN NEWBORN KITTENS. Guenter H. Rose and Jeremiah P. Collins*. Dept. Psychiat., Sch. Med., UCLA, Los Angeles, 90024.

Behavioral responses (squint and blink) and EMG activity from the orbicularis oculi can be evoked by 40 hz light flashes from a Grass PS-1 photostimulator in the newborn kitten and thereafter as a function of flash duration and intensity. Controls eliminated heat and auditory influences. At birth, a threshold flash duration of 500 msec. evokes a 1200-1500 msec. latency EMG response. A dramatic change in light duration threshold and consequent EMG latency is seen over the next several days resulting in a 125-150 msec. latency EMG response to a single light flash (15 μsec.) at 6 days of age (before eye opening). The early occurrence of the light evoked EMG response at birth is contrasted with what is known concerning the developmental onset of other visually evoked electrical activity of the peripheral and central nervous system. (Supported by USPHS Grants HD-04612, HD-05958 and HD-00345).

151 STRUCTURE AND SYNAPTIC CONNECTIVITY OF THE PHOTORECEPTORS IN DAPHNIA RAISED IN COMPLETE DARKNESS. E. R. Macagno* (SPON: C. Levinthal). Dept. of Biological Sciences, Columbia University, New York, N.Y. 10027.

In order to test the requirement of photostimulation for the normal development of photoreceptors, we have grown newly deposited Daphnia magna eggs under various conditions of light and darkness. Seven days after hatching, some of the animals were fixed and prepared for electronmicroscopy and others were put through tests of their behavioral responses to light. The fixed specimens were serially sectioned and photographed on a Zeiss EM-9S at low and high magnification, and three-dimensional reconstructions of the photoreceptors were made. The following parameters were determined: gross and fine structure of rhabdoms, branching pattern of synaptic terminals in the neuropil of the optic ganglion, number and fine structure of synapses, synaptic connectivity to lamina neurons and overall organization of the lamina.

Except for the disorganization and degeneration of microvilli in rhabdomeres of dark-reared animals, we found no significant differences outside of the normal range of variation of the measured parameters, between animals grown in total darkness and in light. The effect on the microvilli, however, could be reversed by putting the dark-reared animals into normal light conditions for at least 48 hours prior to fixation. Our simple behavioral tests for detection of light intensity changes and color discrimination showed no significant differences among the experimental animals. Tests of the optokinetic response and polarization sensitivity are in progress and will be reported. These results indicate that in Daphnia the development of photoreceptors occurs almost normally under complete lack of photostimulation, and that the one abnormal parameter, the precise arrangement of microvilli in rhabdomeres, is not behaviorally significant at the level tested.

152 RESPONSES OF INNER PLEXIFORM SYNAPSES TO DARK-REARING IN THE RETINA OF THE TOAD, XENOPUS LAEVIS. Gail S. Tucker*.SPON: Paul Witkovsky. Ophthalmology Research, Columbia University, 630 W.168th Street, N.Y. 10032.

Retinae from light- (L/D, 8 hr/16 hr) and dark-reared toad larvae were examined in the electron microscope to assess the responses of synapses in the inner plexiform layer (IPL) to total visual deprivation over the course of development. Changes in synaptic density are related both to developmental stage and rearing condition. At all stages examined, dark-reared larvae (DRL) had significantly more conventional synapses per 1000 μ^3 ($\bar{X}$ = 418; $p \geq 0.05$) than did light-reared (LRL; $\bar{X}$ = 241), recovery (RL; $\bar{X}$ = 258) and adult retinae ($\bar{X}$ = 254). DRL had significantly more ribbon synapses ($\bar{X}$ = 124; $p \geq 0.01$) than LRL ($\bar{X}$ = 47), RL ($\bar{X}$ = 87) and adult ($\bar{X}$ = 106) at later stages. The pattern of distribution of ribbon synapses in the IPL (scleral, medial and vitreal portions were examined separately) changes in response to dark-rearing. In LRL (Stage 54), ribbon synapses are concentrated in the medial layer of the IPL. Ribbon synapse density in DRL is elevated throughout the IPL at this stage. A quantitative change in conventional synapse size occurs in response to sensory deprivation in the developing toad retina (Stage 54: LRL, $\bar{X}$ = 373 Å; DRL, $\bar{X}$ = 296 Å; $p > 0.001$). Animals readmitted to a light regimen showed degrees of recovery to LRL condition which were related to the duration of deprivation (i.e., the stage when readmitted to the controlled light regimen). Younger animals recover normal density values at a faster rate than slightly older animals exposed to light .

Thus, formation of the various types of specialized synaptic contacts in the IPL of toad retina is differentially responsive both quantitatively and qualitatively to total sensory deprivation.

153 RETINAL PROJECTIONS OF FROGS AFTER FORCED DOUBLE OR SINGLE INNERVATION OF THE IPSILATERAL OPTIC TECTUM. D. J. Stelzner, L. J. Misantoně and E. Kicliter. Dept. of Anatomy, Upstate Med. Cntr., Syracuse, N. Y. 13210 and School of Basic Med. Sci., Univ. of Ill., Champaign, Ill. 60680

The normal retinal projection in R. pipiens (N=10) was compared with retinal projections of frogs having unilateral optic tectum removal (N=27) with or without an ipsilateral enucleation (N=6; tectum + eye) or nerve crush (N=8; tectum + crush). Using a modified Fink-Heimer method, controls indicated only a sparce amount of degeneration in the optic tract after 4-6 mon. survival. Therefore, the eye contralateral to the tectal lesion was enucleated 5-7 months after the first operation and after 3-8 days survival, the brains were processed for Fink-Heimer staining. The retinal projection in the unitectal frogs was anomalous. Besides the normal projection to the thalamus, there was a dense projection to the contralateral middle thalamic neuropil. Also, in all unitectal animals there was a projection throughout the ipsilateral tectum usually traveling to the tectum through the region of the posterior commissure. The projection to the tectum appeared less distinctly laminated, although it went to the same region of the tectum, in the doubly innervated unitectal and tectum + crush groups than in the tectum + eye group which was similar to normal. No marked differences were found comparing the cytoarchitecture of the tecti of the different groups. However, preliminary results in the unitectal and tectum + crush groups indicate an increase in depth of the superficial tectal layers suggesting that the tectal neurons which are doubly innervated by the contralateral and ipsilateral optic nerves have expanded their dendritic surface. This may explain the slower onset of abnormal visual response in these two groups of unitectal frogs when compared with the tectum + eye group (Exp. Neurol 45: 364-376).

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154 THE PRENATAL DEVELOPMENT OF CENTRAL OPTIC PROJECTIONS IN ALBINO RATS. R. D. Lund and A. H. Bunt. Depts. of Biological Structure, Neurological Surgery, Ophthalmology, Univ. of Washington, Seattle, Washington 98195.

The course of development of the central projection of the optic nerve in fetal rats has been studied using the Fink-Heimer technique after eye lesions made in utero. Supplementary information of the retinotectal pathway was obtained using the electron microscope and horseradish peroxidase tracing technique. The first optic axons arrive at the chiasm on fetal day 15 (birth at day 21). By early day 16, they have reached the epithalamus, having grown through both parts of the lateral geniculate nucleus. A growth rate of 80-100 μm/hr was calculated. By later in day 16 the axons have entered the front of the superior colliculus and occupy all but the borders by day 17. Within the colliculus the first synapses, some of optic origin, are formed by early day 17. Tritiated thymidine studies on the retina indicate that the last ganglion cells to undergo their terminal division do so after the first axons have reached the colliculus. Unlike nonmammalian species, these latest cells are not uniquely restricted to the peripheral retina. Cells of the geniculate and colliculus have undergone their terminal division before the first optic axons have arrived. The relative timing of axonal growth and cell divisions stands in marked contrast to descriptions of the retinotectal pathway in chick and Xenopus. Supported by NIH grants EY 00596, 0311 and by a grant from Fight For Sight, Inc., New York City.

155 ANOMALOUS IPSILATERAL VISUOTECTAL PROJECTION IN GOLDFISH IS FUNCTIONAL. Dean Yager and S.C. Sharma. S.U.N.Y. College of Optometry, New York, N.Y., 10010, and Department of Ophthalmology, N.Y. Medical College, New York, N.Y., 10029.

A conditioned respiration technique was used to demonstrate visual function and to estimate visual thresholds in goldfish that had undergone unilateral tectal ablation 125 days earlier. There was no measurable difference in thresholds for the two eyes, tested separately. Threshold for the anomalous eye-tectum pair was unchanged when the eye contralateral to the remaining tectum was removed. Ipsilateral projection was confirmed with an electrophysiological mapping technique.

The anomalous ipsilateral visuotectal projection in goldfish is, then, able to mediate visual behavior with sensitivity as great as the normal projection in the same animal.

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156 FETAL COLLICULAR LESIONS - THEIR EFFECT ON THE ORGANIZATION OF THE RETINOTECTAL PROJECTION. Bruce F. Miller* and Ray D. Lund. (SPON: P. Coates) Depts. of Biological Structure, Neurological Surgery, Univ. of Washington, Seattle, WA. 98195.

A transverse slit was made across the left superior colliculus (SC) in albino rats on fetal days 15-21 (birth on day 21). The animals were allowed to survive at least six weeks postnatal before mapping the central projections of the right eye using degeneration methods in combination with the autoradiographic method for pathway tracing. The projection of nasal retina was of particular interest since it projects caudal to the slit. In all operated animals there is a nasal retinal projection to posterior SC similar to that in normal animals. In many animals there is an aberrant projection from nasal retina, usually located immediately anterior to the slit. In other animals the aberrant projection is distant from the presumed site of the slit. In a third group of animals besides the normal projection to the lesioned colliculus, some axons recross the midline and innervate the opposite colliculus. In many animals in which obvious slits were made, no abnormal projection was subsequently found. In no case is there sprouting into nucleus lateralis posterior of the thalamus. The various effects of the lesion are presumed to relate to the age at which it was made and to the extent and rate of recovery. It is clear from studying the projection of the nasal retina that while an aberrant retinotectal projection can be induced, the forces which govern the normal spatial distribution of the pathway have a strong tendency to persist. Supported by USPHS Grants EY-00596 and GM-00136 from the National Institutes of Health and by an NSF Traineeship.

157 COMPETITION BETWEEN NASAL AND TEMPORAL HETERONYMOUS HEMIRETINAL FIBERS IN ADULT GOLDFISH TECTUM. Y. L. Tung* and S. C. Sharma. Dept. Ophthalmology, New York Medical College, New York, N.Y. 10029.

Two hypotheses exist to explain the observed retinotectal plasticity in the adult goldfish. One postulates the respecification in the cytochemical properties of the retina or tectum following surgery, and the other suggests a "system matching" mode where competition among remaining fibers occurs to fill the available tectal space. The present experiments test both proposed assumptions. In adult goldfish, the ablation of the temporal half-retina of either the right or left eye was followed by ablation of the nasal half-retina of the other eye. The entire left tectum was removed either one week or one month following retinal surgery. The visual projections from both eyes were mapped to the remaining tectum one to six months postoperatively. Animals mapped one month postoperatively showed the expansion of the half-retina over the entire extent of the dorsal tectum, while the ipsilateral projection was absent. Animals mapped two to three months postoperatively showed a relatively small, unorganized ipsilateral projection in addition to the expanded contralateral one. However, animals mapped five to six months postoperatively showed that each half-eye projected only to the appropriate half-tectum. The results indicate that each half eye does not regulate into a whole and that respecification apparently does not occur. It is suggested that the affinities of the optic axons for tectal loci vary with time during regeneration. Furthermore, the process leading to the stability of retinotectal connections may be dependent upon the similarities of the retinal substrates in their affinities towards the appropriate tectal loci and the density of the substrates available. Whether axons will share a tectal locus or be displaced by each other may be determined by the substrate competitive inhibition of the first order enzyme kinetics. (Supported by N.S.F. Grant G.B. 43506 and N.I.H. Grant EY 01426.)

158 STRUCTURE OF VISUAL CORTEX OF THE REELER MUTANT MOUSE: A GOLGI ANALYSIS. Verne S. Caviness, Jr. (Spon: M. LaVail). Harvard Med. Sch. Boston 02115.

The visual cortex of the reeler mutant mouse may be subdivided radially into three structural zones comparable to those in the normal despite relative malposition of different neuronal classes in the mutant. Terminals of the geniculate projection are concentrated in the centrally lying granule cell zone. Callosal terminals, laterally in the visual cortex, are most densely concentrated in a zone of small and medium pyramids which form the supragranular zone of normal, the infragranular zone of the mutant. A zone of large pyramids and polymorphic cells in infragranular position in the normal but supragranular in the mutant receives a light callosal projection in both. Spiny stellate cells, impregnated by the rapid Golgi method, are concentrated in the granule cell zone in the mutant as in the normal. Pyramidal cells are found in all three zones of both mutant and normal. The pyramidal cells of reeler, like those of the normal, are in general radially aligned though the polarity of many is inverted in the mutant. By exception in the mutant many superficially lying pyramidal cells are horizontally aligned. In the mutant, as in the normal, dendrites of spiny stellates and of pyramidal cells, regardless of polarity, are most richly branched in the zone of the cell body. In both, axons of spiny stellates are deployed through the granule cell zone and to some extent the adjacent pyramidal cell zones. In the mutant as in the normal axons of small and medium pyramids and those of the star pyramids in the granule cell zone collateralize richly in the zone of large pyramidal cells. Those of the large pyramids, by contrast, exit from the cortex with few collaterals to the other two zones in both mutant and normal. Thus axons and dendrites of neurons of the reeler visual cortex appear to be deployed in such a way as to provide for normal intercellular connections despite malposition of different cell classes.

159 PHYSIOLOGIC PROPERTIES OF CELLS IN THE PRIMARY VISUAL CORTEX OF THE REELER MUTANT MOUSE. Ursula C. Dräger* (SPON: P.B. Dews). Dept. Neurobiol., Harvard Medical School, Boston, 02115.

Anatomically the visual cortex in the reeler mutant appears highly abnormal, as do all other cortical structures in this mouse. Geniculocortical fibers enter the cortex of the reeler from above instead of from below; all cell elements present in normal mice are found, but the cell types are arranged in an inverted order through the depth of the cortex[1]. I recorded from single cells with the object of learning to what extent three characteristics of the normal visual cortex are achieved in the reeler: an ordered topography, several specific receptive field types and binocular convergence. (1) The topographic projection of the field of vision was normal in the reeler, the nasal field projecting laterally, the upper field posteriorly within area 17. (2) Cells recorded closest to the cortical surface tended to be abnormal in having unusually large receptive fields and responding in a rather sluggish way to visual stimulation. Deeper in the cortex all receptive field types commonly seen in the normal mouse were found: cells with circularly symmetric receptive fields (on-center, on/off, off-center) and cells with oriented receptive fields (simple and complex). There were more simple cells than complex in the reeler, whereas normally the complex cells predominate. (3) The majority of cells recorded in the binocular part of area 17 responded to stimulation through either eye, and the ocular dominance distribution was about normal. Cells that were influenced by the two eyes always had their receptive fields on corresponding parts of the two retinas and were always of the same physiological type. It is concluded that most cells in the reeler visual cortex are capable of making the right connections with each other regardless of their position within the cortex. (NIH grant EY00605; Rowland Fnd.grant).

[1] Frost, D.O. and V.S. Caviness, 4th Ann. Meet. Soc. Neurosci. (1974) 217.

160 NEURAL CONNECTIONS OF AREA 17 IN AN ANOPHTHALMIC MOUSE STRAIN. Ita R. Kaiserman-Abramof, Ann M. Graybiel and Walle J. H. Nauta. Dept. Anat., Case-Western Reserve U. Sch. Med., Cleveland 44107, and Psychol. Dept., Mass. Inst. Technol., Cambridge, Mass. 02139.

In bilaterally anophthalmic specimens of mouse strain ZRDCT-AN (Chase and Chase '41)the optic cup regresses before the optic tract has formed.In such eyeless specimens the cell population of the small but well-defined dorsal nucleus of the lateral geniculate body (LGd) is about 76% of normal; likewise markedly hypoplastic (but sharply defined by its high acetylcholinesterase content) is the stratum griseum superficiale (SGS) of the superior colliculus. Despite the anophthalmia the occipital cortex includes a major central region that is cytoarchitecturally indistinguishable from area 17 of the normal mouse.

The results of horseradish-peroxidase (HRP) experiments in 15 such eyeless mice indicate that LGd projects to area 17 in a topographic fashion closely resembling the normal pattern; e.g., small HRP deposits in the area's medial one-quarter labeled cells near the LGd-LGv border, whereas HRP deposited near the area's lateral boundary labeled cells in the normally "binocular" LGd region. Labeling of numerous cells outside LGd suggests that area 17 in the anophthalmic mouse may receive more than the normal number of additional afferents from the nuclei lateralis posterior (LP) and lateralis intermedius. In a parallel autoradiographic study the subcortical efferents of area 17 were found distributed to LGd, LGv, SGS, LP, the pretectal region and the pontine gray. These findings indicate a. that the distribution of area 17's efferents in the eyeless mouse is qualitatively normal, and b. that a topographically organized geniculocortical connection can develop despite absence of any link with the retina.

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161 A QUICK CHANGE IN BINOCULARITY OF KITTEN VISUAL CORTICAL NEURONS. Takuji Kasamatsu and Otto Creutzfeldt*. Dept. Neurobiology, Max-Planck Inst. for Biophysical Chemistry, 34 Göttingen-Nikolausberg, W. Germany.

Despite accumulation of evidence which favors the plasticity of neuron networks in the developing visual cortex during the "critical period," few attempts have been made to test, on the on-line basis, the validity of the competition hypothesis (Wiesel and Hubel, J. Neurophysiol. 28: 1029, 1965) which concerns the convergence of binocular inputs to the same cortical neurons.

Kittens which were raised in the normal visual environment were prepared for acute experiments after 1 month of age. The animal was paralyzed by Flaxedil and maintained under N_2O-anesthesia. Single units were recorded from area 17 (medial bank of the posterolateral gyrus) by Insl-X coated steel microelectrodes. The ocular dominance of individual neurons was evaluated quantitatively by constructing post-stimulus time histograms while their receptive fields were stimulated monocularly by function generator-controlled moving visual patterns. A small amount of local anesthetic was injected directly to the contralateral optic nerve to block briefly the transmission of tonic retinal discharges.

This treatment brought about long-lasting enhancement of neural responsiveness to ipsilateral eye stimulation, whereas responses to contralateral eye stimulation were suppressed. The ocular dominance of kitten striate neurons in the "critical period" was found to be dependent on the presence of tonic retinal discharges. Results are interpreted as demonstrating primarily the binocular competition in visual cortical neurons during the "critical period" and also suggesting a role of tonic background activity in the afferent pathway for consolidation of synaptic contacts formed temporarily during the "critical period." T.K. was supported by a research fellowship from the Alexander von Humboldt Foundation.

162 HISTOGENESIS OF VISUAL CORTEX IN THE MIDTRIMESTER HUMAN FETUS. Mark C. Whitehead* and Dorothy G. Flood* (SPON: Paul D. Coleman). Dept. Anat., Sch. Med. & Dent., Univ. Rochester, Rochester, NY 14642.

In 15-20wk human fetuses, increasing amounts of whole brain DNA may represent the phase of neuronal proliferation (Dobbing & Sands, Nature, 1970). In rhesus monkey, neurons of the visual cortex are generated during the middle period of gestation (Rakic, Science, 1974). We have investigated the development of visual cortex in human fetuses of 10-20wk by measuring DNA synthetic activity of supravitally labeled tissue (Rakic & Sidman, J. Neuropath. exp. Neurol., 1968). Blocks of occipital cortex, 1mm square and intact from pia to ventricle, were incubated for 1hr in an oxygenated culture medium containing ^3H-thymidine. Nissl and Bodian stained material facilitated the determination of the boundaries of the 5 embryonic zones. Adjacent blocks were impregnated by the Golgi-Cox or rapid Golgi methods. During midtrimester, the ventricular zone (VZ) decreased slightly in thickness while its proliferative activity diminshed more markedly. Golgi material demonstrated two cellular populations, radial glia and ventricular cells. The subventricular zone (SVZ) had a profile of DNA synthetic activity similar to that of VZ, except for a peak of labeling in one fetus aged 14 1/2wk. Between 14 and 15 1/2wk the thickness of SVZ increased threefold. Migrating neuroblasts and cell bodies of radial glia characterized this zone in the Golgi sections. A rapid rate of increase in the thickness of the cortical plate (CP), which began at 14wk, coincided with the period of increase in SVZ. Neuroblasts migrating to or within CP either possessed differentiated axons extending towards the intermediate zone or were spindle-shaped with primitive processes. By 19 1/2wk differentiated cells with elaborate dendritic and axonal fields (pyramids, stellates with pericellular baskets, and large fusiforms) were prominent in the middle to lower CP. Supported by NIH Grants NS 07870 and GM 01782.

163 LIGHT-EVOKED EXTRACELLULAR POTENTIALS OF THE NECTURUS RETINA: CURRENT SOURCE DENSITY ANALYSIS OF THE ELECTRORETINOGRAPHIC b-WAVE AND THE PROXIMAL NEGATIVE RESPONSE. Luis M. Proenza and John A. Freeman. Dept.of Psychology, Univ. of Georgia, Athens, Ga. 30602 and Dept. of Anatomy, Vanderbilt University, Nashville, Tennessee 27232.

Analysis of the origin of light-evoked extracellular retinal potentials was undertaken in the Necturus eyecup. Extracellular potentials are produced by the flow of current in three dimensions through the resistance of the extracellular medium. The resistivity of the mudpuppy retina was thus measured in three dimensions and found to be anisotropic and inhomogeneous and to vary inversely with the primary orientation of core conductors in each of the three principal axes as shown in Figure 1.

Laminar current distributions in response to diffuse and focal light stimulation were computed using current source density analysis (CSD).[1] CSD's of the electroretinographic b-wave support the suggestion of Miller and Dowling that this response arises from the glial cells of Müller[2] and further indicate that the rising and falling phases of the b-wave are the result of two spatially and temporally separate sinks of current along the Müller cell membrane: an early distal sink followed by a slow proximal sink of current, both having corresponding sources which are widely distributed along the radial dimension. Analyses of the d-wave (off response) indicate that it is generated more proximally than the b-wave but also by the Müller cells. The proximal negative response (PNR)[3] elicited by small spots of light ($\leq$ 250 μm) was associated with a prominent sink in the proximal retina whose corresponding source was located in the tangential plane suggesting that a component of the PNR is produced by laterally oriented amacrine cell processes of the inner plexiform layer.

That the b-wave of the ERG results from the summation of extracellular currents primarily associated with temporally and spatially distributed Müller cell sinks is further supported (1) by CSD of light-evoked $[K^+]_o$ flux, as would be expected from the well known potassium sensitivity of glial cells; and (2) by intracellular recordings which show that the Müller cell response is much more sustained than the b-wave and shows a continuous depolarization for the duration of these sinks.[4]

A mathematical model was developed to express the potential and CSD produced by a specifiable distribution of current generators in a non-uniform resistive medium. Results of the model closely matched the above findings and enabled the effective resistance, electrical characteristics, and position of the recording electrode to be independently estimated.[5]

[1]C. Nicholson & J. A. Freeman, J. Neurophysiol., 38:356-368, 1975.
J. A. Freeman & C. Nicholson, J. Neurophysiol., 38:369-382, 1975.
[2]R. F. Miller & J. E. Dowling, J. Neurophysiol., 33:323-341, 1970.
[3]L. M. Proenza & D. A. Burkhardt, J. Neurophysiol., 36:502-518, 1973.
[4]C. Karwoski & L. M. Proenza, in preparation.
[5]Supported by PHS grants EY00973 (LMP) & EY40117(JAF).

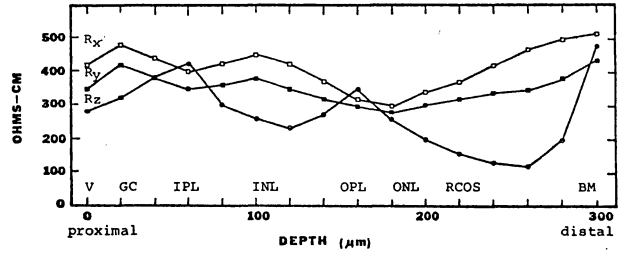


Figure 1. Specific resistance for each of the three principal axes of the Necturus retina as a function of depth (z).

164 SYNAPTIC TRANSMISSION BETWEEN PHOTORECEPTORS AND BIPOLAR CELLS IN THE MUDPUPPY RETINA. Robert F. Miller and Ramon Dacheux*. Neurosensory Laboratory, Dept. Physiol., SUNYAB, Buffalo, New York, 14214.

Present evidence suggests that photoreceptors release a transmitter in the dark and that light stimulation reduces the rate of transmitter release as receptors are hyperpolarized. Support for this view has thus far been restricted to studies of horizontal cells. We have studied synaptic transmission between receptors, horizontal cells and bipolars in the perfused mudpuppy eyecup. Divalent cations were used as synaptic blocking agents while changes in membrane potential and light evoked activity were monitored with intracellular recordings. A brief, 2-3 min. exposure to 2 mM Co^{++} had relatively little effect on receptors, while light evoked responses of post-receptor neurons were usually abolished within 1 minute. The loss of horizontal cell activity was associated with a hyperpolarization, a finding consistent with the observations of other workers. Bipolar cells were differentially affected by Co^{++}. Hyperpolarizing bipolars were hyperpolarized as their response amplitude declined, but depolarizing bipolars were depolarized by the action of Co^{++}. These findings support and extend the view that receptors release a transmitter in the dark; this transmitter depolarizes horizontal cells and hyperpolarizing bipolars but it has a hyperpolarizing action on the depolarizing bipolar.

165 SPECIFIC EFFECTS OF SYNAPTIC TRANSMITTER ANTAGONISTS ON GANGLION CELLS IN THE RABBIT RETINA. Harry J. Wyatt* and Nigel W. Daw (SPON: A.L. Pearlman). Physiology Department, Washington University, St. Louis, Missouri 63110.

Recordings were made from ganglion cells in the rabbit retina, and their receptive fields analyzed in detail while various antagonists of synaptic transmitters were infused into the internal maxillary artery, with other arteries not leading to the eyeball clamped off. The three most frequent classes of cell found in the rabbit retina are on-centre, off-centre and directionally sensitive. Other classes found less frequently are orientation sensitive cells, local edge detectors, uniformity detectors and large field cells. Picrotoxin had a specific effect on directionally sensitive cells: they lost their directional sensitivity and increased their spontaneous activity. Strychnine altered the spontaneous activity but not the receptive field characteristics. Haloperidol and hexamethonium had little effect. On the other hand, strychnine reduced the extent of lateral inhibition in local edge detectors but picrotoxin did not. Some effects were noted for the cells with center-surround receptive fields but the basic arrangement of their receptive fields was not altered by any of the drugs administered.

166 ARE ANATOMY AND PHARMACOLOGY COMPATIBLE IN THE ORGANIZATION OF Y-TYPE CAT RETINAL GANGLION CELL RECEPTIVE FIELDS? Albert W. Kirby. Ophthal. Dept., Kresge Eye Institute, Wayne State University, Detroit, Michigan, 48201.

X-, Y-, and W-cells can be readily differentiated through various means, and have recently been correlated with particular morphological types. Direct bipolar to ganglion cell contacts were previously thought to determine the properties of the receptive field center, while amacrine cells were most often thought responsible for the receptive field surround

Because amacrine interneurons have been reported between bipolars and ganglion cells, and gamma-aminobutyric acid (GABA) is reported in association with amacrines in several species, this study was undertaken to investigate the importance of GABA to receptive field organization of Y-type cells. Picrotoxin and bicuculline tremendously reduce or abolish the surround response of Y-cells, and as expected if rod bipolars communicate with the Y-cell through GABA-containing amacrines, the scotopic center response was also reduced, but never abolished. Using a simple on line determination of center size, eight Y-cells had their centers reduced an average of 60% following a GABA antagonist. Photopic center responses from the same cells behave quite differently; they are little affected.

This study implicates GABA amacrines in mediating the surround response and a portion of the center response of Y-cells. That a scotopic center response is reduced but not abolished and a photopic center response is little affected following GABA antagonism agrees well with a rod pathway reaching the ganglion cell through two types of amacrines (GABA and non-GABA) and a cone pathway reaching the ganglion cell either directly or through non-GABA intermediates. Preliminary evidence suggests that glycine amacrines are involved with X-cells much like the involvement of GABA amacrines with Y-cells.

167 SPATIOTEMPORAL CHARACTERISTICS OF CAT RETINAL RECEPTIVE FIELDS. John K. Stevens, James P. Barrett* and George L. Gerstein. School of Medicine, University of Pennsylvania, Phila., Penna. 19174

Spatial and temporal properties of retinal receptive fields were studied by flashing a small bar of light across the field in 28 discrete steps. The flashes at each of the spatial positions were used to produce 28 PST histograms. These histograms were in turn displayed as a plane, with space on the X axis, time on the Y axis and probability of firing on the Z axis.

Using these response planes it is possible to divide retinal receptive fields into four major categories. 1) Homogeneous ON: ON center receptive fields which have a spatially homogeneous arrangement of excitation and inhibition; 2) Homogeneous OFF: OFF center receptive fields which have a spatially homogeneous arrangement of excitation and inhibition; 3) Heterogeneous ON: ON center receptive fields which have a spatially heterogeneous arrangement of excitation and inhibition; and 4) Heterogeneous OFF: OFF center receptive fields which have a heterogeneous arrangement of excitation and inhibition. It is also demonstrated that our Homogeneous and Heterogeneous retinal fields correspond to Enroth-Cugell and Robson's "Y" and "X" fields respectively. The retinal receptive fields that we describe are remarkably similar to those exhibited by neurons of the lateral geniculate nucleus under identical stimulus conditions.

Supported by N. I. H. grant # NS 05606.

168 TEMPORAL PROPERTIES OF LATERAL INHIBITION OF X (SUSTAINED) AND Y (TRANSIENT) RETINAL GANGLION CELLS IN THE CAT. Ray Winters and Duco Hamasaki. Dept. of Psychology and Dept. of Ophthalmology. University of Miami, Coral Gables, Fla. 33124.

The temporal characteristics of surround inhibition of X and Y cells was assessed by varying the temporal relationship between a flashing spot in the receptive field center and a flashing annulus in the receptive field surround of on-center cells. The greatest amount of suppression of the excitatory response generated by the central spot was produced when the annulus preceded the spot by about 7 msec for X cells and by about 38 msec for Y cells. The time course of peripheral inhibition was analyzed quantitatively and found also to differ for these two types of cells.

169 QUANTITATIVE CHARACTERISTICS OF LATERAL INHIBITION IN TRANSIENT AND SUSTAINED GANGLION CELLS IN THE CAT RETINA. Jay G. Pollack* and Ray Winters* (Spon: Sidney W. Fox). Dept. of Psychology, U. of Miami, Coral Gables, Fla. 33134.

Recently, cat retinal ganglion cells of at least two types have been identified in several laboratories. These experiments examined the quantitative characteristics of the interaction between the center and surround regions of the receptive field (RF) of transient (Y) and sustained (X) optic tract fibers. The effects of three manipulations of lateral inhibitory processes on the response to a 500 msec., 0.5^{o} spot placed in the RF center were examined. In the first experiment, the adaptation level (AL), and hence lateral inhibition, was modified by changing the whole field illumination. Transient cells were shown to have lower spontaneous firing rates (no stimulus condition) than sustained over a wide range of ALs and both transient and sustained cells showed similar increment sensitivity functions for the early high frequency response component. The second and third experiments assessed the affects of lateral inhibition at one AL by simultaneous presentation of annuli which varied either in size or luminance. Analysis of the peak firing rates revealed minimal differences between transient and sustained cells. Transient cells as a group exhibited more off-excitation than sustained cells. Results were interpreted in terms of the time course of excitation and inhibition in the RF.

170 THE SIZES AND DISTRIBUTION OF GANGLION CELLS IN THE RETINA OF THE OWL MONKEY (AOTUS TRIVIRGATUS). Sarah Webb* and Jon H. Kaas. Dept. of Psychology, Vanderbilt Univ., Nashville, Tn. 37240.

The sizes and distribution of ganglion cells in the retina of the owl monkey were determined from whole-mount preparations stained with cresyl violet. Within a central area identified by converging blood vessels, ganglion cells were fairly homogeneous in size. Over the rest of the retina, ganglion cell size varied widely. In the central area, cell body area ranged from 22 to 160 μ^2, with a mean of 65 μ^2. Outside the central area, the mean cell size was approximately 100 μ^2, ranging from 24 to 480 μ^2. The periphery of the retina contained 3-25 ganglion cells/.01 mm^2. In contrast, in an area corresponding to about the central 40^o of visual angle, the ganglion cell density increased rapidly to a peak of over 100 cells/.01 mm^2. Isodensity contours of the distribution of ganglion cells were roughly concentric, with a slight horizontal elongation.

Electrophysiological studies have shown that the representation of the retina in primary visual cortex (V I) is distorted, with proportionally more tissue devoted to the central regions of the retina (Allman and Kaas, 71). When maps of ganglion cell density are compared with the representation of the retina in V I, it can be seen that the distribution of ganglion cells closely reflects the distortion of the representation in V I. In both the central and peripheral parts of the retina, a given number of ganglion cells relates to approximately the same amount of cortical tissue. (Supported by NSF grant GB-36779).

171 NONLINEAR-LINEAR TRANSITION IN ERG OF RANA PIPIENS COMPARED WITH ON/OFF - OFF SWITCHING IN RETINAL GANGLION CELL RESPONSE. F. S. Knox III, Dept. of Physiology, LSU School of Medicine in Shreveport, Shreveport, Louisiana 71130 and J. Levett*, Dept. of Biomedical Engineering, Rush Medical College, Chicago, Illinois 60612.

The electroretinogram (ERG) represents the integrated activity of many cells in the retinal network while the retinal ganglion cell response (single or multi-cell) represents the activity of specific output cells. Previously, Levett (Vision Res. Vol. 12, p.1301-1305) showed that the ERG can be thought of as generated by a linear-nonlinear-linear system. Further study (Levett, Proc. I.S.A. Conf. U. of R. Aug. 22-24, 1973, p.163) revealed that the last linear element is composed of two elements with different adaptational characteristics. Studies of the ON/OFF ganglion cell response (Knox, Soc. for Neuroscience Fourth Ann. Mtg. St. Louis 1974) showed that there is a switching from an ON/OFF to an OFF system as modulation frequencies are increased above 4-6 Hz, and that the ON and OFF information may be carried over two separate channels. These two studies prompted us to postulate that the two information channels are each active to varying degrees over the range of physiological stimulus conditions. We investigated this hypothesis by recording ERG and ganglion cell responses to stimulation with sinusoidally or square-wave intensity modulated light in curarized frogs. Several stimulus paridigms were employed. The results support a model in which the ON channel is active at low to middle ambient light levels and at low stimulus frequencies while the OFF channel is active at middle to high ambient light levels and at higher stimulus frequencies. Thus in the midrange the system is an ON/OFF system in which the ERG has a high level of second harmonic. The retinal model proposed to explain the results suggests that the locus of the channels and their inhibitory interaction is in the horizontal - bipolar - amacrine cell network.

172 GOLGI STAIN AND ELECTRON MICROSCOPY OF THE GROUND SQUIRREL RETINA

Roger W. West. Dept. Psychology, Memorial University of Newfoundland, St. John's, Newfoundland.

Electron microscopy of Golgi-impregnated ground squirrel retinas has revealed a relatively complete picture of the various neuron morphologies. An earlier report (West and Dowling, Science, 178, 1972) indicated at least 15 morphological types of ganglion cells. A careful comparison of levels of arborization with striations in the IPL has now confirmed the discreteness of these classes.

Subsequent work on the other cell types has revealed a similar diversity of morphologies. There are at least 7 different classes of bipolars. These follow a general rule that if their cell bodies are high in the INL their axon terminals are found low in the IPL and vice versa. In all cases the bipolars with high axons (3 types) make non-ribbon-related contacts with receptor bases, whereas, of the bipolar types with low axon terminals (4), three make ribbon-related receptor contacts while only one makes non-ribbon-related contacts.

There are at least 5 morphological types of amacrine cells. The two classes which Golgi stain reveals most frequently are a broad-field unistratified cell with a dendritic spread in excess of 1000 um and a narrow-field diffuse neuron with a dendritic spread of only about 50 um. The broad-field cell has the greatest proportion of bipolar input of all the amacrine types (57% bipolar vs. 43% amacrine). The narrow-field cell has one of the lowest bipolar inputs (4% bipolar vs. 96% amacrine).

There are at least two classes of horizontal cells. The most frequently stained type has an axon while the minority type probably does not.

173 SYNTHESIS, RELEASE, AND ELECTROPHYSIOLOGICAL EFFECTS OF ACETYLCHOLINE IN THE MAMMALIAN RETINA. Richard H. Masland, Adelbert Ames, III, and Carol J. Livingstone*. Massachusetts General Hospital, Boston, MA 02114.

Rabbit retinas were isolated from the pigment epithelium and spread out in a 1.5 ml heated chamber, under a flowing perfusate similar in electrolytes to cerebrospinal fluid. Ganglion cell activity was recorded extracellularly, and receptive fields were established by stimulation of the retina with stationary or moving disks or annuli. The receptive fields appeared identical to those seen in vivo: of 270 classified, 81 were ON-center, 114 were OFF-center, and 74 were directionally selective. Receptive field properties were stable throughout a 6 hr incubation. Two types of experiment were performed, using identical preparations. (1) The release of acetylcholine (ACh) into the perfusate during stimulation of the retina with light was measured. (2) The ganglion cells' electrophysiological responses to ACh and related agents was observed.

Release of ACh was evaluated by incubating the retina for 40 min in the presence of 5 μM 3H-choline (10.1 ci/mmole), after which labelled choline was removed from the perfusate and the retina left in the dark for 90 min. Twenty μM physostigmine was added to the perfusate to inhibit acetylcholinesterase, and radioactive ACh was identified by high voltage paper electrophoresis. Stimulation with light (50 ms flashes, 3 Hz, 1-3 log units above ganglion cell threshold) caused a threefold increase in the rate of appearance of labelled ACh in the perfusate. The light-induced release of ACh was entirely prevented by raising the concentration of Mg++ in the perfusate to 20 mM and lowering the concentration of Ca++ to 0.2 mM. Light-induced release and its Ca++ dependence could both be confirmed in the absence of physostigmine by measuring the rate of appearance of labelled choline in the perfusate.

When ACh (40-400 μM) was added to the perfusate, ganglion cells with ON-center or directionally selective receptive fields were strongly excited. More importantly, physostigmine at 1 μM enhanced the ganglion cells' light-evoked response and at 20 μM strongly increased the spontaneous activity as well. Cholinergic antagonists blocked both the response to exogenous ACh and the light-evoked response of these cells. To determine whether the cholinergic agents were acting directly on the ganglion cells, rather than on some neuron afferent to them, we also studied the ganglion cells of retinas incubated in a medium containing 20 mM Mg++ and 0.2 mM Ca++. At these concentrations of the divalent cations synaptic transmission is apparently blocked with little effect on the ganglion cells' ability to generate action potentials. Twenty-eight of 32 ON-center or directionally selective cells responded to 20 μM ACh under these conditions.

Ganglion cells with OFF-center receptive fields formed a more heterogeneous group. Although some responded to ACh, others were unaffected by 3 mM ACh, by 100 μM physostigmine (a level which should inactivate virtually 100% of the retina's cholinesterase), or by cholinergic antagonists. Only 2 of 20 OFF cells responded to ACh in the high Mg++, low Ca++ medium.

Because photoreceptors and horizontal cells hyperpolarize in response to light, increased release of ACh during stimulation implicates an inner plexiform layer synapse in the release; and the response of ganglion cells in the presence of 20 mM Mg++ and 0.2 mM Ca++ indicates that one such synapse is upon the ganglion cell itself. Our results thus suggest that for ON-center and directionally selective cells ACh is used at the amacrine-ganglion and/or bipolar-ganglion cell synapse.

Although this is probably true for some OFF cells as well, it seems that there is a population of OFF cells which uses ACh neither at the ganglion cell level nor in the distal retina.

174 STRATEGIES FOR CIRCUMVENTING AVASCULARITY AND HYPOXIA IN THE MAMMALIAN RETINA. Julia Chase* (SPON: A. Kling). Livingston College, Rutgers Univ.,New Brunswick, N.J. 08903.

Five characteristics of avascular retinae are described, namely: 1. thinness, 2. lack of taper, 3. lack of a tapetum, 4. heavy choroidal vascularization and 5. large glycogen-filled Muller fibers. All are considered functional adaptations to avascularity rather than indications of poor visual function per se. The role of the Megachiropteran choroidal papillae in circumventing the limitations of avascularity is discussed. D iving mammals, which have a low arterial pO_2 when under water and diseased retinae whose vascular supply is disrupted face problems analogous to those of the avascular retina and appear to adopt some of the same strategies to insure adequate retinal nutrition.

175 SEPARATION OF PHOTOPIGMENT FROM MEMBRANE ADAPTATION BY VITAMIN A DEPRIVATION IN THE DROSOPHILA RETINA. William S. Stark, Glenn S. Pransky* and William G. Zitzmann* Department of Psychology, The Johns Hopkins University, Baltimore, Md. 21218.

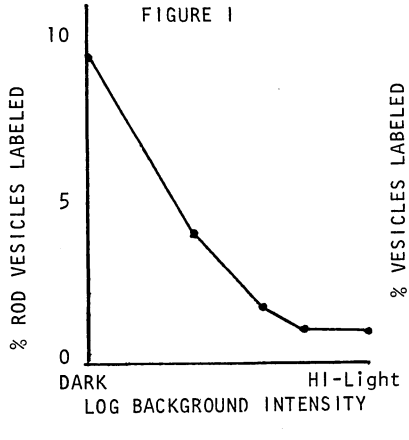
The predominant photoreceptor system in the Drosophila compound eye (R1-6) utilizes a 470 nm absorbing rhodopsin (R) which does not bleach but is reversibly photoconverted to a 570 nm absorbing metastable metarhodopsin (M). Flies do not rely on metabolic resynthesis of visual pigments but rather on photoreconversion of M to R to control sensitivity and adaptation levels. An intense 470 nm adapting stimulus causes a net photopigment conversion of R to M and causes a maximal long-lived receptor depolarization which eliminates further depolarizing transduction. Intense 570 nm light adaptation immediately repolarizes and reactivates these receptors (Stark, J. comp. Physiol. 96: 343, 1975; Minke, Wu and Pak, J. comp. Physiol. in press, 1975). First generation flies vitamin A deprived during larval development are about 2 log units less sensitive than flies raised on the same diet enriched with beta-carotene, indicating that photopigment levels are about .01 times normal levels. Moreover, vitamin A deprivation separates membrane - from photopigment-mediated adaptation mechanisms since it: 1) protects receptor membranes from 470 nm induced inactivation and the associated prolonged depolarizing afterpotential; and 2) replaces receptor inactivation with a diminished photoreversible 470 vs 570 nm adaptation of only up to 0.8 log units. A week of beta-carotene replacement in the adults' diet does not reverse these effects, showing that complete metabolic competence for rhodopsin synthesis has a larval critical period and that a non-bleaching photopigment may be synthesized only once in life. Assuming R to M conversion accounts for most of the 0.8 log unit adaptation in deprived retinas, maximal 470 nm adaptation decreases R to 16% (lower bound) of the 570 nm adapted level. No adaptation conditions would be expected to completely eliminate R since the overlap of R and M absorption spectra means that any maximal adapting stimulus would establish a steady-state combination of R and M. Elimination of receptor inactivation and prolonged depolarization can be explained by a model with the following assumptions: 1) R-M metabolism and membrane channels mediating depolarization are separate (probably linked by an internal transmitter); and 2) vitamin A deprivation decreases photopigment levels without changing the number of membrane channels. The model implies that 470 nm adaptation establishes similar R/M levels in both the deprived and enriched conditions, but that only in the enriched condition is the absolute R to M conversion sufficient to also cause total membrane inactivation. Constant proportion near maximal depolarizing afterpotentials in enriched retinas occur at the same intensity level which produces equivalent proportion photochemical adaptation in deprived retinas. This intensity would produce similar R/M ratios in both retinal types but would convert more R to M in vitamin A enriched retinas. In summary, decreasing photopigment levels by vitamin A deprivation decreases sensitivity and eliminates receptor depolarization blockade caused by substantial R to M conversion; the underlying photopigment adaptation is still present and can be used to: 1) estimate the proportional R-M conversions induced by adapting stimuli; and 2) demonstrate the experimental separability of membrane- and photochemical-mediated receptor adaptation.

Supported by NSF grant GB/MS74-12817 and NIH Biomedical Sciences Support Grant 3 S05 RR07041-0HS1.

176 HRP UPTAKE MEASURES SYNAPTIC ACTIVITY OF VERTEBRATE PHOTORECEPTORS. Samuel Schacher*, Eric Holtzman and Donald Hood*. Dept's of Biol. and Psychol., Columbia Univ., New York, 10027.

The uptake of horseradish peroxidase (HRP) into small vesicles at nerve terminals provides a quantitative method for evaluating synaptic activity by the terminals. We have used this approach to study the synaptic activity of rod and cone photoreceptors in isolated frog retinas under various illumination conditions. We previously reported (Nature, 249: 261, 1974) that "saturating" background intensities, which dramatically reduce rod responses to additional light increments, markedly reduce HRP uptake into rod synaptic vesicles. Our more recent findings include the following: (1) At intermediate light intensities, intermediate levels of HRP uptake into rod synapses are observed (Fig. 1). (2) Rod synapses recover their "dark" levels of uptake, during dark adaptation following exposure to light intensities that are saturating but bleach very little visual pigment. This recovery is not observed after high light intensities (HI-light) that bleach much of the pigment (Fig. 2). (3) The effect of increasing light intensities on HRP uptake into cone synaptic vesicles is not as dramatic as that observed for the rods. Retinas exposed to HI-light for sixty minutes decrease uptake of HRP into cone synapses only to approximately 50% of the "dark" level of uptake (Fig. 2). The cones recover their "dark" levels of uptake if dark adapted, even after this HI-light exposure (Fig. 2).

HRP labeled vesicles in the terminals seem to be randomly distributed. Multivesicular bodies containing HRP, become more numerous with time, and appear to undergo retrograde transport to the myoid region of the photoreceptors. This suggests that some of the vesicle membrane is being degraded. On the other hand, labeled vesicles also accumulate along the ribbon complexes, which suggests reuse of some of the vesicles for transmission.



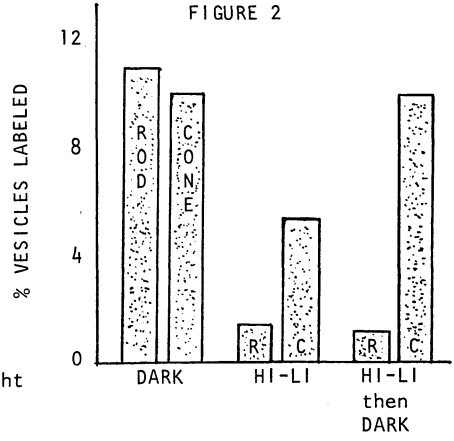


Fig. 1: Effect of light on HRP uptake into rod synapses; each point is average of 4 expts.; HRP exposures were 30-45 min.
Fig. 2: Comparison of rod and cone uptake during 60' exposures to HRP in dark, in HI-light and in dark following an initial 60' HI-light exposure.

177 ULTRASTRUCTURE OF CRAYFISH PHOTORECEPTOR MEMBRANES.
E.E. Nickel* and H.R. Fernandez* (SPON: R.B. Freeman) Univ. of Konstanz, 7750 Konstanz/Germany and Univ.of Southern Calif., Los Angeles, Calif. 90007.

The ultrastructure of photoreceptors of the dark adapted crayfish (Procambarus clarkii) has been examined by means of thin sections and freeze-etched preparations. The retinula of a crayfish compound eye consists of seven retinula cells which form a fused rhabdom. The rhabdom is composed of orthogonally oriented layers of microvilli. These microvilli are continuous with the plasma membrane and are the sites where the visual photopigments are located. The freeze-etch studies reveal particles associated with the cytoplasmic side of the microvillus membrane. These particles are randomly distributed. Occasionally they appear to be arranged in rows. The particles have a mean diameter of 85 Å. There are about 3900 particles per square μm of microvillus membrane surface. Treatment of the retina with 1 % digitonin - a substance capable of extracting visual photopigments - causes a breakdown of the microvilli into smaller segments and a gradual removal of the intramembranous particles. From microspectrophotometric measurements the estimated photopigment concentration of the microvillus is about 4000 molecules per square μm and agrees with the particle density. It is suggested that the particles observed in freeze-etched replicas represent the photopigment molecules in situ.

178 DOES LIGHT-ACTIVATED PHOSPHORYLATION CONTROL PHOTORECEPTOR RESPONSE?
Julie A. Miller*, Ann E. Brodie*, Reinhard Paulsen* and Deric Bownds. Lab. Molec. Biol. and Dept. Zoology, Univ. Wisconsin, Madison, 53706.

Light-activated phosphorylation of the opsin moiety of rhodopsin may be one link between rhodopsin photochemistry and control of the rod receptor potential. The reaction meets several criteria for participation in visual function. It has been observed in living frogs (Kühn, H., Nature 250, 588, 1974), in isolated retinas, and in rod outer segment suspensions. The reaction is sensitive to low levels of light, which bleach as little as 0.1% of the rhodopsin present. It is reversible in two different ways. After illumination, a gradual inactivation of the phosphorylation reaction occurs, and the incorporated phosphate groups dissociate from opsin.

An important characteristic of the reaction is an apparent amplification of the effect of light. After small amounts of illumination, we measure 10-40 X as many phosphate groups incorporated as rhodopsin molecules bleached. This result suggests a parallel with physiological studies which have shown that illumination of small amounts of rhodopsin has a large effect on the light sensitivity of rod outer segments.

To determine whether phosphorylation and sensitivity control might be linked, we have established conditions under which the light-activated phosphorylation can be assayed simultaneously with the permeability changes of the rod outer segment plasma membrane. By using inhibitors to selectively interfere with the light-dependent processes, we have observed an increased permeability response to low levels of light in the absence of phosphorylation. Therefore, the phosphorylation reaction may regulate the sensitivity of photoreception. [Supported by NIH grant EY 00463 and NIH Training Grant GM 01874.]

179 SIMILAR EFFECTS OF LIGHT AND CALCIUM ON THE VISUAL TRANSDUCTION PROCESS IN RAT RODS. Barry S. Winkler. Institute of Biological Sciences, Oakland University, Rochester, Michigan 48063.

Latency, defined as the period of time between photon capture and the generation of the photoreceptor potential, is a measure of the time course of the visual transduction process in rod cells. If, following photon capture, latency is primarily due to the kinetics of release and movement of calcium from an intracellular storage site and its binding to a site on the plasma membrane of the outer segment then changes in its intracellular concentration might be expected to alter latency, as do changes in the intensity of light. Effects of varying the extracellular concentration of calcium from 10^{-3}-10^{-8} M with Ca-EGTA buffers on the amplitudes and latencies of photovoltages to pulses of light from threshold to nearly saturating intensities were studied in isolated rat retinas. In comparison to behavior of dark-adapted rods in 2 mM calcium (control), reductions in the concentration of calcium to 10^{-6} M result in increases in the absolute amplitudes of the photovoltages without changes in latencies. However, when the concentration of calcium is lowered to 10^{-8} M not only were the amplitudes of the photovoltages decreased but there were significant increases in latencies. In these instances, the latencies could be shortened by either superimposing the test pulse of light upon a background light or adding back calcium. On the basis of this similarity it is suggested that calcium is involved in the light-induced process that leads to rod excitation.

180 RECEPTIVE FIELDS OF OMMATIDIA IN THE LIMULUS EYE. Ehud Kaplan* and Frederick A. Dodge. Rockefeller University, New York, 10021

Each photoreceptor unit shows an excitatory center, an inhibitory surround, and sometimes an additional disinhibitory surround. Taking advantage of the remarkable linearity of the response of this retina to incremental stimuli, we have measured the spatial parameters of these visual fields using a TV-like display where the intensity of simple bar patterns was modulated around the mean intensity of the background. The excitation center is fit by a Gaussian about 8^{o} wide at half heighth (range 4^{o}-10^{o}). In the horizontal plane the optic axes of adjacent rows of ommatidia diverge uniformly by about 6^{o} per row. The inhibitory field is typically flat and broad extending more than 45^{o} either side of the test ommatidium. In the vertical plane the divergence of optic axes is nonuniform such that the eye samples visual space below the horizontal with three times the density as above. Consequently, the inhibitory fields show a marked asymmetry that depends on the position of ommatidium in the eye. By fitting the observed fields to solutions of the time-dependent Hartline-Ratliff equations modified to take account of the distribution of optic axes, we can map the strength of inhibition converging onto the test ommatidium. We find this map is generally a simple Gaussian whose sigma is quite variable in the range of 4 to 10 ommatidial diameters. Neither the total amount of inhibition nor its space scale appear to be correlated with position of the unit. Disinhibition is predicted and observed for the shorter space scale.

181 PUTATIVE SYNAPTIC MECHANISMS OF LATERAL INHIBITION IN LIMULUS EYE. Alan R. Adolph and Berndt Ehinger*. Eye Res. Inst. of Retina Fdn., Boston, 02114 and Dept. Histology, Univ. of Lund, Sweden.

Lateral inhibition is mediated by putative serotonergic synapses in the neuropil of the lateral plexus. Serotonin(5-HT) applied directly to the eye produces a potent spike inhibition which mimics the action of natural lateral inhibition. Low concentrations(ca. 1 to 10uM) of the indoleamine cause hyperpolarization by several millivolts of eccentric and retinular cell resting potential, but no changes in receptor or generator potential responses to light. 5-HT also desensitizes the receptors to subsequent 5-HT applications and cross-desensitizes light-evoked synaptically-mediated, lateral inhibition. The dynamics of response recovery following 5-HT suggest that its inhibitory effect is bimodal: a direct action and a blockade of synaptic action. LSD and brom-LSD, which antagonize 5-HT effects in some nervous systems, directly inhibit spike responses in addition to blocking the action of applied 5-HT and light-evoked lateral inhibition in Limulus eye. Histochemical studies by the Falck-Hillarp technique show yellow, rapidly-fading fluorescence within eccentric cell bodies and neuropilar fibrils and varicosities. The fluorescence is strengthened by in-vivo injection or in-vitro incubation of the eye with 5,6-DHT or 6-HT, substances other than 5-HT that are strongly taken up by known 5-HT uptake mechanisms.

182 THE SYNAPTIC ORGANIZATION OF THE PRINCIPAL EYES OF JUMPING SPIDERS. M.D. Oberdorfer* (SPON: W.I. Welker). Anat. Dept., Univ. of Wisconsin, Madison, WI 53706.

The general organization of the first optic ganglion of the antero-medial (principal) eyes of Salticus scenicus and Habrocestum pulex has been described elsewhere (Oberdorfer, M.D., Anat. Record, 178:428, 1974). Pre-fixation in 1% formalin, 1% gluteraldehyde and 3% sucrose in a 0.1M phosphate buffer followed by postfixation in 1% osmium tetroxide (Fahrenbach, W.H., Z. Zellforsch., 93:451, 1969), provided the most consistent preservation of the synaptic ultrastructure within the ganglion. On the basis of morphological appearance, the neuropile of the first optic ganglion has been divided into three regions; the terminal zone, the fibrous zone, and the chiasm. All of the synapses occur within encapsulated zones in the terminal zone of the ganglion. Here the retinal terminals of the principal eyes contact second-order fibers, which are branches of unipolar cells whose cell bodies surround the ganglion, and form both dyad and triad synapses. Second-order fibers also synapse back onto the retinal terminals as well as forming serial synapses with each other. Golgi preparations reveal that the retinal terminals have very fine processes which extend away from the main portion of the terminal and these processes receive synapses from adjacent retinal terminals. All of the synapses are ribbon synapses with a mushroom-shaped presynaptic bar surrounded by synaptic vesicles. The retinal terminals contain spherical vesicles and the second-order terminals contain flattened or pleomorphic vesicles.

183 INFLUENCE OF CUTANEOUS INPUT ON THE PRETERMINAL POLARIZATION OF PRIMARY AFFERENTS AS MEASURED AT THE DORSAL ROOT AND INTRACELLULARLY. R.S.Drake* and E.L.Gasteiger, Dept. of Physical Biology, N.Y.S. Vet.Col., Cornell University, Ithaca, N.Y. 14853

Experiments in low spinal cats anaesthetized with urethane were performed in order to analyze the action of cutaneous and muscle volleys on the polarization of preterminal endings of muscle afferents. Control experiments in anaesthetic-free cords revealed little or no effect of urethane. By measurement of dorsal root potentials a survey was made of the distribution of primary afferent depolarization (PAD) and hyperpolarization (PAH) caused by these stimuli. These recordings provided evidence that both PAD and PAH can be evoked by stimuli of near threshold for group I fibers (3-10xT) to ipsilateral and contralateral muscle and cutaneous nerves. The contralateral responses were of longer latency and smaller amplitude.

Intracellular recordings obtained in the dorsal cord from 20 group I afferents of the gastrocnemius-soleus (GS) nerve (conduction velocities from 70-100 m/sec, $\bar{x}$ = 81.4 m/sec.) showed that cutaneous afferent volleys in the sural nerve produced predominantly PAH. The minimal latency for PAH was 11 msec and the average duration was 175 msec. When the stimulus intensity was increased to 10xT for group I, maximal effects were obtained with essentially no increase at 100xT. Maximal conditioning volleys (100x T) in posterior biceps semitendinosus nerve also had predominantly hyperpolarizing effects, but of smaller magnitude, on GS primary afferents in 10 of 14 fibers tested.

The response to cutaneous conditioning provides direct evidence for a hyperpolarizing effect on type I afferents, an effect essential to the view of Rudomin et al,1975, that PAH reduces monosynaptic reflex variability through decreased excitability fluctuation in IA preterminals.

184 PERCEPTION OF VARIABLE CUTANEOUS STIMULI IN SUBJECTS WITH CHRONIC PELVIC PAIN: THE "OUT OF SIGHT - INTO MIND" EFFECT. Karen L. Barnes and John P. Conomy. Cleveland V.A. Hospital., Cleveland Clinic Foundation, and Case Western Reserve University Med. Schl., Cleveland, Ohio, 44106.

The perception of quantitatively regulated cutaneous stimuli was recorded in a series of individuals with chronic pelvic pain of unknown cause. Subjects were drawn from a special gynecologic clinic dealing with refractory pelvic pain. Prolonged analyses had determined that either 1) a cause for complaints could not be demonstrated, or 2) the sustained level of complaint of pelvic pain was disproportionate to the degree of demonstrable underlying pathology. Testing employed methods previously described by the authors involving a subject-operated recording system which simultaneously quantifies electrical stimulus parameters and the subject's perceptual responses. The chart records yield a non-verbal, permanent record for computational analysis. Four skin sites were evaluated in each subject: non-preferred hand dorsum, lateral mid-calf, paramidline low abdomen, and center back. These sites were selected because they include skin areas both overlying and at distances from regions of pain complaints. At each site absolute perceptual threshold, threshold of cutaneous discomfort, and ability to match experimenter-presented stimuli were evaluated. Subjects with chronic pelvic pain without demonstrable organic cause differed from other patients and from controls by their performance when skin areas overlying their internal sources of discomfort were tested, or when tested in areas inaccessible to their vision. Their performance in non-painful, visually accessible areas was indistinguishable from that of normal individuals. These subjects show heightened sensitivity to stimuli in areas inaccessible to vision or overlying areas of prolonged somatic concern.

185 SHAPE-RECOGNITION RESPONSE LATENCY AND ACCURACY IN A DICHOTOMOUS TACTILE TASK. Elizabeth B. Gardner, J. Kevin McCormick*, Mildred E. Brunow*, Barbara Bunk*, Brian M. Flannery* and Anne C. Graham*. Dept. of Psychology, Fairfield University, Fairfield, Connecticut 06430.

Right-handed, ambidextrous and left-handed subjects simultaneously felt two unfamiliar shapes, one with each hand. Subjects were then asked to visually recognize one of the shapes, choosing it from an array of pictured shapes. There were three response modes: point with right hand, point with left hand, or say the number of the chosen shape. Response latency was recorded. Each subject's score for each of the six conditions (two feeling hands, three response modes) was averaged over 12 trials.

Subjects in all three handedness groups were more accurate at recognizing shapes felt with the left hand, indicating a right-hemisphere superiority in shape recognition. In left hand or right hand response trials, recognition accuracy was greater when information input and output were confined to the same hemisphere than when information was required to cross interhemispherically. In addition, response times were 300+ ms longer for trials which involved interhemispheric transfer of information than for intrahemispheric trials. This finding is taken to support the suggestion that callosal transmission time increases with the complexity of the discrimination.

186 THE FINE STRUCTURE OF THE DORSAL HORN OF THE MONKEY SPINAL CORD Henry J. Ralston III. Department of Anatomy, University of California, San Francisco, California 94143

The organization of laminae I-IV (Rexed) of the lumbar cord of the macaque monkey has been examined by light and electron microscopy. Lamina I has in addition to synaptic profiles containing clear vesicles, a great many synapses with large numbers of 1200 Å dense-cored vesicles. These latter profiles primarily form axodendritic synapses. Laminae II and III have numerous long axons which make multiple synaptic contacts with dendrites and other axons. These axons run primarily parallel to the long axis of the spinal cord. Lamina IV demonstrates a more conventional array of axodendritic, axosomatic and axoaxonal contacts.

Several monkeys have had unilateral dorsal root lesions with contralateral injections of tritiated amino acids into the dorsal root ganglia. These two techniques have been used to identify dorsal root terminals in the dorsal horn by either their appearance during degeneration or by their radioactivity, utilizing electron microscopic autoradiography. The particular synaptic profiles of dorsal root origin and their quantitative distribution in the dorsal horn will be described. (Supported by grant NS-11614 from NIH.)

187 INPUT-OUTPUT RELATIONS AT THE CUNEATE NUCLEUS. K. Krnjević and M.E. Morris. Dept. Anaesthesia Research, McGill Univ., Montreal, Canada.

When afferent fibres and terminals in the cuneate of the cat are directly stimulated, by single shocks applied through a microelectrode, the integrated monophasic antidromic and orthodromic responses recorded from peripheral nerves (in the forelimb) and the medial lemniscus respectively provide estimates of the neural input and output. The relation between these variables is consistently quite non-linear, climbing very steeply for very small values of input, but tending towards a linear slope with large inputs. It can be fitted by a power function with an exponent < 1 that varies somewhat with the position of the stimulating microelectrode and in different preparations. The mean value of the exponent observed in a number of penetrations in the same animal, or in a series of 14 different cats (in most cases decerebrate, unanaesthetized, and breathing spontaneously) did not differ significantly from 0.50. Such a square root relationship - which is very similar to the stimulus-response relation of some cutaneous mechanoreceptor fibres - indicates that transmission through the cuneate nucleus has a gain that is inversely proportional to the output. This not only ensures a very high sensitivity to small signals, but also allows the system to operate over a wide range of inputs without saturating. Although the input-output relation can be significantly altered by a number of experimental manipulations (changes in temperature, afferent conditioning, anaesthesia, etc.), its general character is preserved as long as there is no gross interference with the blood supply.

188 HYPALGESIA INDUCED IN THE HUMAN DENTAL PULP BY ELECTRICAL STIMULATION OF NEARBY ORAL MUCOSA. Richard B. Tacke, R. Wayne Fields, P.C. Sakellaris, and Bhim S. Savara, School of Dentistry, University of Oregon Health Sciences Center, Portland, Oregon 97201.

Several laboratories have demonstrated that local analgesia or hypalgesia can be induced by the electrical stimulation of cutaneous receptive fields or peripheral nerves. The central objective of the present experiments was to test whether the electrical stimulation of a distributed area of oral mucosa (Electroanalgesia or EA stimulation) could produce detectable attenuation of experimentally induced pain in the pulps of nearby teeth. Human subjects were used to provide a direct measure of perceptual effects (verbal report), and Signal Detection Theory formats of data collection and analysis were employed for optimal interpretive power. The tooth pulp was chosen as a model of oro-facial pain because the perceptual consequences of its stimulation by any means is painful (except very near threshold). Three distinct experimental series involving various EA presentation strategies were successively conducted as the forthcoming data dictated protocol modification. It became increasingly apparent that prolonged EA stimulation was necessary to permit induction of hypalgesia. The final experimental series involved successive EA stimulation episodes of several minutes duration, during which repeated perceptual assessments were conducted. The results indicated progressive increase in the magnitude of hypalgesia (probability of reporting a lower perceptual category than expected) with each successive stimulus episode which was highly significant statistically. Remarkably, EA was most effective at an intensity which was just subthreshold, thereby ruling out distraction and placebo effects. To our knowledge, this is the first report of significantly effective electrically-induced hypalgesia using subthreshold stimulus intensities.

189 SOMATOSENSORY FUNCTION AND CORTICAL UNIT ACTIVITY IN CATS WITH ONLY DORSAL COLUMN FIBERS. G. P. Frommer, B. R. Trefz and K. L. Casey. Dept. Physiol., Univ. Michigan, Ann Arbor, 48104, and Dept. Psychol., Indiana U., Bloomington, 47401.

Wall (Brain, 93, 505, 1970) reported that rats failed to orient to somatic stimuli below cord lesions isolating the dorsal columns (DC). We tested the capacity of cats with similar thoracic cord lesions to discriminate the side on which they were touched. Discriminant stimuli included touch (4 gm. spring), vibration, and various diameter discs. Hungry cats placed their heads through a panel opening and were trained to turn to the positive side for liquid food. Performance was at chance on trials where tactile stimuli were not delivered. All cats, found by anatomical and/or electrophysiological study of the cord to have any functional continuity restricted to the DC, learned or relearned to discriminate stimuli delivered below the level of the cord lesion. This occurred despite only partial DC sparing in every case. Cats with complete cord transections failed to discriminate. Lesioned cats lacked orienting responses to any unrewarded stimuli below lesion level and showed paroxysmal licking and biting of the body below the lesion. A total of 530 units were recorded from hindlimb cortex in these and similarly prepared cats under light pentobarbital anesthesia. Pre-lesion, 74% of the units were driven by hindlimb stimulation. Post-lesion, only 34 of 138 units (24.6%) were driven in 5 cats with intact tactile discrimination; none of 18 units were driven in 2 cats lacking discriminative performance. Of 67 driven units in cats with spinal lesions, 15 (22.3%) were excited by forepaw stimuli; no forepaw units were found in intact cats. The results show that a fractional, isolated DC input profoundly alters cortical function but is sufficient to support a somatosensory discrimination. Supported by NIMH Grant MH24951.

190 APPARENT LACK OF A CORNEAL REPRESENTATION IN THE RAT'S $S_{M}I$ CORTEX. Robert D. Hall and James A. Doubler*, Research Laboratory of Electronics, M.I.T., Cambridge, Mass. 02139.

Single-unit and evoked-potential techniques were used to search for a representation of the cornea in $S_{m}I$. Almost 500 penetrations, most of them in an area of a few square millimeters that included representations of the eyelids and adjacent parts of the face, revealed no cortical cells that responded to tactile stimulation of the cornea. In penetrations immediately adjacent to sites where eyelid units were isolated, units were found whose receptive fields included the skin or hair adjacent to the lids, consistent with the general topographical organization of $S_{m}I$, but never the cornea. This discontinuity in the representation of the body surface was apparent also in some receptive fields that completely surrounded the eye but did not include it.

Electrical stimulation through bipolar electrodes on the surface of the cornea evoked a cortical potential of relatively long latency. It was largest in or near the eyelid area, and several lines of evidence suggested that it reflected stimulus spread to the eyelids or other tissues. It was not always possible, however, to abolish the evoked potential by denervating the eyelids and other orbital tissues. Consequently, the peripheral origin of this response was not known for certain in every preparation.

Corneal afferents in the rat terminate in the descending and principal sensory nuclei of the trigeminal complex, and there is no suggestion in this projection that a corneal representation in $S_{m}I$ should be lacking. That it is, however, seems consistent with the specialized nature of the cornea and its innervation and with the preoccupation of the rat's $S_{m}I$ cortex with inputs from hair receptors.

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191 THE PERIPHERAL FIBER POPULATION MEDIATING THE THERMAL EVOKED RESPONSE TO SKIN COOLING IN MAN. Allen B. Chatt* and Dan R. Kenshalo. Dept. Psychology, Florida State University, Tallahassee, Florida 32306.

Thermal evoked responses have been recorded from the scalp specific to cutaneous cooling of the lip and hand (Fruhstorfer, et. al., 3rd International Congress Event Related Slow Potentials of the Brain, Aug. 1972) and hand and forearm of man (Duclaux, et. al., Brain Research, 78, 1974). In the latter study, these evoked responses to skin cooling were shown to be primary in nature, since they comprised the shortest latency, greatest amplitude response at scalp derivations consistent with the hand and arm projection areas of the contralateral post-central gyrus. Smaller and later occurring responses were recorded from the ipsilateral surface while there was no time-locked response specific to skin cooling over occipital sites. The peripheral receptor origin of this cool evoked response may have been specific cold receptors, SA1 mechanoreceptors, or both. The dynamic sensitivity of these two fiber populations to cool stimuli differ widely over adapting temperatures (AT) with cold fibers demonstrating a dynamic sensitivity maximum at 29°C AT decreasing at both higher and lower adapting temperatures while the phasic activity of SA1 mechanoreceptive fibers peak at 40°C AT showing decreasing activity as the adapting temperature is lowered (Duclaux and Kenshalo, J. Physiol., 224, 1972). By utilizing this differential dynamic sensitivity between the two fiber populations, we have been able to clarify the nature of the fiber population responsible for the cool evoked response in humans.

Cool evoked responses were recorded to a 10°C cooling pulse of 19°C/sec onset applied to the thenar eminence of the right hand when presented from a 29°C AT. The response demonstrating the shortest latency (160-210 msec.), highest amplitude (9-17μv) surface positive was recorded from the contralateral scalp site approximating the hand projection area of the post-central gyrus. The response was highly reproducible from session to session and subject to subject. A less reliable response, smaller in amplitude and of slightly longer latency (generally 5-10 msec.) was recorded from the ipsilateral scalp site. Negative results from the stimulus control sessions reaffirmed that the response was due to skin temperature change and not to some artifact of stimulation. No reproducible time-locked responses were recordable from 40°C AT.

It would appear then that the thermal evoked response to peripheral skin cooling in man is mediated by specifically sensitive cold fibers which are known to have conduction velocities in the low Aδ range. Their dynamic sensitivity maximum occurs at a 29°C AT where cool evoked responses are readily recorded from human scalp. In support of this, small Aδ primary afferents have been shown to have access to the cortex in the cat through the spinocervical pathway (Handwerker and Zimmerman, Brain Research, 36, 1972). Also, some cortical cells specifically sensitive to peripheral skin cooling have been found in cortex of squirrel monkeys (Kreisman and Zimmerman, Brain Research, 55, 1973). Scalp responses to cooling from 40°C AT were not demonstrable to any reliable degree. This result would appear to exclude SA1 mechanoreceptive fibers as contributors to the cool evoked scalp response. (Supported by PHS grant NB-02992.)

192 BILATERAL TOOTH PULP REPRESENTATION IN THE SPINAL TRIGEMINAL COMPLEX. Samuel G. Nord, Ph.D. Dept. of Neurol. Upstate Med. Ctr. Syracuse 13210

We reported previously that the canine tooth pulp of the cat projects ipsilaterally to the ventromedial trigeminal nucleus caudalis where cells receiving afferents from other, homolateral, pain-related, trigeminal receptors are situated. Subsequently, other investigators demonstrated that the trigeminal nerve distal to its ganglion also contains a small population of afferents which originate in the contralateral canine pulp. The present experiments were designed to localize bulbar neurons innervated by the contralateral canine afferents, to determine their response characteristics and to compare these findings with data obtained from neurons which receive ipsilateral canine projections. The medullae of lightly anesthetized, immobilized cats were explored with microelectrodes for cells responsive to electrical stimulation of each of the maxillary canine teeth. Single pulses were delivered directly to the pulps through embedded, concentric, bipolar electrodes. Sixth-three of 88 pulpal neurons studied were exclusively responsive to ipsilateral and four to contralateral stimuli. The remaining 21 cells could be fired by stimulation of either tooth. However, thresholds were always higher for contralateral stimulation, minimum response latencies were longer and spike densities were lower. Inhibition of the response to stimulation of either canine typically occurred if a preceding, conditioning stimulus was applied to the other canine at various inter-stimulus intervals. In contrast, simultaneous stimulation of the teeth ordinarily resulted in response enhancement above individual tooth levels or in evoked spikes which could not be produced by stimulation of either tooth alone. Post-experimental, histological study of appropriate brain sections revealed that responsive cells were intermingled in ventromedial trigeminal zones without regard to effective site of stimulation. Supported by NIH Grant NS10814.

193 TEMPORAL EFFECTS ON MAGNITUDE SENSATIONS PRODUCED BY SKIN INDENTATION IN HUMANS. Kenneth W. Horch, Paul R. Burgess and Dennis A. Poulos. Dept. Physiol., Col. Med., Univ. of Utah, SLC, 84132.

Subjects lose awareness of maintained, static indentations of the skin of their forearm with a time course that is dependent on indentation depth, but largely independent of stimulus area. If the stimulator is slowly withdrawn during the period when a sensation of indentation is present, an "after sensation" persists for some time after the stimulator has left the skin. If the stimulator is rapidly withdrawn, such after sensations are not felt. If the skin is indented at rates below 0.05 mm/sec, large (2 mm) indentations of the skin can be made without the subjects being aware that an indentation is present. Although the velocity of indentation affects the apparent magnitude of an indentation, subjects generally do not feel small, rapid indentations as being greater than larger, slower ones. Simply decoding the impulse frequency of cutaneous mechanoreceptors would produce apparent reversals of this type. Thus the CNS must integrate velocity information in order to decode skin position. This integration process may be involved in the after sensations which persist when a stimulus is slowly withdrawn. Supported by grants NSF GB42643 and NIH NS08769.

194 PROPERTIES OF CUTANEOUS TACTILE UNITS OF THE BULLFROG (R.catesbeiana)

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Electrical and natural stimulation of cutaneous receptors evoke an excitatory effect on frog dorsal root ganglion (DRG) cells. Extracellular microelectrode recordings were obtained from the ninth DRG of decerebrate bullfrogs. Frog skin touch receptors were shown to be discrete structures which appear as dome-shaped translucent elevations of the epidermis. These domes are particularly responsive to direct touch with vonFrey hairs having measured binding forces of 50-100 mg. Tactile stimulation of adjacent skin never caused impulse activity, except when skin distortion disturbed the domes. Threshold stimulation of all units studied, whose receptive field was on the plantar surface and back of the ipsilateral hind leg, evoked a response that always consisted of a single impulse upon application of mechanical or electrical stimulation. A few units also discharged one impulse upon mechanical stimulus release (on-off response). No after discharged was seen at stimulus strengths of three or four times threshold, nor after repetitive stimulation up to 1000 pps. The range of distribution of active domes/receptive field was 1-14. Generally the most sensitive domes appeared to be in or near Center field. Twice as many of the tactile units responded more readily to cold than to warmth. The impulse frequency of units responding to thermal stimulation ranged from 3-11/sec. The conduction velocity of units measured was within a range of 9-25 m/sec. with a mean of 12 m/sec. The results suggest the following: 1) the existence of discrete structures which respond to tactile stimuli. 2) cold/touch receptors which appear to be present in greater quantity than warm/touch receptors. 3) touch units which should be considered primarily as mechanoreceptors with a possible secondary function as thermoreceptors. (This work was supported by NIGMS Training Grant 1 TO 2 GM 05010-01 MARC).

195 INPUTS FROM THE BRAINSTEM ONTO SPINOTHALAMIC AND SPINOBULBAR NEURONS OF THE LUMBO-SACRAL CORD OF THE CAT.

James R. Bloedel and Douglas B. McCreery.* Depts. Neurosurg., and Physiol., Sch. Med., U. of Minn., Mpls., Minn., 55455.

In cats anesthetized with alpha-chloralose, responses of spinothalamic and spinobulbar neurons to natural stimulation of the hindlimbs was investigated. It was found that electrical stimulation of the lower brainstem could inhibit the responses of these cells to mechanical pressure intense enough to be considered noxious. The latency of the onset of the inhibition indicates that it is mediated via a pathway having a conduction velocity of at least 25 m/sec. It was found that electrical stimulation of the face and pinna could evoke a similar response inhibition, but at a latency about 10 msec longer than that produced by the direct brainstem stimulation.

A possible neuronal substrate mediating these interactions between the segmental and trigeminal sensory systems was also investigated. This is a population of neurons activated antidromically from the spinal cord with cell bodies in the pontine and medullary reticular formation. These neurons are strongly excited by electrical stimulation of the face and pinna and also by natural stimuli (a light tap was most effective). The conduction velocity of their axons, latency of their response to pinna and face stimulation, and location of the cell bodies all suggest that these neurons may be part of the pathway mediating the trigemino-segmental interactions described above.

196 COLUMN-LIKE ORGANIZATION OF COMMISSURAL AND CORTICO-CORTICAL FIBERS IN THE SOMATIC SENSORY CORTEX OF PRIMATES. E. G. Jones and H. Burton. Departments of Anatomy and Neurobiology and Physiology and Biophysics, Washington University Medical School, St. Louis, Missouri, 63110.

In an anatomical study of the first somatic sensory (SI) and certain related cortical areas in rhesus and squirrel monkeys, commissural and ipsilateral cortico-cortical fibers have been found to terminate in discrete vertical groupings. The cells of origin of the commissural and to a lesser extent those of the cortico-cortical fibers are arranged in clusters.

After ablation of SI on one side, axon degeneration methods "label" the full complement of commissural fibers. Degenerating commissural fibers are found only in the contralateral face, trunk, tail and proximal limb representations, but within parts of these regions the degeneration is discontinuous. Bundles of degenerating axons some 500 μ wide are aligned in register in layers V and VI so as to form a band along the posterior border of SI but, particularly in the face area and in the second somatic area (SII), the bundles are separated by gaps of 300-500 μ. Each bundle of axons gives rise to a separate focus of terminal degeneration 500-1000 μ wide in layers I through IV.

The terminal distribution of these axons is best seen in autoradiographic experiments in which commissural fibers are labeled by axoplasmically transported ^{3}H amino acids injected into the opposite SI. Each commissural bundle forms a band of terminals c. 500 μ wide in layer IV and in layer II and the deep half of layer I, with a narrower intervening "stalk" in layer III. No terminals are found in layers V and VI.

Experiments involving retrograde labeling of commissurally projecting cells with horseradish peroxidase (HRP), show that despite large injections that coalesce so as to label virtually all of SI, commissural cells of the opposite SI are labeled in groups with intervening zones of non-labeled cells. All commissurally projecting cells are large pyramidal cells situated only in layer IIIb. The precise topographic ordering would suggest that commissural fibers arise from and terminate on exactly homotopically situated pyramidal cells and the laminar pattern of termination would suggest that they end mainly in relation to the apical and basal dendritic sprays of these cells.

Cortico-cortical axons arising in area 3 of SI and terminating in areas 1, 2, 5 and SII are also organized in precise vertical arrays. Small injections of isotope in area 3 cause labeling of cortico-cortical terminals in separate foci in the other areas. Each focus is consistently about 500-1000 μ wide and the grains form a vertical band extending from layer IV up to the deep half of layer I. Correlative HRP experiments show that cortico-cortical fibers arise from pyramidal cells particularly in layers III and V, with fewer from layer II and virtually none from layers IV and VI. Commonly, single injections of HRP in SI lead to retrograde labeling of discontinuous groups of pyramidal cells in SII and in area 4.

The vertical bundles of commissural and cortico-cortical axons demonstrated here are topographic in nature and cannot necessarily be considered functional units in the sense of the electrophysiological "column" but it is felt that their organization reflects a basic vertical patterning upon which such columns must depend.

197 ALTERATIONS IN THALAMOCORTICAL PROJECTIONS TO THE RAT SOMATOSENSORY CORTEX FOLLOWING NEONATAL VIBRISSAE REMOVAL. H.P. Killackey, G. Belford*, R. Ryugo* and D.K. Ryugo*. Dept. of Psychobiology, Univ. of Calif., Irvine, 92664.

The normal development of the "barrels" which compose the posteromedial barrel subfield (PMBSF) of the mouse somatosensory cortex has been shown to be dependent on the integrity of the mystacial vibrissae in the newborn (van der Loos and Woolsey, Science, 1973). In the present experiments we have investigated the effect of neonatal vibrissae removal on the pattern of thalamocortical projections to the PMBSF in the adult rat. A row of vibrissae was removed on the day of birth. As adults, some animals received lesions of the ventral posterior nucleus, and their brains were processed with the Fink-Heimer technique for staining anterograde degeneration. The brains of other animals were stained for succinic dehyrogenase (SDH). In sections of normal brains cut tangential to the surface of the PMBSF and stained with either SDH or the Fink-Heimer technique the thalamocortical terminals in layer IV are arranged in rows of discrete clusters which replicate the arrangement of the mystacial vibrissae (see A). In experimental animals with a row of vibrissae removed at birth the corresponding row of thalamocortical projections is no longer organized in discrete clusters but forms instead a uniform band (see B).

The results of this study suggest that early peripheral damage can influence the formation of thalamocortical connections. These results, in conjunction with those of van der Loos and Woolsey, suggest that thalamocortical projections play an important role in determining the organization of cells in the neocortex.

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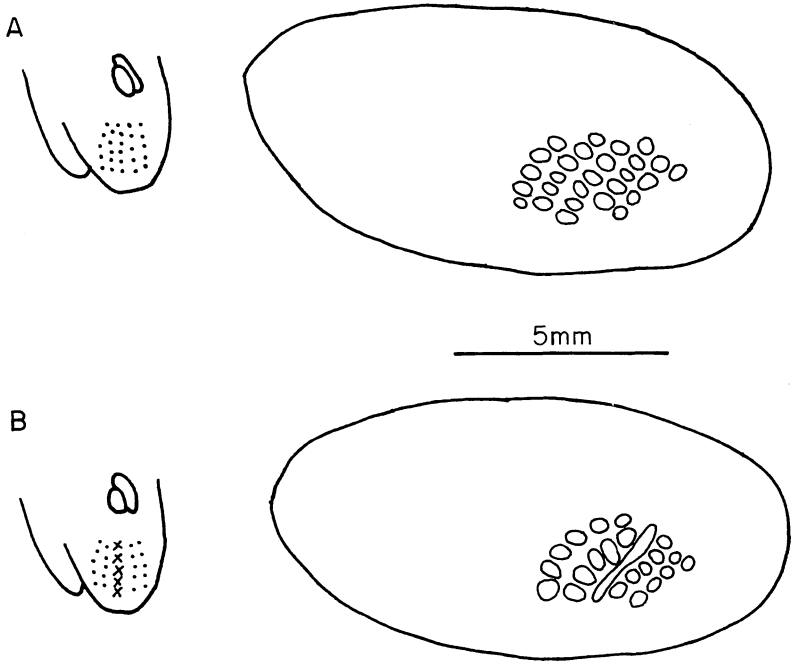

Figure 1. Projection drawings of single tangential sections through the PMBSF of a normal (A) and experimental rat (B).

198 CORTICOCORTICAL CONNECTIONS OF THE BARREL FIELD OF RAT SOMATOSENSORY CORTEX. R. Ryugo* and H.P. Killackey (SPON: R.A. Giolli), Dept. of Psychobiology, University of California, Irvine, 92664.

Portions of the rat somatosensory cortex representing the mystacial vibrissae and sinus hairs are composed of discrete aggregates of cells within layer IV which have been termed "barrels" (Welker and Woolsey, 1974). We have examined the corticocortical connections of this barrel field utilizing the Fink-Heimer technique. Three types of lesions were made in adult agouti rats: 1) complete sections of the corpus callosum; 2) lesions involving the supragranular layers of the barrel field cortex; and 3) lesions extending into the infragranular cortical layers. Following section of the corpus callosum, the barrel field is essentially free of degeneration, but is bordered by narrow zones of dense terminal degeneration. After either deep or superficial cortical lesions within the barrel field, degenerating fibers can be traced ipsilaterally to these same narrow zones of cortex, where degenerating terminals are localized primarily in the superficial layers (I-III). In addition, degenerating fibers can be traced to ipsilateral terminal fields in the superficial layers of motor cortex (as defined by Hall and Lindholm, 1974) and the second somatosensory area (as defined by Welker and Sinha, 1972). Finally, following lesions restricted to the supragranular layers, fascicles of vertically oriented degenerating fibers can be traced through layer IV to a uniform terminal field in layer V. This finding is of particular interest in light of the discrete nature of the thalamic projections to the superjacent layer IV (Killackey, 1973). This suggests that the discreteness of the thalamocortical input to the barrel field cortex is not maintained in the organization of the intracortical circuitry.

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199 THALAMOCORTICAL PROJECTIONS AND BODY REPRESENTATION IN THE VENTROPOSTERIOR NUCLEUS OF THE MACAQUE THALAMUS. P. R. Loe, D. A. Dreyer, C. B. Metz*, A. Rustioni, and B. L. Whitsel. Depts. Anat. and Physiol., Sch. Med., U. of N. C., Chapel Hill, N. C. 27514

The ventroposterior nucleus (VP) can be viewed as consisting of a series of cellular lamellae. Neurons within a given lamella have similar receptive fields, and the receptive fields of a medial-to-lateral sequence of lamellae shift continuously to describe an orderly sequence along the body.

In order to determine the relation between the body representation in VP and that in the primary somatosensory cortex (S-I) we employed a combination of electrophysiological and histochemical techniques. Extracellular microelectrode recording was used to identify the receptive fields and submodality properties of the neurons within a localized region of S-I. Horseradish peroxidase (0.05 to 0.2 µl. of 50% solution) was then injected into the region of S-I whose characteristics had been determined. Subsequent examination of VP sections stained for peroxidase activity gave an indication of the distribution of VP cells projecting to cortical neurons with identified receptive fields and submodality characteristics. The results suggest a topographical relation between given restricted S-I areas and vertical bands of cells within single VP lamellae.

These data validate a scheme of thalamocortical projections which was suggested by single unit mapping studies of VP described previously. (Supported in part by NS11737, NS12440, and DE00011 from the National Institutes of Health.)

200 THE ULTRASTRUCTURE OF PHYSIOLOGICALLY STUDIED CORTICAL CELLS. B. N. Christensen and F. F. Ebner. Brown Univ., Prov., R. I. 02912.

The objective of these studies is to correlate the synaptic connections of cortical neurons with the physiological responses elicited by peripheral or thalamic stimulation. The hand area of somatic sensory-motor (SSM) cortex in the opossum is being studied because each locus in this area receives dual sensory and motor thalamic inputs, and gives rise to pyramidal tract fibers. Opossums were anesthetized with Fluothane for surgical procedures, were given long acting local anesthetic, and maintained for recording under a mixture of N2O and O2. The bone over SSM cortex was removed, the dura was reflected to expose the hand area, and the brain was covered with agar. Bevelled glass micropipettes with tip diameters of less than 0.5um and + 50 Mohm impedence when filled with 4% procion brown in water were introduced into hand area cortex. Cortical cells were located by a shift in resting membrane potential (RMP), characterized by their spontaneous electrical activity and response to peripheral or thalamic stimulation, and then injected intracellularly with procion brown. After 10-20 cell injections the animal was perfused for electron microscopy (EM). Blocks of cortex containing individual injection sites were embedded in plastic. The entire blocks were cut at 5um and mounted on glass slides. Sections containing the procion filled cell body and processes were removed from the glass slide, glued to the face of a plastic block, and sectioned for EM. Both the overall dendritic pattern and the details of synaptic contacts could be derived from serial sections through the injected cell. The physiological responses of cortical cells revealed two clearly different types. One type of cell shows a low RMP, typically around 30 mV. Action potentials and other signs of synaptic activity are seen when the electrode enters the cell. Action potentials can be generated in these cells by passing depolarizing current and inhibition by reversing the current flow. Electrical stimulation of the contralateral forepaw frequently produces responses in these cells with a latency range of 12-20 msec. Examination of these cells with the EM shows that they are neurons with numerous synaptic contacts on their dendrites and some on their soma. One unusual morphological feature of several injected neurons is that nearly all of the presynaptic terminals are found invaginated into the postsynaptic cell membrane. Frequently the membrane differentiation is still intact and the synaptic vesicles appear normal when the terminal is completely engulfed by injected cell cytoplasm. Another surprising feature of these cells is the advanced stage of phagocytic reaction to the injected neurons after a survival period of less than 10 hours from injection to perfusion. In some cases phagocytic cells are already deeply invaginating the surface of the injected neuron. A second type of frequently encountered cortical cell is first identified by its high RMP, typically around 60 mV. These cells are electrically silent. They show no spontaneous or current induced action potentials or PSP's, and never show any response to contralateral forepaw stimulation. Several of these presumed glial cells were located in layer II. Light microscopic examination of their dendritic morphology suggests that some of these cells are small neurons with sparse apical and basal processes rather than glial cells. Examination of a large number of sections through these cells with the EM showed only a few synaptic contacts on these cells. The few presynaptic elements located contained small round plus flattened vesicles and formed symmetrical membrane differentiations on the cell processes. Several interpretations of these findings are possible and will be discussed. (Supported by PHS grant #NS-06551).

201 THALAMIC AND TRANSCALLOSAL INPUTS TO SIII IN CAT. M.H. Feldman, A.M. Doyle*, and D.P. Purpura. Depts. Neuroscience and Neurol., Albert Einstein College of Medicine, New York, N.Y. 10461

The organization of SIII (ant. suprasylvian gyrus) elements responsive to ipsilateral ventrobasal (VB) thalamic and contralateral SIII transcallosal stimulation (TC) was studied in encephale isole cats. Extracellularly recorded units are more responsive to VB than TC stimulation. Approximately 15% of units respond to both inputs whereas only 4/75 units are activated by TC alone. Mean latency of TC-responsive units is longer than VB-activated elements. Patterns of unit discharges were variable but were shared by both modes of stimulation. Intracellular recordings confirm minimal convergence of VB and TC inputs to SIII neuron. In general TC stimulation elicited graded prolonged EPSPs (latency 5-25 msec) with latency and effectiveness dependent upon stimulus intensity. In some cells short latency (6-8 msec latency) EPSP-IPSP sequences were observed. IPSPs in isolation were not noted. VB stimulation elicited similar graded EPSPs with one or more spikes dependent upon stimulus intensity. EPSP-IPSP sequences were also common in VB-activated SIII elements. Short latency IPSPs effectively reduced spontaneous discharge frequency. TC-VB inhibitory interactions were noted frequently involving elements presynaptic to the neurons examined. IPSPs were additive following TC and VB stimulation. The data indicate that a small proportion of SIII elements share similar response properties to VB and TC inputs. The majority of recorded neurons in SIII are more intimately related to specific thalamocortical than transcallosal afferent projection systems.

202 EFFECTS OF TOPICAL BICUCULLINE ON EVOKED POTENTIALS AND SINGLE NEURONS IN ANTERIOR CEREBRAL CORTEX OF DOMESTIC CATS. F. A. Harris* and A. L. Towe, Dept. Physiol. & Biophys., Univ. of Wash. Sch. of Med., Seattle, WA 98195

Surface-recorded evoked potentials and extracellularly recorded spikes from single neurons were studied in pericruciate and precoronal cortex of chloralose-anesthetized, immobilized cats. Responses to skin stimulation were studied before and after application of bicuculline crystals to each recording site. Bicuculline increased both the amplitude and duration of the potentials produced in each area by contralateral forepaw (CF) shock, and sometimes increased the potentials produced by off-focus (IF, CH, IH) paw stimulation. Often, an 'off-focus bicuculline spike,' similar to the 'off-focus strychnine spike,' developed during an otherwise normal evoked potential. In precruciate cortex, the potential evoked by IF stimulation was also markedly enhanced. The amplitude of the potentials evoked by CF stimulation increased 4-fold in precoronal, 2.5-fold in postcruciate, and 2-fold in precruciate cortex. The changes were similar to those observed with topical strychnine. The time course of bicuculline action on single neuron responsiveness was followed in 31 cases. The changes were similar to those observed with topical strychnine, though slower to develop. The responses of 334 single neurons to footpad shock were observed at various time after bicuculline application, and compared with the response of 300 strychninized and about 3000 normal neurons. Again, the changes observed were similar to those seen with topical strychnine. With few exceptions, neuron responsiveness was markedly enhanced, the average spikes/discharge to CF stimulation increasing 1.6-fold for sa and sb neurons, and 2.3-fold for m neurons. Many neurons showed no enhancement of responsiveness, but many showed a striking increase, some responding with an average of 12-20 spikes/discharge. Response thresholds were lower and frequency-following capacities were higher than normal. Many m neurons showed an increase in responsiveness to off-focus stimulation. (Supported by NS 396 & NS 5136)

203 A RETICULO THALAMIC SYSTEM REGULATING PROPIOCEPTIVE ATTENTION IN MAN. Francisco Velasco, Marcos Velasco and Héctor Maldonado*. Division of Neurophysiology, Sci. Res. Dept., Natl. Med. Ctr., I.M.S.S., México, D. F.

Electrophysiological studies performed during stereotaxic procedures in man have furnished evidence that in the thalamus and subthalamus there are two different systems mediating propioceptive impulses: I.- Lemniscal system (median lemniscus and specific somatosensory thalamic nuclei): a) Its electrical stimulation elicited circumscribed sensory responses in the contralateral extremities. b) Multiple unit activity (MUA) showed somatotopical organization for tactile and propioceptive stimuli. c) Somatic evoked potentials (SEP) showed prominent early components not modified during various attentive situations. d) Lesion produced blocking of all components of cortical SEP and sensory deficit of contralateral extremities. II.- Extralemniscal system (prelemniscal radiations, ventralis lateralis). a) Electrical stimulation increases amplitude of involuntary movements. b) MUA showed spontaneous 5 c.p.s. rhythmic cellular discharges. c) SEP showed only late components whose amplitude changed during various attentive situations. d) Lesions blocked late components of cortical SEP and produced a state of inattention to propioceptive stimuli of contralateral extremities. It is concluded that the extralemniscal system is engaged in the process of selective attention and perhaps in the initiation of motor exploratory behavior.

204 SOMATOSENSORY CORTICAL ACTIVITY FOLLOWING VIBRISSAE MOVEMENTS IN CATS. Wolfram Schultz*, Christian Hellweg* and Otto D. Creutzfeldt* (SPON: S. Axelrod). MPI für biophysikalische Chemie, Göttingen, Germany.

The hair follicles of mystacial vibrissae in cats contain mainly four types of mechanoreceptors that project to a somatosensory cortical area where virtually no input from other primary afferents can be found. By controlled mechanical stimulation, it is possible to nearly exclusively excite the two slowly adapting afferents and thus give rise to a known peripheral input to cortical cells. Under moderate barbiturate anaesthesia responses to this selective mechanical stimulation were recorded in thalamo-cortical projection fibers and intracellularly from cortical cells. Thalamic precortical fibers showed the two classes of responses typical for the two kinds of slowly adapting peripheral input. Their response properties were very similar to the phasic-tonic patterns described for primary afferents. The activity of cortical cells, however, was dominated by the appearance of one to three IPSP's of 40-90 msec duration at the beginning of a response, usually after a brief phasic excitation. This characteristic alternation of excitation and inhibition changed significantly with different stimulus intensities. After this initial pattern, only a few cells showed an increased discharge activity during a maintained stimulus, although an increased firing threshold could not be detected during that time. This study shows that primary afferent impulse patterns reach the precortical level rather unchanged, but are transformed in a characteristic way by the cortical circuitry.

205 SOMATOSENSORY AND ACOUSTICALLY-DRIVEN RESPONSES FROM HUMAN SCALP DURING SUBJECTIVE UNCERTAINTY ABOUT STIMULUS MODALITY. Hilton Stowell. Research Division (Sensory Neurophysiology), Central Georgia Regional Hospital, Milledgeville, GA 31062.

Stimulus-time-locked electrical activity was computer-summed for 256 ms post-stimulus-onset from two scalp electrodes, approximating respectively contralateral hand region of post-central gyrus and frontal Area 6 at 2 cm contralateral to the midline; reference was contralateral earlobe. Stimulation at 2.6 Hz was either (1) brief indentations of middle finger pad (tap); (2) equally brief free-field clicks; (3) both of these simultaneously; (4) short trains of clicks pseudorandomly interrupting longer trains of either (1) or (3); or (5) short stimulus absences interrupting as in (4). Binaural background noise masked unwanted airborne stimulation always. Seven non-patient subjects (ages 8-42), initially naive, showed inter-individual differences for condition (4) at both electrodes; some gave the same stable modality-specific waveforms as in (1), (2), and (3), while others showed slight variability of somatosensory (SER) and acoustically-driven (AcER) activity during sequential combinations of (1), (2), and (3), and in condition (4) very marked variability of the AcER, which at times approximated the waveform of their SER. One subject (aged 42) gave an AcER at times insignificantly different from her SER during mixed modality stimulation, and tended to evolve this AcER in a predictable temporal sequence during (4). Unstable responses could be restored to their individual modality-specific waveforms by enough trials of (1) and (2) temporally well-spaced. The cortical origin of an AcER cannot be determined using an earlobe reference, but our AcER, whether obtained parietally, frontally, or vertically, was not the auricular waveform originating at the periphery, which could be detected preceding our AcER. The SER was relatively stable in all conditions; subjects had previously given stable SERs to foot and lip tapping, recovered at relevant post-central scalp sites and having the expected parameters for these receptive fields. With the same exception (aged 42), frontal SERs differed from parietal for identical stimulation, while AcERs were similar at both sites; but SERs were still discriminable from AcERs over Area 6, an unexpected result in view of data from superior frontal regions of other primates suggesting Area 6 as a Type II heterotopic, sensory-convergent area (ALBE-FESSARD & BESSON, 1973).

The aim of this work is to establish what parameters of the scalp-recorded evoked responses can be confidently related to different stimulus modalities and subjective sensations, in both non-patient and patient populations. Results to-date do not contradict either a phase-shift model (SCHNEIDER, 1974) - with respect to stimulus onset and a hypothetical central reference -, or a frequency-shift model - with regard to the first 250 ms of the sinusoid -, for the cortical encoding of information related to stimulus modality and subjective sensory quality.

This study was supported by the Department of Planning, Evaluation, Research, and Training of the Central Georgia Regional Hospital, Georgia Department of Human Resources.

206 DEFECTS IN ACCURATE PROJECTION OF THE CONTRALATERAL ARM INTO EXTRAPERSONAL SPACE IN MONKEYS AFTER REMOVAL OF THE POSTERIOR PARIETAL ASSOCIATION CORTEX. Robert H. LaMotte and Carlos Acuña*. Dept. Physiol., The Johns Hopkins Sch.Med., Baltimore, Maryland 21205.

Monkeys were trained on tasks which differed in the degree of visual control permitted during projection of the limb towards one of 30 target lights spaced 6° apart horizontally on a concave screen at arm's length. On each trial a sustained key response by the subject turned on one of the lights and was followed later by a tone which signalled the reach to begin. A correct response required touching the ¼" diam. target light with the finger without touching the screen or a neighboring light. Following training and testing, Brodmann's areas 5 and 7 were removed in the hemisphere contralateral to the hand tested.

Under conditions which permitted a view of the target as well as visual guidance of the limb during the act of reaching, errors in the accuracy of reaching occurred 6-12° away from the target and in a direction toward the side of the lesion. No errors were made to locations above or below the horizontal line of targets. This defect remained during hundreds of test trials and over several test sessions. With continued testing there was considerable recovery of preoperative accuracy. Occasionally, there was a misguidance of fingers toward the target even though the arm was accurately projected. Additional results suggested this to be a visuospatial disorder and not simple ataxia.

When visual guidance of the limb and a view of the target were prevented by lowering a helmet over the head just before onset of the tone, errors in reaching-which occurred in normal animals-were increased in magnitude by 6-12°. In contrast with conditions allowing visual guidance of the limb, the direction of errors was always toward the midline. Furthermore, there was little or no recovery of preoperative accuracy for this version of the task.

Posterior parietal removal, in contrast to lesions of the postcentral gyrus, did not impair performance of simple tactile-guidance tests or tests of the capacity to detect or discriminate between vibratory stimuli.

The defects in accuracy of reaching following posterior parietal removal suggest a visuospatial disorientation which is not due to a loss of vision (e.g. a scotoma) or a motor defect (ataxia).

207 CORTICAL CELLS PROJECTING TO THE DORSAL COLUMN NUCLEI OF CATS. Julie A. Weisberg*, Carol Metz* and Aldo Rustioni. Depts. Anat. and Physiol., Sch. Med., Univ. North Carolina, Chapel Hill, 27514.

Cortical projections to the dorsal column nuclei (gracile and cuneate, DCN) have been demonstrated anatomically with the Golgi method and by impregnation of degenerated fibers in many animal species. In this study the retrograde transport of horseradish peroxidase has been used in adult cats. 0.05 to 0.1 microliter of 50% HRP was injected in the rostral part of the DCN where degeneration is dense after cortical lesion (Kuypers and Tuerk 1964). After 24 hours to three days, animals were perfused with a double aldehyde mixture (0.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1M phosphate buffer at pH 7.2). Serial 40 micra coronal or sagittal frozen sections of the pericruciate cortex were incubated for visualization of reaction product and counterstained with cresylviolet. Labelled cells are mainly found contralaterally to the injected side and are preferentially located in area 4 and in the fore- and hindlimb region of SI. Labelled neurons are confined to layer V and have somata comprised between 25 and 45 micra. None of the "giant" cells in area 4 and 3a contain HRP-positive granules. The question arises whether at least some of the cortical cells which project to the DCN also contribute to the cortico-spinal tract. It is planned to study this question in adult cats and in kittens in which cortical cells projecting to the DCN are labelled with HRP and, in the same animal, cells of origin of cortico-spinal tract are retrogradely affected by spinal hemisection. To this end, a method has been devised by means of which 1 to 2 mm thick brain slabs are incubated for visualization of HRP and subsequently embedded in celloidin. The method achieves excellent staining of cellular details without apparent loss of reaction product in labelled neurons.

208 STATIC AWARENESS OF KNEE JOINT ANGLE IS NOT AFFECTED BY LOCAL ANESTHETIC BLOCK OF KNEE JOINT RECEPTORS. F.J. Clark, K.W. Horch, P.R. Burgess and S.M. Bach*. Dept. Physiol., Univ. Nebr. Med. Centr., Omaha, Ne. 68105 and Dept. Physiol., School of Med., Univ. Utah, Salt Lake City, 84132.

A previous study has shown that human subjects can detect a 3-4° change in knee joint angle when one leg is rotated slowly (less than 1°/min) from a position where both legs are perceived as being aligned. This sense of misalignment does not fade with time and presumably depends upon continuous sensory input from the limbs. The present experiments were done to determine whether sensory inputs from the knee joints are necessary for such static limb position sense.

Tests were conducted at two intermediate knee joint angles (ca. 108° and 130°) in 10 subjects before and after anesthetizing both knee joints with a local anesthetic injected into the joint cavity (8 ml of 2% Xylocaine or 8 ml of ½% Marcaine). Each test consisted of four or five runs where the right leg was moved, at a rate less than 1°/min, either up or down by 5° or else held stationary. The sequences were presented in pseudorandom order. Subjects were able to accurately detect these changes in alignment, their direction, and distinguish them from runs where there had been no change in joint angle. The injection of local anesthetic into both knee joints had no measurable effect on the subjects' performance.

Gross recordings were made from the posterior and medial articular nerves of cats in response to joint bending and twisting before and after injecting 0.2 ml/kg of 2% Xylocaine or ½% Marcaine. The local anesthetic blocked all slowly adapting responses within a few minutes though some rapidly adapting responses persisted for a longer time.

These results provide further evidence that receptors in joints play little if any role in static limb position sense.

209 RESPONSES FROM A MECHANORECEPTOR AFTER ANTIDROMIC STIMULATION OF ITS AFFERENT NERVE FIBRE. Kay-M. Gottschaldt and Adebayo Fakoya+. MPI f.Biophysik.Chemie, 34 Göttingen, West-Germany.

Grandry corpuscles, mechanoreceptors in the beak skin of birds, consist of a pile of specialized cells having synaptic contacts to terminals of a branched afferent nerve fibre. The receptors respond with a phasic discharge at a frequency proportional to the velocity of a stimulus but not to its amplitude. A peculiar phenomenon was observed in experiments where small strands dissected from the ophthalmic nerve were stimulated antidromically while recording afferent impulses of single Grandry units from the same strand. In many preparations the antidromic stimulus elicited an afferent impulse that was apparently fired back from the peripheral receptor. Collision experiments proved that both the antidromic and the backfired impulse were travelling in the same nerve fibre. The probability of "antidromic backfiring" increased with the frequency of antidromic stimulation and was also enhanced by additional mechanical stimulation of the receptor. A comparison of conduction time after anti- and orthodromic electrical stimulation revealed a delay of at least 1 msec in the antidromic response which increased on cooling the peripheral receptive field of the unit. It is suggested that the phenomenon of "antidromic backfiring", which was not observed other than in Grandry units, indicates the intervention of a chemical or unknown biophysical process in the generation of afferent impulses in Grandry units. The finding supports the postulate that release of transmitter substance from Grandry cells mediates between the mechanical stimulus and the response in the afferent nerve fibre.

210 THRESHOLD PROPERTIES OF HAIR AND FIELD MECHANORECEPTORS. R. P. Tuckett*, K. W. Horch and P. R. Burgess. Dept. Physiol., Sch. Med., Univ. Utah, Salt Lake City, 84132.

Most of the mechanoreceptive sensory neurons supplying the hairy skin of the cat with peripheral axons conducting in the Aα range can be divided into two major categories: hair neurons and field neurons. This separation is based on the fact that a hair neuron will respond to small amplitude (less than 2 mm) movement of the tip of an isolated, innervated guard hair, whereas a field neuron will not respond to isolated guard hair movement. Stimuli administered with a hand-held probe were used to further classify each neuron into one of the following receptor types: G_1 hair, intermediate hair, G_2 hair, F_1 field, intermediate field, or F_2 field. Using a mechanical driver, we determined the threshold to three kinds of stimuli: constant velocity ramps, single sine cycle displacements, and mechanical paired pulses (recovery curve). In the last case, instead of exponential decay, a fraction of the receptors in each category displayed a damped oscillation in the recovery of sensitivity to the test pulse. With an electrical (instead of mechanical) conditioning pulse, there were no oscillations. Our data suggests a continuum of properties for both hair and field neurons, with distinctive clusters of properties at the end points. For example, at one end of the spectrum, G_1 and F_1 neurons respond only to high velocity ramps, to high frequency sine cycles, and have short recovery curves.

211 PROPERTIES OF MECHANOSENSITIVE NERVE FIBERS INNERVATING THE CEPHALIC INTEGUMENT OF THE RAT SNAKE. Morris K. Jackson* and Gernot S. Doetsch, Dept. Anat., and Depts. Surg. (Sec. Neurosurg.) and Physiol., Med. Coll. Ga., Augusta, Ga. 30902.

Jackson (Anat. Rec. 181: 382-383, 1975) recently described the structure and distribution of specialized cutaneous corpuscles within the cephalic scales of the rat snake (Elaphe obsoleta lindheimeri). To determine if these corpuscles might be mechanoreceptors, neural responses were recorded from 146 single maxillary nerve fibers of 11 rat snakes.

Fourteen percent of the units were exclusively driven by mechanical stimulation of corpuscles. Each fiber innervated 5 to 13 discrete end organs ($\bar{X}$ = 8). These units were optimally excited by movement of a mechanical stimulus across the corpuscles, with discharge frequency directly related to the number of end organs stimulated. Fibers were relatively insensitive to vertical displacement, adapted rapidly, gave ON and OFF responses, and followed horizontal vibratory stimulation at 256 Hz.

The other fibers studied (86%) were not specifically related to corpuscles, and were subdivided into three classes based on their rate of adaptation to vertical skin deformation: a) 68% were rapidly adapting; these units had the smallest mean receptive fields ($\bar{X}$ = 12.0 mm^2) and the highest mean thresholds ($\bar{X}$ = 652 mg force); the majority followed horizontal skin vibration at 256 Hz. b) 26% were slowly adapting; they had the largest mean receptive fields ($\bar{X}$ = 20.4 mm^2) and the lowest mean thresholds ($\bar{X}$ = 432 mg force); only a few could follow vibratory stimulation at 256 Hz. c) 6% of the fibers had adaptation rates and other properties intermediate between those of the other two classes.

The present data show that the maxillary nerve of the rat snake contains several types of mechanosensitive fibers and support the hypothesis that the cephalic corpuscles are indeed specialized mechanoreceptors.

212 MULTIPLE REPRESENTATION OF TRIGEMINAL MECHANORECEPTORS IN OPOSSUM SOMATIC SENSORY NEOCORTEX. Benjamin H. Pubols, Jr., and Lillian M. Pubols. Dept. of Anatomy, College of Medicine, Pennsylvania State Univ., Hershey, PA.

Organization of somatic sensory neocortex has been investigated in sodium pentobarbital anesthetized Virginia opossums. Use of closely spaced microelectrode penetrations (1/4-1/2 mm grid pattern) and delicate mechanical stimulation of the body surface including facial vibrissae, revealed a somatotopically organized double representation of the contralateral mystacial vibrissae and rhinarium, the two representations being adjacent, orderly, mirror images of each other. Approximately half of the mystacial vibrissa units responded to deflection of a single vibrissa, others responding to deflection of up to five adjacent vibrissae. Properties of the lateral trigeminal area (LTA) and medial trigeminal area (MTA) were similar except for a greater sensitivity to direction of vibrissa deflection in units of MTA, and a larger rhinarial area in MTA but a larger mystacial area in LTA. A small cortical region of bilateral representation, located lateral to LTA, also includes a representation of trigeminal mechanoreceptors.

It is suggested that MTA, and the regions of contralateral head dorsum and postcranial representation, taken together, are homologous with the first somatic sensory area (SmI) of placental mammals, and that the region of bilateral representation is homologous with the second somatic sensory area (SmII) of placental mammals. However, LTA appears to represent a neural correlate of tactile behavioral specialization unique to the Virginia opossum, a "third" trigeminal representation, but not equivalent to SmIII. (Supported by USPHS grant NS-06371)

213 CORTICOFUGAL MODULATION OF SOMATOSENSORY INPUT IN THE DORSAL COLUMN NUCLEI OF THE RACCOON. Mark J. Rowinski*, S. David Stoney, Jr.* (SPON: Stephen H. Hobbs), Dept. Physiology, Med. Coll. Ga., Augusta, Ga., 30902

Electrical stimulation of motor cortex in raccoons anesthetized with sodium pentobarbital produces slow positive waves (P waves) recorded from the surface of the dorsal column nuclei. Using bipolar surface stimulation of the cortex ($\leq$ 2mA) it was found that P wave amplitude was maximal over the contralateral cuneate nucleus approximately 3 mm caudal to the obex. The cortical best points for generating P wave activity were restricted to the posterior sigmoid gyrus near the lateral tip of the cruciate sulcus. This cortical region exhibited the lowest threshold for generating EMG activity in contralateral forelimb muscles. In all cases the stimulus strength required for producing a detectable DCN P wave was greater than that for eliciting threshold EMG activity. Stimulation of the same cortical region also produced an increase in excitability (up to 30%) of superficial radial nerve terminals in the cuneate nucleus as determined by the Wall method. The time course of the excitability change closely matched the P wave time course. In a preliminary sample of cuneo-lemniscal projection cells, forelimb motor cortical stimulation was shown to depress the peripherally evoked activity of some forelimb hair and touch units. Off-focus peripheral stimulation also produced P waves and increased the excitability (up to 50%) of superficial radial nerve terminals in the cuneate nucleus. This data suggests that cortical modulation of DCN activity, especially that mediated by presynaptic inhibitory mechanisms, originates in the forelimb motor region of the raccoon cortex.

214 THE ELECTROPHYSIOLOGICAL ORGANIZATION OF THE CANINE NUCLEUS GRACILIS. Richard J. Schneider, Bruce A. Silver* and Abdul L. Itani*. Division of Neurosurgery, U. Maryland, Baltimore 21201.

Electrophysiological recording was performed on 27 mongrel dogs, anesthetized with pentobarbital, following posterior fossa operations to expose the dorsal funiculus nuclei. Glass-coated, tungsten microelectrodes with uninsulated tip diameters of 1-10 microns were used to record single unit and multiunit receptive fields within a grid of 0.1 mm^2 boxes covering the nucleus. Selected dogs were perfused following the termination of the experiment and histology was performed.

Maps of the receptive field arrangement were constructed from 327 single units which were supplemented by multiunit fields where information was lacking. The nucleus could be separated into two sections divided by a transition area at the level of the obex. Rostral to the transition area, no systematic somatotopy was demonstrated in the dorsoventral plane, while dermatomal arrangement occurred medio-laterally. Units were sparser than in caudal areas, but their potentials were generally greater in amplitude. The modality count of units showed equal occurrence of joint and deep pressure units to touch and hair displacement units. Both types of units from the same receptive field frequently occurred in proximity.

Caudal to the transition area, units were more densely clustered. No feet dorsal-trunk ventral "caninunculus" could be interpreted from the results; instead a dermatomal arrangement similar to that seen in the dorsal funiculi was observed. The modality composition was heavily biased toward touch and hair displacement units, and their potentials were of lower amplitude than those observed rostrally. At the caudal end of the nucleus, neither modality nor arrangement changed, but units again became sparse.

215 EFFERENT PROJECTIONS OF THE DORSAL COLUMN NUCLEI AND THE OVERLYING DORSAL COLUMNS OF THE VIRGINIA OPOSSUM. M.J. RoBards* and D.W. Watkins*. (SPON: DeF. Mellon). Dept. Anat., Sch. Med., Univ. of Va., Charlottesville, Va.

In 21 wild-born Virginia opossums, lesions were made in the dorsal column nuclei (DCN) and the overlying dorsal columns of the medulla. Five to twelve days later, the animals were perfused and the brains were stained with the Fink-Heimer or de Olmos stains for anterograde degeneration. In cases with well restricted lesions, degenerating axons and telodendria were traced both contralaterally and ipsilaterally. The main contralateral projections were traced to the dorsal and medial accessory inferior olive, the intercollicular zone (intercollicular nucleus, adjacent dorsolateral and lateral central gray, and caudal deep layers of the superior colliculus), ventral tegmentum, red nucleus, posterior thalamic area, parafascicular, subparafascicular, and central intralaminar nuclei, lateral ventrobasal complex, and zona incerta. Ipsilateral degeneration, some of which may be attributed to the damage to the dorsal columns, rather than to the DCN themselves, was found in the external cuneate nucleus, portions of the vestibuloacoustic complex (particularly the inferior and medial vestibular nuclei) and cerebellum. Of the three cerebellar peduncles, degenerating fibers were seen only in brachium conjunctivum. Degenerating axons coursed through the deep cerebellar nuclei, and a terminal field projection was suggested in interpositus. Seven slabs of, alternately, degenerating and normal axons gave the cerebellar cortex a striped appearance in silver stained frontal plane sections. With the exception of the projection to the ventrobasal complex and the inferior olive, some bilateral degeneration was found in each of the targets listed above after restricted lesions. Lesions in any rostrocaudal part of the opossum's DCN produce a uniformly widespread pattern of degeneration, suggesting that the opossum's entire DCN are analogous and perhaps homologous with the rostral subdivision of these nuclei in the cat.

216 CLUSTERED ARBORIZATIONS: A FEATURE OF THE FELINE DORSAL COLUMN SYSTEM. Peter J. Hand and Thomas VanWinkle* Department of Animal Biology, School of Veterinary Medicine and *Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, Pennsylvania 19174

Previous anatomical studies of the feline dorsal column system demonstrated the presence of extremely localized and dense terminal axonal arborizations (clusters or patches) in the dorsal column nuclei (n. cuneatus and gracilis) (1,2) and the first somatic sensory cortex (3) following dorsal rhizotomies or small lesions of the thalamic ventrobasal complex (VB), respectively. Because of the large lesions employed, earlier studies did not reveal degeneration clusters in nucleus ventralis posterolateralis pars medialis (VPLm), a subdivision of VB and a target of nucleus cuneatus (NC) efferents. In this investigation micro - or macroelectrodes were used to make small lesions in different zones of NC in seventeen cats. Resultant axonal degeneration was stained according to the Fink-Heimer I technique. All NC zones studied projected to contralateral VPLm in a striking pattern of dense and localized terminal arborizations or clusters (5). Clusters were organized into onionskin-like dorso-ventral laminae. The size of and density of degeneration in a cluster varied according to its dorso-ventral position within VPLm. Dorsal VPLm, where proximal receptive fields and "deeper" units are represented (4) contained small diameter clusters (50-125 um), which possessed moderate amounts of degeneration (Fig. 1A). Clusters in ventral VPLm where distally located receptive fields and "superficial" units are represented (4) were of larger diameter (150-300 um)and contained dense degeneration (Fig. 1B). The clustered arborizations were topographically organized in agreement with the electrophysiologically determined somatotopia (4), i.e. dorsal NC neurons projected their axons to ventral VPLm and ventral to dorsal, lateral to medial, and medial to lateral VPLm. In addition to the classical VPLm connection, NC projected to the following contralateral brainstem and thalamic nuclei: caudal medial accessory olive, dorsal accessory olive, rostral inferior colliculus, ventrolateral superior colliculus, nucleus ruber, medial geniculatus pars magnocellularis, suprageniculatus, medial and lateral divisions of the posterior thalamic nuclear group, zona incerta, and fields of Forel. Brainstem projections of caudodorsal NC were less widespread than were those of ventrocaudal and rostral NC. In conclusion, the neural substratum most likely responsible for the synaptic security, fine grain somatotopia, and possibly the modality specificity so characteristic of the dorsal column system is the discrete and dense terminal arborization or cluster now shown to be present at all levels of this system.

(Supported by USPHS grants NS-08410 and NS-06716)

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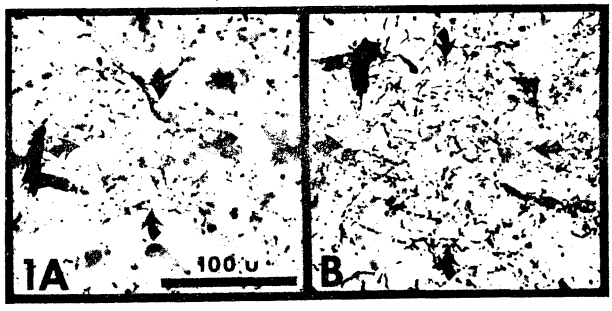

217 CUTANEOUS MASKING: COMPARISON OF PSYCHOPHYSICAL AND ELECTROPHYSIOLOGICAL MEASUREMENTS. S.E. Laskin* and W.A. Spencer*. Dept. of Physiology, N.Y.U., Dept. of Neurobiology, P.H.R.I., and Depts. of Physiology and Neurology, Columbia Univ., N.Y. (SPON: E.P. Gardner)

We have studied cutaneous masking to clarify functions of somatosensory inhibitory circuitry. Masking of cutaneous sensations at the locus of punctate stimulation has been quantitatively examined in human subjects using brief air pulses. Employing a conditioning-test 2 stimulus paradigm, we found that maximal masking occurred at the minimal interstimulus distance at which subjects could discriminate conditioning and test stimuli. Masking effectiveness varied inversely with interstimulus distance up to 10 cm on the forearm. Masking was demonstrable with the test stimulus delivered from 10 msec before, to nearly 70 msec after, conditioning stimulation.

The responses of single neurons, sensitive to hair movement, in cat SI cortex were then examined quantitatively with identical stimuli. Suppression of spikes in the brief impulse train comprising a test response, evoked by a fixed stimulus at the receptive field center, was measured upon systematically delivering conditioning stimuli at successive points along the longitudinal axis of the receptive field. The measured spatial distribution of such in-field "inhibition" was unimodal and highly covariant with the excitation evoked by the conditioning stimuli. In nearly one-half of the units studied, inhibition extended beyond the excitatory receptive field, forming a "surround" inhibitory zone. By varying interstimulus intervals, suppression was demonstrable with test stimuli ranging from 10 msec before to some 70 msec after conditioning stimulation. Peak suppression occurred with test stimuli delivered 10-15 msec after conditioning stimulus onset. Inhibition measured in the "surround" areas had nearly the same time course as the suppression induced by conditioning stimuli within receptive fields. The spatial and temporal patterns of these inhibitory effects revealed at the cortical level thus exhibit many of the parametric features of psychophysical masking studied with paired air pulses in humans. (Supported by USPHS Grants NS 09361 and GM 01668)

218 SINGLE NEURONS OF RACCOON PRIMARY SOMATOSENSORY NEOCORTEX WHICH ARE PREFERENTIALLY RESPONSIVE TO THE LINEAR ORIENTATION OF A TACTILE STIMULUS.

Robert F. Leroy* and Lillian M. Pubols, Dept. Anat., The Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129.

An analysis of single unit responses in the forepaw area of raccoon SmI neocortex was undertaken to gain a better understanding of the basis of cutaneous pattern discrimination.

Animals were anesthetized with methoxyflurane throughout the experiments. Microelectrodes were used to record the activity of single units. Determination of receptive fields of single units was achieved using ordinary von Frey hairs, and von Frey hairs upon which were mounted pieces of acetate plastic, 0.3 mm wide, and 5.0, 7.5, 10.0 or 20.0 mm in length (linear stimuli). Some units were investigated quantitatively, using controlled mechanical pulses (skin indentation of 500μ for 500 to 2000 msec, one pulse every 7 sec) from an automated electromechanical stimulator with either a 1 mm diameter probe (punctate stimulus), or with linear probes, as described above. This report is based upon detailed studies performed on 35 units exhibiting initially negative-going action potentials, and cutaneous receptive fields on the glabrous skin of the forepaw.

The receptive fields (RFs) of these units were of two types. One type (n=21) was small (mean diameter of 2.8 mm) and circular in shape. Units having this RF type were found throughout the forepaw pads and digits. The other type (n=14) was approximately rectangular in shape. The mean dimensions of these fields were 7.3 by 2.0 mm. The long axes of these rectangular RFs were oriented proximodistally when they were located on the digits, but there was no consistent orientation when these RFs were on palm pads (Fig. 1).

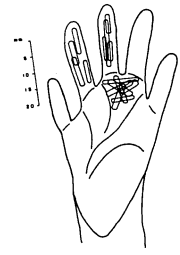

FIGURE 1

An example of the response of a circular RF unit is shown in Fig. 2. This unit exhibited a higher rate of firing to punctate stimuli applied within the receptive field than to linear stimuli applied across the receptive field. When automated linear stimuli were applied to the receptive fields of the rectangular RF units, it was found that punctate stimuli and linear stimuli applied in non-preferred orientations were significantly less effective in evoking an excitatory response than were linear stimuli applied in the preferred orientation (e.g., Fig. 3). It is suggested that these two cortical unit types contribute to somatic sensory form and pattern discrimination. (NIH: NS-06371)

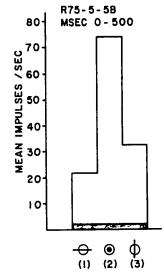

FIGURE 2

Fig. 1: Distribution of Rectangular RFs of SmI neocortical units on a raccoon ventral forepaw.

Fig. 2: Response of a circular RF unit (Ist 500 msec of a 1000 msec stimulus): (2), Punctate stimulus within RF; (1) and (3), linear stimulus across RF. Shaded area: mean spontaneous activity.

Fig. 3: Response of a rectangular RF unit (Ist 500 msec of a 2000 msec stimulus): left to right; linear stimulus within RF, linear stimulus perpendicular to proximal RF, linear stimulus perpendicular to middle RF, linear stimulus perpendicular to distal RF. Shade area: mean spontaneous activity.

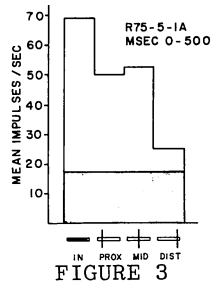

FIGURE 3

219 LOCALIZATION AND DISTRIBUTION OF VARIOUS SENSORY RECEPTORS IN MOUSE PALATAL MUCOSA. Pedro B. Nava, Jr. Dept. of Anatomy, The Milton S. Hershey Medical Center, Hershey, Pennsylvania 17033.

The innervation of the oral mucosa of the hard palate has been investigated in ten CD-1 young adult mice by means of whole-mount silver preparations and histological sections. Gross inspection of the palatal mucosa revealed nine palatal rugae (designated I-IX from anterior to posterior) whose long axis was parallel to the transverse plane of the body. Several periodic swellings were noted along each rugae. The whole-mount technique revealed a neural pattern which closely paralleled the arterial supply demonstrated in rats by Cox and Dorenbros (1974). The major palatine nerve, a branch of the maxillary division of the trigeminal nerve, coursed through the palatine canal at the level of palatal rugae VII and extended anteriorly in line with the nasopalatine duct to the incisive papilla; this provided sensory fibers segmentally to the palatal rugae on the oral side of the hard palate. The minor palatine nerve supplied the palatal area posterior to rugae VII and included a portion of the smooth hard palate. Details of palatal innervation demonstrated by transverse and longitudinal histological endings: taste buds, a complex corpuscular ending, Merkel cells and free nerve endings. Taste buds were noted in two areas: on the medial aspect of the nasopalatine duct (palatal rugae I) and singly or in clusters in the smooth portion of the hard palate. The corpuscular endings were concentrated at the apices of the palatal swellings of each of the nine rugae. Merkel cells were generally observed in the basal epithelial layer adjacent to the corpuscular endings, while free endings were noted throughout the palate. (Supported in part by NIH-NIDR Grant No. 1-DE-22401)

220 UNMYELINATED FIBERS IN THE INFERIOR CARDIAC NERVE OF THE CAT. Dennis G. Emery, Robert D. Foreman and Richard E. Coggeshall. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550.

The inferior cardiac nerve arises from the stellate ganglion and is a major sympathetic nerve to the heart. Receptor fields for myelinated fibers in this nerve have been recorded, but the fiber composition of the nerve is not known. Electron microscopic examination demonstrated that the nerve contains 50-150 myelinated fibers and 25,000-40,000 unmyelinated fibers. The myelinated fibers range from 1.5 - 7 μ in diameter with a mean size of 3.2 μ. The unmyelinated fibers range from 0.2 - 1.7 μ in diameter with a mean of 0.59 μ.

In one cat, C fiber volleys recorded from the nerve indicate that two populations of unmyelinated fibers with different conduction velocities are present. The larger population exhibits velocities from 0.4-0.7 m/sec, while the smaller population conducts from 0.7-1.0 m/sec. According to the fiber diameter-conduction velocity conversion factor of Gasser (J. Gen. Physiol., 38: 709, 1955) the slower conducting of these fibers would be in the 0.2-0.4 μ range, and the faster ones would be larger than 0.4 μ in diameter. Yasumi, et al. (Am. J. Physiol. 226: 1088, 1974) and Uchida and Murao (Am J. Physiol. 227: 753, 1974) report unmyelinated cardiac afferents in upper thoracic communicating rami. These may belong to a population of unmyelinated sensory fibers in the inferior cardiac nerve, with the other population consisting of post-ganglionic fibers. Degeneration studies are underway to determine the fiber types in the nerve. (Supported by NIH Grant # NS 11255 and the Moody Foundation of Galveston.)

221 UNMYELINATED FIBERS IN MAMMALIAN RAMI COMMUNICANTES. R.E. Coggeshall, M.B. Hancock and M.L. Applebaum*. (Spon: D.G. Emery.) Department of Anatomy and Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550.

The majority of axons in the mammalian rami communicantes are unmyelinated. To gain insight into the function of these fibers, ventral rhizotomy or combined ventral rhizotomy and dorsal root ganglionectomy were performed on the left side of segments T_7, T_8 or T_9 in the cat. Operated animals were allowed to survive for one week, then sacrificed by arterial perfusion. The sympathetic chain with the attached communicating rami were removed from the normal and operated side of the operated segment. In 6 cats to date the grey communicating rami showed no decrease in myelinated or unmyelinated fibers after either operation. Thus these fibers are interpreted as being exclusively postganglionic. After ventral rhizotomy, the unmyelinated fibers show a 30% decrease from normal. These fibers are interpreted as preganglionic in nature. After the combined operation (dorsal root ganglionectomy and ventral rhizotomy), there was an additional decrease of 17% in the unmyelinated fibers. These are interpreted as sensory fibers. The 50%+ unmyelinated fibers remaining after both operations are interpreted as postganglionic fibers. (Supported by NIH Grants NS 09018, NS 10161 and NS 11255.)

222 CELLS OF ORIGIN OF VENTRAL ROOT AFFERENTS. C.W. Maynard*, R.B. Leonard, J.D. Coulter, R.E. Coggeshall and W.D. Willis. Marine Biomedical Institute and Departments of Anatomy, Physiology and Psychiatry, University of Texas Medical Branch, Galveston, Texas 77550.

Previous studies have identified a population of unmyelinated fibers in the ventral spinal roots which project towards the spinal cord and arise from cells in the dorsal root ganglion. The present experiments using horseradish peroxidase (HRP), seek to identify the cells of origin of these ventral root afferents and to establish whether the central processes of these fibers enter the spinal cord. Laminectomies were performed on cats and the dorsal roots L_5 through S_1 were visualized through the dissecting microscope and sectioned intradurally on one side. The roots were left intact on the opposite side except for one segment where both the dorsal and ventral roots were cut. One or two segments of the spinal cord were injected bilaterally. 0.2 microliters of 30% HRP were injected slowly at each of two depths (3.5 and 2.5 mm) in the spinal cord. Animals were sacrificed 24 to 36 hours later. In ganglia where the dorsal roots were left intact, the majority of ganglion cells were heavily labelled with fine HRP granules. No labelled cells were found in ganglia where both the dorsal and ventral roots had been cut. In ganglia where only the ventral roots remained in continuity with the spinal cord, a number of ganglion cells were labelled. Most of these neurons were small although a few larger ones were also marked. The labelled cells were situated near the region where the dorsal root ganglion fuses with the ventral root to form the spinal nerve. These findings support the conclusion that the ventral root afferents, presumably including both myelinated and unmyelinated fibers, have their cell bodies in the dorsal root ganglion proper and that the fibers themselves project into the spinal cord. (Supported by NIH Grants NS 12481, NS 11255, Training Grants NS 05743, GM 00459 and the Moody Fdn.

223 VENTRAL ROOT PROJECTIONS OF MYELINATED DORSAL ROOT GANGLION CELLS IN THE CAT. Gerald E. Loeb. Laboratory of Neural Control, NINCDS, NIH, Bethesda, Maryland 20014.

Single unit extracellular recordings were obtained from L7 dorsal root ganglion cell somata in 11 adult male and female cats. The proximal and distal projections of these primary afferents were searched for by electrical stimulation of the sciatic nerve and of the dorsal and the ventral roots of the same and adjacent spinal levels; continuity was confirmed by collision. 124 proprioceptive and cutaneous afferents with myelinated distal processes conducting at 10-120 m/sec were found to have single proximal processes in the L7 dorsal root only. However, five units (3.9%) had L7 ventral root proximal processes, and one (possibly two) of these had the usual dorsal root projections as well. All of the proximal projections in both dorsal and ventral roots conducted at 20-70% of the velocity of the distal projections in sciatic nerve. No "recurrent collateral" or other direct connections to adjacent levels were found in 44 units which were extensively tested for such projections.

224 AN ANALYSIS OF THE BORDER BETWEEN N. VENTRALIS LATERALIS AND N. VENTRALIS POSTEROLATERALIS IN THE CAT THALAMUS. Karen J. Berkley. Dept. Psychol., Fla. St. Univ., Tallahassee, Fla., 32306.

Fibers in the ventrolateral quadrant of the spinal cord (VLQ) that project to the thalamus terminate predominantly in portions of the intralaminar group nuclei. In the monkey, spinothalamic tract fibers also terminate the n. ventralis posterolateralis (VPL). In the cat, however, these fibers appear to bypass VPL, terminating instead in n. ventralis lateralis (VL) near its border with VPL.

Although the evidence is convincing, one of the problems with the interpretation of these data is that it is difficult to determine the exact VL-VPL border on a cytological basis alone. It may be, for example, that VL and VPL, instead of being immediately contiguous structures, either overlap with each other or are separated by some small zone interposed between them. Thus, rather than projecting to VL proper, VLQ fibers in the cat could instead be projecting either to a zone of VL-VPL overlap or to a zone intermediate between VL and VPL.

In the present experiment, an attempt was made to determine the VL-VPL border using the connections of the two nuclei as the basis for their definition. "VL" was defined as the terminal target of the cerebellum; "VPL" as the terminal target of the dorsal column nuclei. A differential labeling strategy was employed in which, in the same cat, "VL" was labeled using degeneration tracing methods after removal of the cerebellum, and "VPL" was labeled using autoradiographic tracing methods after injection of ^{3}H-leucine and ^{3}H-proline into various portions of the dorsal column nuclei.

Analysis of such proparations studied in coronal, saggital, and horizontal planes shows that VL and VPL appear to overlap at the dorsomedial edge of the most rostral portion of VPL. In addition, a small zone, relatively empty of terminals, is interposed between VL and VPL at the dorsolateral edge of the most rostral portion of VPL. Except for these two restricted regions, VL and VPL appear to be predominantly contiguous, rather than overlapping or separated structures.

The region of VL-VPL overlap appears to approximate that region to which the la afferent fibers of the forelimb project by way of the ventral portions of the cuneate nucleus. On the other hand, the small empty zone interposed between VL and VPL appears to be confined within that region to which the la afferent fibers of the hindlimb project by way of the nucleus z. Preliminary study suggests that if the projections of nucleus z are included in the definition of the extent of VPL, then VL and the expanded VPL appear to overlap at both of the la recipient zones located at the dorsolateral and dorsomedial edges of the most rostral portions of VPL. Furthermore, preliminary analyses of the projections of VLQ fibers suggests that the spinothalamic tract fibers projecting to this region may terminate in the vicinity of these small regions of overlap between VL and VPL, rather than in VL or VPL proper.

(Supported by PHS grants NS 11892 and NS 02992.)

225 CONDUCTION PROPERTIES OF TYPE I SKIN AFFERENT FIBERS
Zsuzsanna Wiesenfeld, Arthur Craig, Jr. and Daniel Tapper. Dept. Phys. Biol., N.Y.S. Coll. Vet. Med., Cornell U., Ithaca, N.Y. 14853

Analysis of conduction properties of Type I afferent fibers was made on the posterior femoral cutaneous nerve (PFCn) in adult cats anesthetized with urethane. By use of averaging procedures and selective activation of Type I terminal haarscheiben, single action potentials were monitored in the same preparations at two sites along the PFCn, at the first sacral dorsal root, and at the fiber terminals within the spinal cord. In more than 90 per cent of the cases a single action potential in the single Type I fiber could be evoked with a minimal stimulus current of 0.09 ± 0.06 ma (mean ± standard deviation). An increase to 0.15 ± 0.12 ma was required to elicit activity in adjacent fibers. Conduction speed was relatively slow (29-34 meters per second) between the receptor and the most peripheral recording site; it reached maximum velocity (48-61 meters per second) within a centimeter or less of the skin structure activated. The impulse velocity was dramatically reduced (ca 20-40 meters per second) during its central terminal course within the dorsal spinal gray matter. Our data suggest that the initial slow conduction speed is due to a long utilization time confined to the vicinity of the skin terminal, that the speed along the skin nerve and dorsal root is essentially uniform, and that the apparent increase in conduction velocity with distance from the periphery is due to this utilization time rather than to differential stretching of the nerve during growth as had previously been suggested.
(Supported by USPH Grant NS 07505)

226 DENDROAXONIC SYNAPSES ON PRIMARY AFFERENT AXONS IN THE SUBSTANTIA GELATINOSA GLOMERULI OF THE CAT SPINAL TRIGEMINAL NUCLEUS.
Stephen Gobel, Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, Md. 20014.

The glomeruli in the substantia gelatinosa layer of the spinal trigeminal nucleus contain three kinds of dendritic processes (Gobel, J. Neurocytol. 3,219, 1974). One of these, the type 2 dendrite, contains large, rounded, synaptic vesicles in its spine heads which are about the same size and shape as the synaptic vesicles in the primary trigeminal axonal (C) endings of the glomeruli. The type 2 spines receive an axodendritic synapse from the C ending and in turn form a dendroaxonic synapse on the C ending. These two synapses typically occur in reciprocal pairs with the axodendritic synapse lying in the depths of scalloped depressions in the surface of the C ending while the dendroaxonic synapse is found on the rim of these depressions. The type 2 spines also form dendrodendritic synapses on adjacent dendritic spines which are devoid of synaptic vesicles (type 1 spines). The type 2 dendrite, with its large, rounded, synaptic vesicles, is considered on a morphological basis to be excitatory at its dendroaxonic and dendrodendritic synapses. Based on this hypothesis the type 2 dendrite participates in the following synaptic events. A type 2 dendrite, in response to C axon excitation would activate type 1 spines by releasing transmitter at its dendrodendritic synapses (2→1) and at its dendroaxonic synapses (2→C→1). In addition, the reciprocal pairing of axodendritic and dendroaxonic synapses (C⇄2) may prolong transmitter release from the axodendritic synapses of C axonal endings beyond the time of arrival of incoming action potentials.

227 SPINAL CORD FIELD POTENTIALS EVOKED BY PERIPHERAL NERVE STIMULATION IN THE PRIMATE. J.E. Beall*, A.E. Applebaum, R.D. Foreman and W.D. Willis. Dept. Anat. and Physiol., and Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550.

Field potentials evoked by electrical stimulation of the sural nerve were recorded in the lumbosacral enlargement of the anesthetized monkey (Macaca mulatta). Regularly spaced microelectrode penetrations were made transversely across the spinal cord, and field potentials were recorded at 200 or 500 μm increments in depth. Field potentials were averaged by computer. Histological reconstruction of the grid of electrode tracts and recording sites allowed correlation of areas of negativity and positivity with the structure of the cord. Recordings were also made from dorsal roots or nerve trunks to determine the components of the afferent volley contributing to the field potentials. Potentials corresponding to the phase 2 and phase 3 potentials seen in the cat lumbosacral cord were observed in the monkey. The main focus of negativity was in the lateral portion of the dorsal horn. In addition, another large slow wave followed the phase 2 and 3 waves. This late wave has not been reported in the cat. Stimulation strengths suprathreshold for the activation of the largest Aδ afferents were required to evoke this late wave. The latency for the onset of the late potential corresponded with the arrival time of the peak of the Aδ component of the afferent volley to the cord. After transection of the cord at thoracic levels the late wave was still present, thus eliminating the possibility that it was due to a spino-bulbospinal reflex. The focus of negativity for the late wave was lateral in the dorsal horn and extended into lamina VII. These data suggest that the late negative wave is due to interneuronal activity in response to Aδ stimulation. (Supported by NIH Grant NS 09743, Training Grants NS 05743 and GM 00459, and the Moody Foundation of Galveston.)

228 LOCATIONS AND RESPONSE PROPERTIES OF CAT SPINORETICULAR NEURONS. R.A. Maunz*, N.G. Pitts* and B.W. Peterson. The Rockefeller University, New York, N.Y. 10021.

Neurons in the lumbosacral spinal cord projecting to the medial brain stem reticular formation were identified by antidromic activation of their axons in chloralose anesthetized, decerebellated cats. Stimuli, consisting of 0.1 msec rectangular pulses with intensities from 50-300 uA, were applied through bipolar concentric electrodes located in the medial ponto-medullary reticular formation up to 1.5 mm from the midline at depths of 1-5 mm from the floor of the IVth ventricle. Responses were identified as antidromic by their constant latencies, even with threshold stimulation, their ability to follow stimulus frequencies up to 100 Hz or more, and the presence of collision block. Spinoreticular neurons were found to be located predominantly in medial portions of laminae VII and VIII of Rexed and to project, bilaterally in some cases, to the medial reticular formation. Conduction velocities of these neurons ranged from 18-95 m/sec. Responses of these neurons to electrical stimulation of hindlimb nerves were examined. Neurons were found to have bilateral receptive fields, with low threshold inputs from cutaneous and mixed nerves and high threshold inputs from muscle nerves. With repetitive stimulation, the responses were often seen to diminish at rates of 1/sec as compared to the responses at 1/10 sec, and occasionally the responses were seen to increase.

Supported in part by NSF grant BMS 75-00487 and NIH grants NS 02619 and NS 05463.

229 THE MORPHOLOGY AND PHYSIOLOGY OF SPINOCERVICAL TRACT NEURONS. P.K. Rose, A.G. Brown, C.R. House, and P.J. Snow (SPON: R. Feinstein). Dept. Vet. Physiol., University of Edinburgh EH9 1QH, Scotland.

The physiology and morphology of antidromically identified spinocervical tract (SCT) neurons has been studied in adult cats (decerebrate, decerebrate-spinal, chloralose anaesthetized). Light mechanical stimulation of the hindlimb was used to determine the properties of the afferent input of each neuron and subsequently the neuron was impaled and injected with Procion yellow dye. Reconstruction of 20μm serial paraffin sections of the spinal cord provided a comprehensive morphological description of each neuron. The most striking feature of SCT neurons has been the wide range in the shape and orientation of the dendritic trees. Some neurons had only dorsally directed dendrites but most also had dendrites extending in horizontal and ventral directions. All SCT neurons had complex dendritic trees. Each SCT soma gave rise to up to 12 primary dendrites and some primary dendrites branched as many as 16 times. The small number of neurons studied (22) does not permit a complete study of the correlations between the morphology and physiology of SCT neurons. Nevertheless one consistent finding emerged. Irrespective of the location of their receptive field on the hindlimb, SCT neurons which were located in the lumbosacral spinal cord and which were excited only by light brushing of the hair all had antennae-like dendritic trees with each primary dendrite directed dorsally. All 22 SCT neurons marked with Procion dye were found between 1200μm and 2200μm from the cord dorsum. Extracellular recordings from a further 95 SCT neurons, obtained during a systematic search of the cord to a depth of 3000μm, revealed that SCT neurons are uniformily distributed in the dorsal horn at a depth corresponding closely to lamina IV.

Supported by a grant to A.G.B. from the British M.R.C.

230 SPATIAL ACUITY VS. SPATIAL SUMMATION IN THE WARMTH SENSE. Joseph C. Stevens and Lawrence E. Marks. John B. Pierce Foundation Laboratory and Yale U., New Haven, Ct. 06519.

The warmth sense tends to sum the neural effects of stimulation over large areas (up to hundreds of square cm) of the skin so that the sensed level of a radiant stimulus depends as well on its areal extent as on its intensity. This tendency to sum neural signals seems to limit severely the capacity of the warmth sense to mediate spatial information about the stimulus, e.g., its areal size, its configuration, and its apparent locus on the skin. An inverse relation between acuity and summation can be illustrated by an experiment in which the task is to localize brief warmth sensations as originating either on the distal or on the proximal side of a tactile reference signal delivered to the mid-forearm. Compared with vision and touch the warmth sense performs badly at localization, but the main point is that localization improves steadily with increasing level of stimulus intensity. Correspondingly, spatial summation declines with level from a near reciprocity between area and intensity at the threshold of warmth to virtually zero at the threshold of burning pain. Other experiments on areal size estimation and on "two-field discrimination" (analagous to "minimum separable" in vision and "two-point limen" in touch) also demonstrate the level dependence of spatial acuity and summation in the warmth sense.

231 AN INVESTIGATION OF WARM DETECTION IN THE RHESUS MONKEY BY THE REACTION TIME METHOD. R.E. Beitel, R. Dubner, R. Harris and R. Sumino*, NIDR, NIH, Bethesda, MD. 20014.

We have found that reaction times (RTs) to warm stimuli applied to the monkey's face are correlated with stimulus parameters and provide an index of warm sensation. A spring-loaded, feedback-controlled, contact thermode (1 cm diam.) was applied to the upper hairy lip in water-deprived monkeys (N=2). Each monkey was trained to depress a panel for a random period of time (2-8 sec) preceding the onset of a warming shift. Release of the panel within 2.5 sec from the onset of warming was rewarded by water (0.5 cc). The stimulus parameters investigated were intensity (0.4° to 10°C) and velocity (0.3° to 10°C/sec) of the warming shift from different adapted temperatures of the skin (30°, 35° and 40°C), and interstimulus interval (ISI). RTs were influenced by both stimulus intensity and velocity only when the magnitude of the warming shift was small (<1°C) and the stimulus velocity was fast (>5°C/sec). Under these conditions, larger warming shifts resulted in shorter average RTs. Otherwise, RTs were determined by the rate of stimulus change regardless of the stimulus intensity: faster rates resulted in shorter average RTs. At adapted skin temperatures of 30°, 35° and 40°C, average RTs to warming shifts varied inversely with adapting temperature. For ISIs <20 sec, average RTs to the second warming shift were longer when the preceding shift was relatively large (>3°C). We have analyzed the response characteristics of primary afferent cold and warm fibers innervating the same region of the face in rhesus monkeys. We hypothesize that the suppressed response of cold fibers to warming shifts is insufficient to account for warm detection. The response of warm fibers, however, is influenced significantly by stimulus velocity, adapting temperature and ISI in a manner consistent with our behavioral results.

232 RESPONSES TO CUTANEOUS THERMAL STIMULATION OF SPINOTHALAMIC TRACT NEURONS IN THE MONKEY. R.F. Martin*, A.E. Applebaum, R.D. Foreman, J.E. Beall*, and W.D. Willis. Marine Biomedical Institute and Depts. of Anatomy and Physiol., University of Texas Medical Branch, Galveston, Texas 77550.

Neurons of the spinothalamic tract in the lumbosacral spinal cord of anesthesized monkeys (Macaca mulatta) were antidromically activated by stimuli applied to the contralateral caudal diencephalon. Natural mechanical stimuli to the skin of the hindlimb were used to determine the receptive fields and to classify the responses of the spinothalamic tract neurons. Thermal stimuli were applied by a Peltier device in contact with the receptive fields of the neurons. Calibrated heating and/or cooling stimuli produced static and in some cases dynamic responses in most of the spinothalamic neurons tested. The commonest thermal responses signaled noxious heating. However, some neurons were discharged by non-noxious cooling or warming. No units were found responding only to thermal and not to mechanical stimuli, although some units responding to mechanical stimuli were not sensitive to thermal stimuli. The mechanical stimuli activating cells excited by non-noxious thermal stimuli included hair movement and low or high intensity cutaneous stimulation. Noxious heat activated cells having a comparable variety of mechanical receptive fields. The one spinothalamic neuron located in Lamina I so far investigated had a von Frey threshold of 5.5 g., responded to pinching, and was discharged by warming and also by cooling (below 20°C) and noxious heating (over 46°C). (Supported by NIH Grant NS 09743, Training Grants NS 05743 and GM 00459 and the Moody Foundation of Galveston.)

233 CONVERGENCE OF VISCERAL AND CUTANEOUS INPUT ONTO SPINOTHALAMIC TRACT NEURONS IN THE THORACIC SPINAL CORD OF THE RHESUS MONKEY. R.D. Foreman, M.B. Hancock and W.D. Willis. Marine Biomedical Institute and the Department of Anatomy, The University of Texas Medical Branch, Galveston, Texas 77550.

Recordings were made from spinothalamic tract cells in the thoracic spinal cords of anesthetized monkeys (Macaca mulatta). Spinothalamic tract cells were antidromically activated by stimulating their axons in the contralateral caudal diencephalon. Individual tract cells were examined for responses to electrical stimulation of the ipsilateral greater splanchnic nerve and to natural cutaneous stimulation. Afferent volleys elicited in the splanchnic nerve were monitored in the lower thoracic sympathetic chain. The locations of the stimulation sites in the brainstem and the recording sites in the spinal cord were determined from histological reconstructions. A major finding in this study was that spinothalamic tract cells located in the dorsal horn and intermediate gray responded to both cutaneous and visceral input. Adequate cutaneous stimuli included hair movement, light touch, and pinch. For many tract cells the frequency of discharge to mechanical stimulation of the skin increased as the intensity was graded from non-noxious to noxious levels. The cutaneous receptive fields were located over the lower thorax and abdomen and varied considerably in size. The observed convergence of visceral and cutaneous input onto spinothalamic tract cells may provide a basis for referred pain. (Supported by USPHS Grants NS 09018 and NS 09743, and Training Grant NS 05743, and a grant from the Moody Foundation.)

234 RESPONSE OF NEURONS IN TRIGEMINAL NUCLEUS CAUDALIS TO NOXIOUS MECHANICAL AND NOXIOUS THERMAL STIMULATION OF THE MONKEY'S FACE.

J.W. Hu, D.D. Price and R. Dubner, NIDR, NIH, Bethesda, MD. 20014.

Neurons within the spinal trigeminal nucleus caudalis (1-5 mm below the obex) and subjacent reticular formation were studied in rhesus monkeys anesthesized with chloralose. Each cell was characterized in terms of its antidromic responses to stimulation of ventral posteromedial and/or posterior thalamic nuclei and to three types of stimuli applied to its receptive field: (a) graded 5-sec temperature shifts at a rate of 9°C/sec from 35°C to final temperatures of 20° to 54°C, generated by a contact thermode, (b) graded intensities of electrical stimulation to determine the conduction velocities of converging primary afferent fiber populations and (c) mechanical stimulation ranging from light touch to pinch with serrated forceps.

This analysis yielded 5 classes of units distinguished by the range of responses to mechanical stimuli and by the convergence of different primary afferent fiber populations. Type 1 units exhibited rapidly-adapting responses to hair movement or light touch and received only A-alpha primary afferent input. Type 2 units responded to light touch and pressure with maintained discharges and received only A-alpha primary afferent input. Type 3 units responded maximally to pinch with serrated forceps but also were activated by light touch and pressure. They received A-alpha, A-delta and C fiber input. Type 4 units responded to firm pressure and maximally to pinch with serrated forceps. These units had A-delta and sometimes C fiber input. Type 5 units responded only to pinch with serrated forceps and had exclusive A-delta fiber input. Some cells in all five classes responded antidromically to stimulation of the thalamus. Antidromic action potential latencies of Type 1, 2, and 3 units were shorter (mean=2.67±1.07 msec, n=72) than those of Type 4 and 5 units (mean=5.66±2.89 msec, n=16). Receptive field sizes were usually small (1-2 cm^2) for Type 1, 2, 4 and 5 units, and large for Type 3 units (1-3 trigeminal divisions). The marginal layer of N. caudalis contained mostly Type 4 and 5 units, some Type 3 units, but no Type 1 or 2 units. The superficial portion of the magnocellular layer contained mostly Type 1 and 2 units, while neurons at the base of this layer contained Type 3 units and some Type 4 and 5 units. Cells in the subjacent reticular formation included all 5 types but showed a tendency to have large receptive fields (>1 trigeminal division). Neurons responding to noxious thermal stimuli (44-52°C) included 61% (27/44) of Type 3 units, 22% (6/27) of Type 4 units and only 5% (1/20) of Type 5 units. No Type 1 or 2 units responded to thermal stimuli. No units were found that responded exclusively to thermal stimuli. The response patterns of Type 3 and 4 units to noxious thermal stimuli were similar. Thermal thresholds ranged from 38°C to 50°C and stimulus-response functions were monotonic to final temperatures of 48° to 52°C.

The response characteristics and laminar distribution of trigemino-thalamic neurons and interneurons in N. caudalis and subjacent reticular formation are consistent with data obtained in the spinal cord dorsal horn. The data support the hypothesis that nociceptive input is coded centrally by neurons with a wide dynamic response range as well as by those neurons exclusively responsive to noxious stimuli.

235 PERIPHERAL AND CENTRAL NEURAL MECHANISMS THAT MODIFY FIRST AND SECOND PAIN EVOKED BY NOXIOUS HEAT PULSES. D.D. Price, J.W. Hu, R. Dubner, and R. Gracely*, NIDR, NIH, Bethesda, Maryland 20014.

Abrupt, short-lasting, noxious stimuli are common in every day experience. Often such stimuli produce an initial sharp pain followed by a second burning pain. Psychophysical experiments were carried out on 4 human subjects to determine how first and second pain are influenced by peripheral receptor mechanisms and by central nervous system inhibitory and facilitatory mechanisms. For these experiments, brief painful stimuli delivered to the hands or feet were a train of 4-8 constant waveform heat pulses generated by a contact thermode (1-cm diameter). During the heat pulse, the temperature at the thermode-skin interface was in the noxious range (44-49^{0}C) for 0.7 sec. The interpulse interval (IPI) varied from 1/40 sec to 1/2.5 sec. The dependent variables, perceived intensities of either first or second pain, were measured by the following procedures: 1) magnitude estimation, 2) cross-modality matching using a hand dynamometer, and 3) reaction times to the noxious heat pulses. Subjects were instructed to respond selectively to either first or second pain. The thermode temperature as well as the output force from the dynamometer were monitored continuously.

The perceived intensity of first pain decreased with each successive heat pulse when IPIs were less than 40 sec. Suppression of first pain occurred most noticeably with IPIs of 4, 6, and 10 sec. During these trains the mean reaction time showed a transition from 1.1 sec to 2.1 sec. When first pain was blocked by ulnar nerve compression for 30-40 min, reaction times remained between 2.0-2.3 sec throughout the train. These reaction times of first and second pain are consistent with A-delta and C fiber conduction velocities, respectively. Suppression of first pain did not occur if the location of the thermode changed between pulses.

In contrast to first pain, the perceived intensity of second pain did not change with IPIs of 1/5 sec and 1/10 sec. The perceived intensity of second pain reliably increased with each successive pulse at IPIs of 1/3 sec and 1/2.5 sec. These results were also obtained after blockage of first pain by ulnar nerve compression and when the location of the thermode changed between pulses.

Identical trains of heat pulses also were applied to receptive fields of A-delta heat nociceptors and C polymodal nociceptors. Responses of these afferents were recorded in the L_6 and L_7 ganglia of anesthetized rhesus monkeys. Responses of both groups of fibers decreased with each successive heat pulse at IPIs ranging between 2 and 40 sec. In general, this temporal suppression was incomplete. Sensitization never occurred using these heat pulses.

These results indicate that first and second pain can be evoked reliably by noxious heat pulses. The decrement of first pain produced by successive heat pulses is related, at least in part, to suppression of A-delta heat nociceptor activity. In contrast, summation of second pain produced by successive heat pulses occurs in the face of partial suppression of C polymodal nociceptor activity. Central facilitatory mechanisms that could account for this summation have been demonstrated in the spinal cord dorsal horn.

236 TOOTH PULP INPUTS AND INTERACTIONS WITH INNOCUOUS STIMULI IN BRAIN STEM NEURONES. B. J. Sessle, B. Holmwood, K. MacLeod and L.F. Greenwood. Fac. Dentistry, Univ. Toronto, Toronto, Canada M5G 1G6.

Tooth pulp is innervated only by small fibres, and pain appears to be the only sensation elicited from it. It thus offers a rare opportunity for studying neural mechanisms that may be involved in pain or its control (e.g. acupuncture). In decerebrate or anaesthetized cats the effects of bipolar tooth pulp stimuli were examined on single neurones recorded in histologically verified microelectrode penetrations of the trigeminal (V) main sensory and oralis nuclei and adjacent brain stem regions. Inputs and interacting effects were also tested using electronically controlled mechanical stimuli applied to orofacial tissues and electrical stimulation of intra- and extra- oral nerves. Neurones relaying to the ventrobasal thalamus were identified by antidromic stimulation techniques. Of a total of 300 V neurones recorded, 40% could be activated by pulp stimulation (latency 2-8 msec) but most (75%) of these also had discrete mechano-receptive fields in the mouth or face. Although 60% of all V neurones projected to the thalamus, only 25% of V neurones with a pulp input did so. Responses to pulp stimulation could be suppressed for 500 msec or more by single or vibratory mechanical conditioning stimuli applied to the same tooth or to other orofacial tissues. However, responses of V neurones to tactile stimuli could also often be inhibited by single or repetitive pulp conditioning stimuli; sometimes early facilitation occurred. The late period of facilitation that has recently been reported following the inhibitory phase was not noted in 50 V relay cells and interneurones tested. Evidence of such late facilitation was seen more medially in reticular formation neurones with widespread inputs involving orofacial and limb tissue, but this effect was not restricted to pulp conditioning stimuli. (Supported by the Canadian M.R.C.).

237 MICROELECTROPHORETIC STUDY OF CAT DORSAL HORN NEURONES ACTIVATED BY NOXIOUS STIMULI. Mirjana Randic and Henry Yu.* Department of Biochemistry and Pharmacology, Tufts Univ. Sch. of Med., Boston, Mass. U.S.A.

Using a microelectrophoretic method in conjunction with dye-marking of cells we have studied the chemical sensitivity of the cat dorsal horn neurones activated by noxious stimuli (at the level of Rexed's Laminae I and II) to glutamic acid, bradykinin and 5-hydroxytryptamine.

We have found that a majority of the examined neurones were excited by L-glutamate. Bradykinin, applied either microiontophoretically or systemically had a powerful excitatory effect. 5-Hydroxytryptamine, predominantly depressed the firing of the dorsal horn neurones activated by noxious stimulation, although excitatory effects were occasionally observed.

These results suggest a possible chemical transmitter or modulator role for L-glutamic acid, bradykinin and 5-hydroxytryptamine at the level of spinal neurones that could be excited exclusively or predominantly by noxious stimuli. (Supported by PHS Grant NS11174-01 and NSF Grant GB 37864).

238 THE DEPENDENCE OF DORSAL ROOT POTENTIAL POLARITY AND TIME COURSE UPON ELECTRODE PLACEMENT AND SEPARATION. Arthur Taub and L.M. Kitahata. Yale Univ. Sch. Med., New Haven, Ct. 06510

The polarity and time course of the dorsal root potentials as conventionally recorded with bipolar leads from the cut dorsal root of the cat are critically dependent upon electrode placement along the root and electrode separation. The time course and polarity of the dorsal root potentials recorded differentially against a non-neural "indifferent" electrode resemble those of the dorsal cord potential. Specifically, the major deflection of the dorsal root potential (DR V), is positive with respect to ground, and not negative, as previously described. Correspondingly, the late negative deflection (DR VI) is negative and not positive. The spatial distribution along the dorsal roots of the major positive and late negative deflections of the dorsal root potential suggest that they are the results of processes intrinsic to the dorsal root fibers ("active processes"), in the case of DR V depolarization, and in the case of DR VI, either hyperpolarization or release of depolarization. The early negative deflections (DR I, II, III) show a spatial decrement along the dorsal root corresponding to an origin from a potential generator extrinsic to the dorsal root fibers ("passive processes"). The polarity of either DR V or of DR VI can be made to vary by suitable placement of electrodes from positive, through zero, to negative. This artifactual dependence of the major features of the extracellularly recorded dorsal root potential upon electrode placement and separation may account for the variation in the reported polarity of the dorsal root potential in different laboratories.

239 UNIT ACTIVITY IN THE GASSERIAN GANGLION ELICITED BY CAT TOOTH PULP STIMULATION. R. Wayne Fields, Richard J. Beale, Richard B. Tacke, and Bhim S. Savara, School of Dentistry, University of Oregon Health Sciences Center, Portland, Oregon 97201.

Several laboratories have demonstrated a regular somatotopic arrangement of first-order Gasserian ganglion units, presumably neurons, having cutaneous receptive fields in the facial area (e.g., Kerr and Lysak, Arch. Neurol. 11:593, 1964; Darian-Smith et. al., J. Neurophysiol. 28:682, 1965; Beaudreau and Jerge, Arch. Oral Biol. 13:247, 1968). Units with receptive fields lying within the Opthalmic dermatomal division (I) were found to occupy rostromedial positions within the ganglion, Mandibular (III) units were found to occupy anterolateral positions, and Maxillary (II) units were found to occupy regions intermediate to those of I and II. These previous reports have also described unit activity exhibiting intraoral, but not pulpal receptive fields. The present information summarizes characteristics of unitary activity recorded in the Gasserian ganglion elicited by bipolar stimulation of the maxillary canine tooth of anesthetized (Halothane) cats. The peripheral origin was verified to be in the test pulp by control tests following pulpal extirpation. The initial units which we have identified have exhibited latencies ranging from 1.1 - 3.6 ms which, judging by estimated conduction velocities and allowances for conduction time in the sidearm bridge of the unipolar ganglion cells, are of the A-delta class. Unitary thresholds ranged to 0.4 - 4.0 v, and there was an inverse relationship apparent between latency and threshold. Response waveforms were predominantly monophasic of 30 - 100 μv. amplitude. At present, no C-fiber units have been observed, but a systematic search for such units is continuing and will be discussed in view of a recent report of their presence (Anderson and Pearl, Exp. Neurol. 47: 357, 1975).

240 FUNCTIONAL ROLE OF DIFFERENT NEURONE TYPES IN THE ROSTRAL TRIGEMINAL NUCLEI OF THE CAT. Douglas W. Young and Kay-M. Gottschaldt. Dept. Neurobiol., MPI f. Biophysik. Chemie, 34 Göttingen, West Germany.

Neurones in the rostral trigeminal nuclei receiving tactile input from the face, especially the vibrissae, were investigated electrophysiologically. Continuous infusion of pentobarbitone ensured stable anaesthesia throughout the experiment. Under these conditions primary afferent, relay and inter-neurones could be distinguished using the criteria of receptive field size during natural stimulation, characteristics of the discharge plus the latency and excitability following electrical stimulation of the cutaneous receptive field and the contralateral ventrobasal thalamus. Second order relay neurones exhibited different degrees and often changeable patterns of convergence and in most cases their responses showed clear deviations from responses in first order neurones. Interneurones fell into two classes, those with responses proportional to the intensity of natural stimulation and those which discharged a burst of impulses, independent of the strength of the mechanical stimulus. As a means of influencing the input-output relationship at relay neurones we suggest that the two types of interneurones participate in the control of 1) the degree of convergence onto relay cells and 2) the capacity of relay neurones to transmit tactile information coded in the responses of individual mechanoreceptive afferents. It is not yet clear whether or not these control mechanisms operate jointly or independently.

241 DEAFFERENTATION OF ONE SIDE OF THE TRUNK IN CATS. Michael E. Goldberger and Marion Murray, Dept. Anat., Medical College of Pa., Phila. Pa. 19129.

The contribution to locomotion made by the dorsal roots of one side of the trunk was compared to the contribution of ipsilateral hindlimb afferents. Dorsal roots T5-T13 were cut extradurally and, in some cats, their ganglia were also removed. The immediate post-operative effects were striking and consisted of a marked curvature of the vertebral column toward the deafferented side, 'forced' turning of the head and eyes to the side contralateral to the lesion, loss of righting from the same side, hyperextension of the ipsilateral hindlimb and hyperflexion of the contralateral hindlimb with both legs abducted. These effects may be attributed, in part, to an asymmetry of body-on-body and body-on-head righting reflex input. In contrast, hindlimb deafferentation and/or ganglionectomy (L1-Cd8) produces an ipsilateral hindlimb flaccid paralysis with no demonstrable effects on trunk or head postures. The time-course of recovery was examined by determining the speed and accuracy with which the animals performed a simple conditioned locomotor task: crossing 9-foot runways ranging from 2"-12" in width. Initially, the animals can cross only the wide boards, due to the postural asymmetry, hindlimb abduction, and loss of pelvic fixation. These show compensation in time and the animal's speed increases. During the second week, accuracy in locomotion is first seen and the animals can now cross the 2" runway, but slowly. Later, speed increases and a plateau is reached by 5 weeks. The same pattern of recovery follows hindlimb deafferentation. Thus, locomotor recovery appears to take place in three stages: first, uncontrolled movements recover and their speed increases; second, accurate placement of the limbs recovers and, third, as accuracy increases, so does the speed of accurate movements. During the recovery period movements of the ipsilateral hindlimb appear important in compensation for the loss of postural control. If the hindlimb is deafferented in animals who have recovered from trunk deafferentation, the performance decompensates and many of the original postural abnormalities reappear. The stages of recovery are then repeated in a similar time course, although the degree of recovery is not as great as before. Thus, although the ipsilateral hindlimb plays a role in compensation for trunk deafferentation, it is only partly responsible.

Locomotion is thus disrupted following either thoracic or lumbosacral deafferentation but the reason for the disruption is different in each case. Both hindlimb and trunk afferents contribute to locomotion but apparently, their contribution is qualitatively different. The normal central distribution of trunk afferents was therefore mapped using standard degeneration methods, and compared with the lumbosacral root distribution. The most caudal extent of lower thoracic root projection is L2, thus direct control over limb movements is unlikely. The intraspinal distribution is similar in pattern to that of lumbosacral roots except that the projection to medial lamina VIII and VII from thoracic roots is much greater. In the dorsal column nuclei, the projection to the cell cluster region is proportionately smaller than that to the ventral cell groups (unlike the lumbar roots) where it overlaps the pyramidal and non-primary afferent projections. The behavioral results suggest that the recovery of locomotion mediated by the ipsilateral hindlimb afferents when postural mechanisms have been disrupted may be related to the compensatory use of hindlimb movements rather than to recovery of postural functions *per se*. (Grant No. NINDS 1R01 NS 11919-01)

242 FORELIMB DEAFFERENTATION IN RHESUS MONKEYS: PRECISE MOVEMENTS WITHOUT VISUAL GUIDANCE. Gil C. Allen*, J. Orbach, D. Berman and A. J. Berman. Dept. of Psychology, Queens College, Flushing, N.Y., Dept. of Neurosurgery V.A. Hospital, Bronx, N.Y. and Mt. Sinai School of Medicine, New York, NY

Monkeys with dorsal roots C2-T3 sectioned bilaterally can be trained so that they use their deafferented forelimbs to execute precise movements between two spatially separated targets without visual guidance. For this purpose, a 3 by 3 display of touch-activated buttons was mounted in front of chaired animals and training was conducted in darkness. The approach used was to train a button pressing response involving a sequence of two buttons. Naive intact monkeys took 2-7 days, with an average of 300 trials per day, to respond reliably to a first, briefly lit button (100 msec). By comparison, deafferented monkeys, tested four weeks after surgery and after shaping in the light to reach for food, could not be made to respond to the briefly lit button but did respond if this button remained lit until pressed, providing some visual guidance of the limb to this first location. Three to four days of training, with an average of 200 trials per day, were required. This procedure was utilized as a means of providing the deafferented animals with a precisely defined start position for the two-button task. Touching the lit start button resulted in its offset and the onset of the target button. This second button was dimly lit (0.78 mL, 100 msec) to eliminate the possibility of visual guidance of the responding limb. Intact monkeys needed only a few training days to reach 90% criterion of success. Deafferented monkeys, on the other hand, were not able to perform the task. Shaping, by allowing the monkeys to respond in light and then gradually reducing the light, or with the target button lit for 1 sec and then reducing its duration, resulted in the animals making the response, but only under the conditions of shaping. When testing was carried out in darkness and the target button only briefly lit, the monkeys again could not make the response, even after many thousands of shaping trials. It was found however that pulsing of the target button (100 msec, 2/sec) until it was pressed, followed by a systematic decrease in the number of pulses provided, was effective in shaping the response under the required conditions. By this method, deafferented monkeys achieved an 80% criterion of success with the target button briefly lit, after 16-25 days of training. It should be noted that, even after considerable training, the responses made by the deafferented monkeys were not of the smooth, direct nature of those seen in intact animals. Instead, the limb was raised, the elbow flexed and the hand thrust toward the target button.

Once the two button response was established, monkeys could respond to the target button even when it did not light. Furthermore, they could reach the same target position, briefly lit, from new start positions with little additional training and no pulsing of the target light. Thus, they could not only perform an already learned response without visual guidance, but they could perform a new movement to a spatially defined location without visual guidance during training or testing. This task, which required performance without visual guidance, could only be trained under circumstances in which visual monitoring of the responding limb was minimal or absent. It appears therefore that training with visual guidance, while appropriate to prepare the animal for performance that includes vision, may in fact interfere when the final task requires performance without visual guidance.

Our findings may be interpreted in several ways. It is unlikely that the trained response reflected simply a conditioned movement pattern, as deafferented monkeys were able to reach a previously trained target position from new start positions. It is possible, however, that some form of brain representation of the spatial location of the target button is established (eg. memory trace, cognitive map). It is also possible that the required movement of the deafferented limb is correlated with aspects of movements of intact body parts, such as head or eyes.

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243 WHITE NOISE ANALYSIS OF MAMMALIAN MUSCLE RECEPTORS. G. P. Moore, D. G. Stuart, R. M. Reinking* and E. K. Stauffer. Dept. of Electrical Eng., Univ. Southern California, Los Angeles, Calif. 90007 and Dept. of Physiology, School of Medicine, Univ. of Arizona, Tucson, Arizona 85724.

The dynamic response properties of typical group Ia, Ib and II afferents from the medial gastrocnemius muscle of the cat have been studied using recently developed techniques which employ random stimulation. A random signal having a Gaussian amplitude distribution and a flat spectrum from 0-250 Hz was used as the input to an electromechanical device attached to the tendon of the muscle and designed to execute controlled length (or force) changes in a follow-up servo mode. Resulting changes in force (or length) and the spike train of the receptor resulting from the random stimulus were recorded as the output variables. In various experimental runs the length of the muscle was varied by several mm around the standard length, and in length-servo mode, peak-to-peak random length changes were varied from 200-800μ. The resulting data were analyzed using special computational algorithms appropriate to the view that the receptor output is a sequence of delta functions. Accordingly, the first- and second-order Wiener kernels for all three receptor types have been calculated. In the absence of reflex activity, the dynamic properties of muscle receptors can be characterized by two time functions which describe the mean and variance of the input signals in the 10 msec epoch prior to each receptor spike. Further analysis of this pre-spike epoch furnishes important information about those input signals to which each type of receptor is maximally sensitive. (Supported by U S P H S grants NS 07888 and RR 05675).

244 LONG-TERM NERVE FIBER RECORDING AND STIMULATION IN MAMMALS. J.A. Hoffer and W.B. Marks. Biophysics Dept., Johns Hopkins Univ., Baltimore 21218 and Laboratory of Neural Control, NIH, Bethesda 20014.

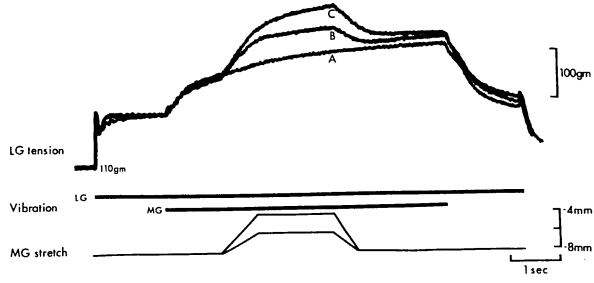
The tenuissimus muscle of rabbit (a functional knee flexor) is supplied by a single nerve 250μm in diam. that has about 100 myelinated fibers and innervates only that muscle. We captured several mm of nerve in 2 consecutive insulating cuffs, with original connectivity and blood supply kept intact, following our technique described earlier (Soc. Neurosci. Abstr. 4:300, 1974). We recorded differentially between the midpoint and shorted ends of each cuff. Time delays of spike appearance allowed us to classify afferents and efferents as we monitored single-unit activity in alert, freely-moving rabbits. We correlated unit activity with limb position as determined from videotaped locomotion on a treadmill. Unitary spikes, initially 10-40μV, reached 100μV during the first days post-implant, but over several weeks became smaller again. We attribute this to connective tissue growth in the cuffs, which at first perhaps raises the resistance by replacing fluid, but later coats the Pt contacts and separates them from the nerve. In two long-term animals, acute recording under pentobarbital after 2½ and 4½ months showed spindle primary afferents which could be driven by vibration. Further, squeezing the ipsilateral foot caused contraction of the tenuissimus muscle, showing that at least part of its motoneuron supply was intact. Lastly, stimulation through contacts in the cuffs caused contractions that could be graded from single twitches through sustained tetanus for stimulus currents in the 20-30μA range. Histology showed that not less than one quarter of the original fibers had survived. We submit that this method of chronic recording and stimulation --as anticipated by R.B. Stein et al (Can. Fed. Biol. Soc. 18, 1975)--may be suited for interfacing with the original nerve supply and establishing direct control of prosthetic devices or paralyzed limbs in humans.

245 An Estimate of the Gain of the Secondary Spindle Afferent Pathway in Extensor Muscles of the Decerebrate Cat. W.Z.Rymer and K.Kanda, Lab. of Neural Control, NINCDS,NIH,Bethesda,Md, 20014

When the cat medial gastrocnemius(MG) is subjected to combined longit--udinal vibration and slow extension, there is typically an increase in tension of its synergist, the lateral gastrocnemius(LG) which is greater than that induced by vibration alone. We have examined this effect in 10 decerebrate cats, in which medial and lateral gastrocnemius muscles were separated and attached to two independent muscle pullers, each carrying a tension myograph. Since longitudinal tendon vibration of appropriate amplitude is known to preferentially excite primary endings, and to'clamp' their discharge frequency, the observed LG tension increase was probably a result of increased secondary spindle afferent discharge from the MG muscle. This excitatory effect varied considerably with animal condition, but proved to be approximately proportional to MG extension amplitude in stable preparations.

The figure below illustrates the effect of simultaneous MG vibration and extension on LG tension. The LG tendon was also vibrated as indicated, to prevent interference from transmitted vibration or fusimotor reflex effects. The three superimposed tension traces are ensemble averages (10 trials each) in which the onset of MG vibration and MG stretch was successively delayed to allow the specific effects to be measured. Trace A represents the effects of simultaneous independent vibration of the MG and LG tendon on LG force, and was used as a baseline for measurement of the additional force increment produced by added MG extension, as represented in traces B and C. In trace B a 2mm stretch produced a 51gm LG tension increase, whereas trace C demonstrates a tension change of 94gm in response to a 4mm stretch. These values suggest that MG secondary discharge contributed excitation producing 24gm mean LG tension increase per millimeter MG extension.

The importance of this secondary effect can be judged by comparing it with the tension induced by MG stretch or vibration, applied separately. MG stretch alone was found to produce a linear increase in LG tension of 35-50 gm(LG)/mm(MG). MG vibration alone produced an increase in LG force which was proportional to vibration frequency, with an estimated slope of 1.0-1.9 gm/hz. Since stretch induced primary ending discharge has rarely been shown to exceed 10 impulses/mm (when measured during maintained stretch) the primary endings would have contributed no more than 10-19gm/mm to the stiffness of the synergist stretch reflex. The estimated stiffness resulting from the secondary afferent stimulation was typically 18-25gm/mm and this was usually greater than the calculated primary contribution. If we assume that the pattern of primary and secondary spindle afferent projection is similar for homonymous and heteronymous motoneurons, then these experiments support the view that secondary spindle afferents are excitatory to extensor motoneurons (Matthews,P.B.C., J.Physiol. 204, 365-393, 1969) and indicate that their contribution may be at least as powerful as that made by the primary spindle afferents.



246 ON THE SPINAL STEPPING GENERATOR. V.R. Edgerton, S. Grillner and A. Sjöström[x], Dept. Physiol. University of Göteborg, Sweden

Hindlimb walking movements are generated by interneurones within the spinal cord. This network operates even after curarization and a spinal cord transection if e.g. noradrenergic precursors are administered (Grillner and Zangger, Acta Physiol. Scand. 1974, 91, 38-39A). The aim of this investigation is to study how this network operates by recording (1) spinal interneuronal activity, correlated to the simulataneous rhythmic efferent activity in filaments to ipsi- and contralateral flexors and extensors and (2) intracellular motoneuronal activity. Unlike previous studies all phasic activity from supraspinal structures was eliminated. In thirteen adult cats, the spinal cord was transected (Th 12) and 10-12 ipsi- and contralateral hindlimb nerves dissected and mounted for stimulation. Several natural filaments on both sides were isolated for recording efferent activity. The intact ventral roots of L 7 and the ipsi- and contralateral ventrolateral funiculi could be stimulated for purposes of identification of the neurones. The cats were curarized and given Nialamide (50 mg/kg) and DOPA (10-50 mg/kg) i.v. Extra- and intracellular recordings were performed conventionally with glass microelectrodes filled with sodium or potassium chloride or citrate. Rhythmic activity of 78 interneurones in the L 7 segment of the spinal cord were studied. The onset and termination, peak frequency and the general shape of the frequency curve of the interneuronal bursts was related to the onset and termination and intraburst variations in efferent activity. Approximately half of the interneurones had an activity that paralleled the entire period of either ipsi- or contralateral flexor or extensor activity. Other neurones had a peak activity or entire period of activity tightly related to only the onset or termination of flexor or extensor activity. Mean variations in time between two highly correlated events in the interneurone and efferent activity in most cases represented less than 1 % of the cycle duration. Mean correlation ratios between such points was 0.96 ± 0.06 (SD). All neurones tested have been possible to activate in the longlasting reflex discharges occurring after DOPA (Jankowska et al. Acta Physiol. Scand. 1967, 70, 389-402). Short trains of stimulation of peripheral hindlimb nerves in most cases failed to disrupt the interneuronal and efferent synchrony that existed during spontaneous rhythmic activity even though the phase of the efferent cycle could be altered markedly. Five cells have been monosynaptically activated from group I afferents and two from the ventral roots, i.e. Renshaw cells, and three antidromically from the contralateral ventrolateral funiculus which could serve as coordinating neurones between fore- and hindlimbs. In each step cycle a burst activity or large depolarization could be recorded in α-motoneurones which was followed by an abrupt hyperpolarization and then a subsequent gradual depolarization until a new burst was initiated. The period of hyperpolarization that occurs during spontaneous efferent activity is due not only to disfacilitation but also to active postsynaptic inhibition, which was shown conventionally by chloride injection and hyperpolarizing currents.

247 PROJECTION PATTERNS AND AXONAL BRANCHING OF RETICULOSPINAL NEURONS. B.W. Peterson, R.A. Maunz*, N.G. Pitts* and R.G. Mackel*. The Rockefeller University, New York, N.Y. 10021.

Extracellular microelectrodes were used to record the activity of reticulospinal neurons within the medial ponto-medullary reticular formation in the cat. In one series of experiments, reticulospinal neurons were activated antidromically from electrodes placed in the ventro-medial reticulospinal tract (RST_m) and in the ipsi- and contralateral lateral reticulospinal tracts (RST_i, RST_c) at spinal levels C_{1-2}, C_4, Th_1 and L_1. Stimuli consisted of 0.1 ms monopolar constant current pulses with amplitudes of 0.05-1 mA. Responses were identified as antidromic by their constant latencies even with threshold stimulation, their ability to follow stimulus frequencies up to 100 Hz or more and the presence of collision block. An individual reticulospinal neuron was classified as projecting in one of the three reticulospinal tracts if it could be driven from that tract by stimuli less than half as intense as those required to drive it from the other two tracts. Of 120 neurons that satisfied this criterion, 76 were found to project in RST_i, 38 in RST_m and 6 in RST_c.

Locations within the brain stem of all reticulospinal neurons were determined by reference to dye marks made with the recording electrodes. RST_m neurons were found primarily in n.r. pontis caudalis and the rostro-dorsal part of n.r. gigantocellularis, areas which correspond to the region from which reticulospinal excitation of motoneurons can be evoked (Grillner & Lund, 1968; Wilson & Yoshida, 1969). 71% of these neurons projected as far as the lumbar spinal cord. Their axons had a median conduction velocity of 101 m/sec, which is consistent with the short latencies of reticulospinal excitation in lumbar motoneurons. RST_i neurons projecting to C_4 and beyond were clustered in the caudo-ventral part of n.r. gigantocellularis, which corresponds to the region from which reticulospinal inhibition of motoneurons can be evoked (Llinas & Terzuolo, 1964). 63% of the entire population of RST_i neurons projected as far as the lumbar spinal cord. Axons of RST_i neurons had a median conduction velocity of 68 m/sec, which may in part account for the relatively long latency of reticulospinal inhibition in lumbar motoneurons. RST_i neurons projecting only to the first three cervical segments formed a spatially distinct group rostral and dorsal to other RST_i neurons, suggesting that they may comprise a functionally specialized subpopulation within the reticulospinal system. RST_c neurons were found throughout n.r. gigantocellularis and had a median conduction velocity of 70 m/sec.

In a second series of experiments, microstimulation with 0.1 ms current pulses of 50 μA or less was used to activate branches of reticulospinal neurons within the gray matter of the cervical enlargement. Measurements of the range of stimulus action as a function of stimulus intensity indicated that 33 neurons were antidromically activated from within the ventral horn of C_{6-8} by stimuli too weak to spread to the spinal white matter. Twenty-two of these 33 neurons also sent axons to the lumbar spinal cord, indicating that reticulospinal neurons may act at more than one segmental level, as do lateral vestibulospinal neurons (Abzug et al., 1974). In several cases, movable microstimulating electrodes were used to trace the precise course of reticulospinal axon branches within the cervical gray matter. The arborizations of such axon branches were quite extensive and in one case included large areas of laminae VII and VIII on both sides of the spinal cord.

Supported in part by NSF Grant NSF GB 36927 and NIH grants NS 02619 and NS 05463.

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248 THE ORGANISATION OF MUSCLE AFFERENT AND CUTANEOUS INPUT TO THE UPPER CERVICAL CORD IN THE CAT. V. C. Abrahams and F. J. R. Richmond*. Dept. of Physiology, Queen's University, Kingston, Ontario K7L 3N6.

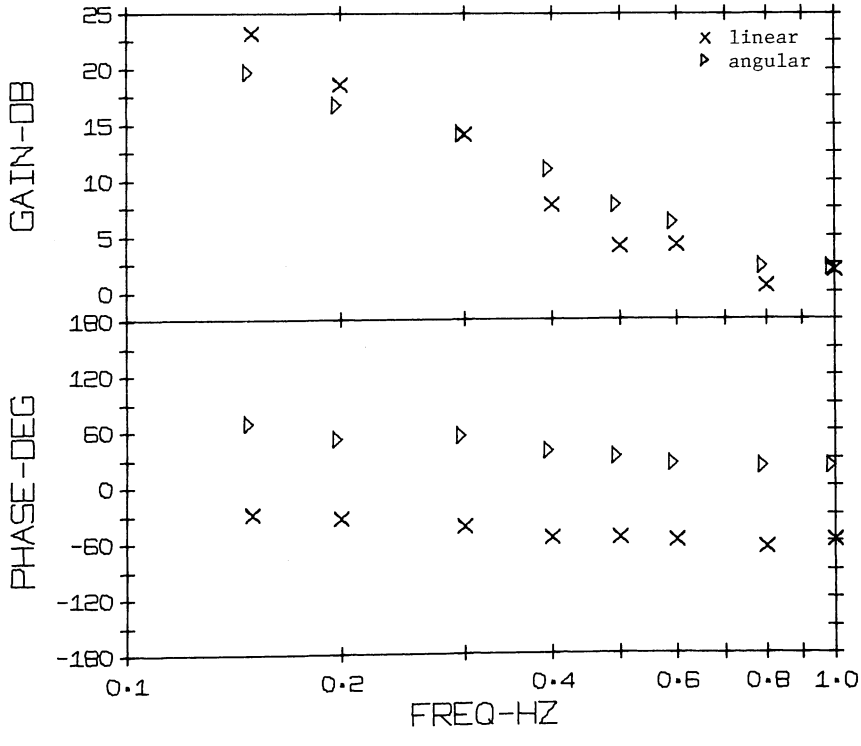
The motor functions of the upper cervical cord are mainly confined to head movement. The segmental organisation of this part of the spinal cord would therefore be expected to reflect the intrinsic organisation and specialisation of the head movement system. Certain unique specialisations have already been found in these segments, for example, the absence of a monosynaptic reflex (Abrahams, Richmond and Rose, Brain Res. 92, 130, 1975). We have now made a microelectrode study of the distribution of units in C1 to C3 activated by skin and neck muscle afferent stimulation. In general the distribution of such units is similar to that found elsewhere in the spinal cord in that cutaneous units predominate in the more dorsal region of n. proprius and both muscle and cutaneous afferents project to the deeper regions of the nucleus. At these deep levels convergence and interactions between cutaneous and neck muscle afferents can be demonstrated. As expected the most effective cutaneous stimuli are those applied to areas of the face supplied by the trigeminal nerve, and only a minority of cutaneously activated units could be excited by stimulation of neck skin. It may be concluded that the laminar organisation of afferent projections to the upper cervical cord is similar to that of the lumbosacral cord with one important exception. Segmental relations are displaced and trigeminal input substitutes for segmental cutaneous inflow at least in C1 and C2. Such an arrangement would seem appropriate for a segmental organisation concerned with head movement, for facial stimuli can be prepotent in leading to head movement. It also explains the projection of facial afferents into upper cervical segments, and the fact that at this level there is no morphologically distinct spinal nucleus of the trigeminal nerve. Supported by MRC and Queen's University.
*Studentship of the MRC.

249 HUMAN MUSCLE SPINDLE RESPONSE PATTERNS RECORDED DURING VOLUNTARY MUSCLE CONTRACTION. Doris Burg*, Alfred J. Szumski[1] and Albrecht Struppler*. Neurol. Clinic, Tech. Univ. Munich, W. Germany, and Dept. of Physiology[1], Medical College of Virginia, Richmond, Virginia, 23298.

Muscle spindle receptors were isolated, identified and recorded in the median nerve of unanesthetized human subjects, using tungsten semimicroelectrodes (110-150 kOhm). Single receptor units were isolated in the nerve by minute manual manipulations of the electrode; the receptor was identified by criteria which included passive stretch and response during the electrically induced twitch and the phasic reflex; the muscle of receptor origin was identified by electrical stimulation through the recording electrode located at the muscle nerve fascicle. During an isometric voluntary contraction, the majority of muscle spindles were coactivated with alpha activity in spite of muscle contraction spindle unloading (silencing). However, spindles were isolated which were unloaded during minimal contraction and coactivated with increasing strengths of contraction, and some spindles were effectively unloaded at all contraction strengths. Further, spindles which revealed through testing a low dynamic sensitivity were coactivated during the contraction phase and were minimally influenced to not influenced during relaxation. Spindles with a high dynamic sensitivity were mostly silenced during the contraction and fired in a burst-like manner during relaxation. The results suggest differences of spindle involvement in voluntary contraction depending on the specific gamma drive, and the importance of alpha-gamma coactivation during isometric contraction.

250 VESTIBULAR INFLUENCES ON TRICEPS BRACHII IN DECEREBRATE AND DECEREBELLATE CATS. J. H. Anderson, J. F. Soechting and C. A. Terzuolo. Lab. of Neurophysiol., University of Minnesota, Minneapolis, 55455.

The dynamic relations between vestibular inputs and the motor output of forelimb extensor muscles were studied during natural vestibular stimulation. Decerebrate cats were immobilized with plaster casts, and then subjected to sinusoidal, horizontal accelerations so as to excite either afferents from semicircular canals or from otoliths. For angular accelerations the head of the cat was placed directly over the center of rotation, ipsilateral acceleration being positive. For linear accelerations two directions were used: along the cat's long axis (forward acceleration being positive) and perpendicular to it (ipsilateral acceleration being positive). For both inputs, the phase of the motor output relative to acceleration differs significantly from the phase relations between primary afferents and acceleration as well as vestibular nuclei neurons and acceleration (see figure below). Furthermore, data obtained in chronic decerebellate cats indicate that this phase difference between vestibular afferents and motor output is not due to cerebellar activities. However, the absolute value of the gain is reduced by approximately 7 dB after cerebellectomy, over the frequency range investigated. Finally, modulation of the motor output was only slightly affected by dorsal rhizotomy and abolished by bilateral labyrinthectomy. These results demonstrate that there is considerable processing of primary vestibular afferent activity at the brain stem level for the organization of the motor output responsible for postural adjustments. The data appear to be adequate to provide a block diagram of the logic by which vestibular inputs are utilized for the control of posture. Supported by USPHS Grants NS-2567 and NS-5494.



251 MOTOR CORTEX PYRAMIDAL TRACT NEURON (PTN) DISCHARGE IN MONKEYS IS ENHANCED BY MISMATCH BETWEEN INTENDED AND ACTUAL DISPLACEMENT. E. V. Evarts and J. Tanji*. Lab. Neurophysiology, NIMH, Bethesda, MD 20014.

Phillips (1) has proposed that one factor controlling motor cortex PTN discharge is a signal of mismatch between an intended and an actual displacement. In making this "servo" hypothesis, Phillips referred to an experiment by Evarts (2) showing a relation between PTN discharge frequency and the load opposing a movement with which the PTN was active. In addition to this "servo" control of PTN activity, of course, there is "centrally programmed" control which can give rise to PTN discharge in the absence of feedback from the periphery.

The present experiment on monkey motor cortex PTN activity was designed to dissociate the "servo" and the "centrally programmed" factors in motor cortex PTN discharge, and to provide additional observations relevant to Phillips' hypothesis that PTN discharge would be favored by mismatch between an actual and an intended displacement.

The key element in the design of this experiment was to independently vary 1) the monkey's "intended movement" and 2) the displacements of the arm which opposed or assisted this intended movement. To achieve this dissociation, monkeys were trained to respond to externally produced handle displacements in either of two ways according to a prior instruction (PULL or PUSH). Execution of either of the two instructions could be elicited by either of two possible directions of torque-motor produced displacement. Thus, the intended movement (determined by the instruction) could be varied independently of the displacement, which could either match (assist) or mismatch (oppose) the intended movement. In this experiment, motor cortex PTNs in arm area were independently classified according to their relations to active ("centrally programmed") movement and passive (externally produced) movement. In a group of 20 PTNs which were reciprocally related to both the active and to passive movement, there was a striking tendency for PTNs which discharged for a given direction of active (i.e., intended) movement to be excited by the opposite (i.e., mismatching) direction of passive movement. This relation was observed for 18/20 PTNs. When the external displacement assisted (i.e., matched) the intended movement, its initial effect was to suppress PTN discharge. In spite of this initial suppression by the "matching" input, PTN activity due to the "central program" would nevertheless occur, but the intensity of "centrally programmed" PTN discharge was reduced when the peripheral displacement assisted the movement.

In analyses of the timing of PTN discharge following the displacement serving to trigger the intended movement, it was apparent that this PTN discharge had two components: 1) a relatively short-latency (25 msec) component which depended on the nature of the input to the arm, and 2) a longer-latency (50 msec) component which depended on the movement that the monkey had been instructed to perform. While the first component was input-related and the second component was output-related, interaction of input and output was seen for both components. Thus, a perturbation which was excitatory for the first (or input) component speeded the occurrence of the second (or output) component and an intended movement which involved output of a given PTN enhanced the response of the PTN to an excitatory perturbation.

These findings provide confirmation for Phillips' hypothesis, and show that PTN discharge associated with "centrally programmed" or "intended" movements is enhanced by peripheral inputs indicating mismatch between the intended and the actual displacement.

1. Phillips, C.G. (1969), Proc. Roy. Soc. B. 173: 141-174.
2. Evarts, E.V. (1968), J. Neurophysiol. 31: 14-27.

252 CONNECTIONS BETWEEN MOTOR CORTEX CELLS AND MOTONEURONS REVEALED BY CORRELATION TECHNIQUES IN AWAKE MONKEYS. Eberhard E. Fetz, Dwight C. German, and Paul D. Cheney. Regional Primate Research Center and Depts. of Physiol. and Biophysics, and Neurol. Surg., Univ. of Wash., Seattle 98195

Previous investigators have demonstrated monosynaptic connections to motoneurons by summing the intracellularly recorded membrane potentials following single spikes in the presynaptic cell. We investigated the statistical effects of single motor cortex cells on the firing probability of motoneurons by summing rectified EMG activity following action potentials of precentral cells. Unitary EPSP's produced by pyramidal tract (PT) cells in motoneurons are subthreshold, but would be expected to transiently augment the firing probability of an active motoneuron. Monkeys were trained to alternately flex and extend the wrist against a load and hold for 1-2 seconds. Averages of full-wave rectified EMG activity following 2-12 thousand action potentials were compiled with a LAB-8 computer. Of 70 cells related to the wrist movements, 8 were followed by transient increases in firing probability of covarying motor units in the multiunit EMG record. These increases in firing probability began abruptly at latencies between 6 and 11.5 msec following the cortical spikes and had a time course similar to monosynaptic EPSP's evoked by electrical stimulation of cortex. For the remaining cells, the mean level of average EMG activity either remained constant (54) or changed steadily (8) over the entire analysis interval (5 msec before to 25 msec after the cortical spike). We conclude that the transient increases in mean EMG activity were probably produced by monosynaptic connections between the cortical cells and relevant motoneurons. Simultaneous recordings from synergistic muscles suggest that a single PT cell may contact motoneurons of more than one muscle.

253 COORDINATION OF LONG-LATENCY (FSR) REFLEX RESPONSES AMONG MUSCLES OF THE LEG DURING STANCE POSTURE CONTROL IN HUMANS. Lewis M. Nashner and C. Curtis Boylls*. Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, Oregon 97210.

The EMG activity of four proximal and distal leg muscles was measured during the evocation of FSR responses in order to test the idea that the FSR is a "group" response, coordinated within a number of muscles during performance of a stance task. The previous study showed that the "Magnitude" of an FSR response in the ankle joint muscles is either augmented or reduced, depending on its utility as a stabilizer during specific stance tasks. Although the FSR stimulus was direct rotation of the ankles (either extension or flexion), FSR responses also appeared in quadriceps and hamstring muscles approximately 5-10 msec preceding those in the stretched ankle muscles (tibialis anterior or gastrocnemius). Unlike those in tibialis anterior or gastrocnemius, FSR responses in quadriceps and hamstring were cocontractive and were evoked by extension or by flexion of the ankles. When FSR "Magnitudes" were altered in the stretched ankle joint muscles, those in the two proximal muscles were also altered in approximate proportion. Hence the FSR actually involves a group of leg muscles, with modifications being applied proportionately to all muscles in the group.

The characteristics of the FSR relationship among muscles is termed the FSR "Structure". Constancy of FSR "Structure" was measured in several tasks during which FSR "Magnitude" was altered. The "Structure" of the FSR appears to be a property which is determined independently of FSR "Magnitude". Currently being explored are task conditions during which FSR "Structures" may be modified.

254 FIELD POTENTIALS IN TELEOSTEAN SPINAL CORD. EVIDENCE FOR PAD. H. VANEGAS* and P. RUDOMIN. Centro de Investigación del IPN , México D.F. and IVIC, Caracas, Venezuela.

A comprehensive understanding of the role of presynaptic modulation of synaptic transmission in vertebrates requires comparative studies in groups other than mammalian and amphibian. Preliminary observations reported herein suggest the existence of primary afferent depolarization (PAD) in the spinal cord of the carp (Ciprinus carpio). Adult carps were anesthetized with tricaine,paralized with gallamine and artificially respirated. The exposed cord dorsum was stimulated with platinum ball electrodes and field potentials were recorded 1-3 segments caudally with 3M NaCl micropipettes. The responses consist of an early negative wave with two components (20 and 10 m/sec conduction velocity) followed by an early slow wave (ESW) which peaks at about 4-8 msec, lasts 10-15 msec and merges with a late slow wave (LSW) which lasts 80 to 100 msec. Field potential profiles indicate that the ESW is maximally negative 600 to 800μ below the cord dorsal surface at 300 to 400μ lateral to the midline. The corresponding source is located 800 to 1100μ below the dorsal surface and 200μ lateral to the midline, thus suggesting an horizontal dipole. The LSW is maximally negative at 800 to 1100μ below the surface at about 400μ lateral to the midline. This negativity may be associated to a medial source or may itself extend towards the midline. Histology has shown that the neuronal somata are located near the central canal at 800 to 1000μ from the dorsal surface while the dendrites extend laterally. Therefore, the ESW is interpreted as arising from synaptic depolarization of distal dendrites by afferent fiber collaterals, and the LSW, which is picrotoxin sensitive, as being due to depolarization of such afferent fiber terminals. Studies are in progress to further establish the nature of these potentials.

Partly supported by NIH grant NS 9196-05 and by CLAB.

255 MOTOR UNIT DISCHARGE PATTERNS DURING TREADMILL WALKING AND TROTTING IN THE CAT. F.E. Zajac and J. Young. Dept. of Elec. Eng., Univ. of Maryland, College Park, Maryland 20742.

It has been found that the initial interval in a pulse train must be a "doublet" though later ones may be quite variable to produce maximum tension area from single units (Young and Zajac, this meeting). We have studied the behavior of units to assess whether such pulse patterns occur during locomotion controlled by CNS stimulation (Shik et al, Biofizyka 11:659-666, 1966). Single units were isolated from ventral root filaments (L7-S1) and were presumed either extensor (53 units) or flexor (24 units) by correlating their discharge with electrical activity in ankle extensor and flexor muscles during walking. Some units were presumed γ-motoneurons by their high discharge rate during rest which was modulated only slightly during locomotion. Extensor α-motoneurons fired in bursts with average rates (31 pps; range 13 to 59 pps) which did not depend on treadmill speed though the number of discharges decreased with faster speeds. Flexor units fired at higher rates (43 pps; range 30-59 pps) with numbers which remained the same or increased with speed. Sychronization of unit discharge with brain stimulation was weak and observed infrequently. All flexor and most extensor units (86%) began firing in each step cycle with doublets followed by quite variable discharge bursts. We conclude that a unit's discharge pattern is organized to produce quick development of the muscle unit's active state. (Supported by NIH grant NS 11518).

256 POSTCONTRACTION ACTIVATION OF MOTOR NEURONS IN SPINAL ANIMALS. Robert S. Hutton and Shuji Suzuki*, School of Physical and Health Education, University of Washington, Seattle, 98195.

Postcontraction sensory discharge (PCSD) occurs in hindlimb muscle following ventral root (VR) stimulation or reflexive activation and is believed related to afterdischarge from spindle Ia afferents (Hutton, Smith, Eldred, 1973, 1975). Suzuki and Hutton (1975) recently reported that, of 62 motoneurons isolated in the L_7VR, 26 of 36 units facilitated by ramp stretch also responded with an increased frequency in spontaneous discharge ($P < .01$) following isometric contraction. No significant change was noted in frequency response from units inhibited or uninfluenced by stretch. As these observations involved intact cats (chloralose-urethane anaesthesia), the segmental potency of PCSD on motoneurons without supraspinal bias could not be assessed. Therefore, identical experiments were performed on spinal animals with cords transected at T12.

Four cats were denervated to isolate the innervation of the triceps surae. The Achilles tendon was attached to a force transducer mounted to a displacement manipulator. Tetanus was produced by stimulating the cut S_1VR at 100 Hz and 5 times twitch threshold. Isolated motoneurons were recorded from filaments in the cut L_7VR. This activity was displayed on a Tektronix oscilloscope and filmed with a Grass C4 camera for later frequency analysis.

Sixty-one motoneurons (MNs) were divided into four functional groups according to their stretch reflex response. These groups were MNs excited by stretch (MN-S+), MNs inhibited by stretch (MN-S-), MNs not influenced by stretch (MN-S_o), and MNs showing mixed or unpredictable responses to stretch (MN-S_m). Changes in postcontraction motoneuronal discharge (PCMD) are shown in the table that follows.

SUMMARY OF POSTCONTRACTION CHANGES IN DISCHARGE OF MOTONEURONS GROUPED ACCORDING TO THEIR STRETCH REFLEX RESPONSE

	$PCMD_{\uparrow}$	$PCMD_{\downarrow}$	$PCMD_o$	TOTAL
MN-S+	22	1	10	33
MS-S-	0	0	2	2
MN-S_o	0	2	10	12
MN-S_m	6	1	7	14
TOTAL	28	4	29	61

These results are similar to those found in intact animals with the exception that postcontractile changes in MN frequency (significant ($P < .01$) for the MN-S+ group only) were lower. More phasic units were identified in spinal animals (39%) than in intact animals (16%). In summary, muscle afferents are sufficiently excited during PCSD to activate MNs presumably serving the contracted muscles. On the basis of the earlier findings by Hutton, Smith, and Eldred, we conclude that these changes in MN firing represent the functional outcome of an increased spindle Ia discharge following an isometric contraction.

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Hutton, R.S., Smith, J.L., and Eldred, E. Persisting changes in sensory and motor activity of a muscle following its reflex activation. Pflügers Arch., 353:327-336, 1975.
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Supported by BSSG, RR-07096, NIH and GSRF.

257 IDENTIFICATION OF FAST AND SLOW TYPES OF MOTONEURONS. Dale A. Harris and Elwood Henneman. Dept. Physiol., Harvard Medical School, Boston, Mass. 02115.

These experiments were designed to identify different types or species of motoneurons by means of size-independent differences in their firing patterns. Single units belonging to the plantaris pool were isolated in ventral root filaments of decerebrate cats. The plantaris nerve, cut distally, was stimulated electrically at 500 pps to elicit repetitive firing of these motoneurons. The maximal firing rate of each isolated motoneuron was measured. In order to minimize the effects of differences in motoneuron size, which are known to influence firing rate, the plantaris units selected for study were those whose critical firing levels were limited to the 0-8% range. An analytical method was devised which indicated that the maximal firing rates of these motoneurons were not sampled from a single population. The distribution of rates was clearly bimodal suggesting that within the 0-8% range there are two populations of motoneurons, a fast firing type and a slow firing type. These two types of motoneurons appear to be distributed coextensively throughout the 0-8% range. (Supported by a grant from the National Institutes of Health).

258 THE POTENTIAL DEPENDENCE OF THE AFTER-HYPERPOLARIZATIONS OF EXTRAOCULAR MOTONEURONS. N. H. Barmack. Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, Oregon 97210.

The frequency of discharge of extraocular motoneurons varies over a large dynamic range (10-800 imp/sec). Following an action potential, there are two distinct after-hyperpolarizations (AHP). The first, AHP_1, immediately follows the action potential and has a duration of 2.5-3.0 msec. The second, AHP_2, follows AHP_1 and has a duration in excess of 50 msec. The potential dependence of these AHP's in trochlear motoneurons was studied in anesthetized and paralyzed cats. The membrane potential of impaled motoneurons was biased by sub-threshold intracellular current injections. The effects of these membrane potential displacements on the amplitudes of antidromically evoked AHP_1 and AHP_2 were measured. AHP_1 had a reversal potential of -70mV and an average compensation for displacements from the reversal potential ("compensation gain") of .36. AHP_2 had a reversal potential of -80mV and a compensation gain of .08. These different reversal potentials and compensation gains predict that the interspike interval would be controlled by the conductance underlying AHP_2 at low levels of membrane depolarization, and by the conductance underlying AHP_1 at more depolarized levels. This prediction is confirmed for repetitive discharges evoked by intracellular stimulation. Measurements of input resistance of these motoneurons demonstrate a 30-50% decrease in slope resistance at depolarized membrane potentials. This rectification might represent an additional shunt for currents generated at or near the soma, and would be expected to decrease selectively the amplitude of AHP_2 which is thought to be generated primarily at the soma. In the case of repetitive discharges at high frequencies, this shunting might prevent excessive hyperpolarization caused by summation of AHP_2 conductances. Such summative hyperpolarizations occur in spinal motoneurons, but are absent in extraocular motoneurons.

259 SINGLE MOTOR UNIT CHARACTERISTICS IN MONKEY JAW MUSCLE DURING CONSTANT FORCE HOLDING. William Clark and Erich S. Luschei (SPON: T.C. Ruch). Reg. Prim. Res. Ctr. and Dept. of Physiology and Biophysics, Sch. Med., Univ. of Wash, Seattle, Washington 98195.

The activity of single motor units was recorded in the temporalis muscle of monkeys trained to maintain a constant force on a bite bar between their front teeth for 1-2 seconds for an applesauce reward. The force required for reinforcement was altered by changing the position of a visual force target. Twitch contraction times and twitch tensions were obtained for each unit by triggering a computer of average transients from the action potential of the motor unit and averaging the force record in the interval immediately following the spike (Stein et al., Brain Res. 40: 187, 1972). Motor units were also characterized by the lowest force at which steady firing is seen, average frequency of firing as a function of force and the degree to which the firing of one motor unit is synchronized with the firing of other units. Most of the units tended to increase their firing frequency abruptly over a narrow range of forces just above threshold and then to fire at a more or less constant rate at higher forces. The twitch contraction times of motor units in this muscle were found to be considerably shorter than those reported for human hand muscles and for cat hindlimb muscles. Motor units which were recruited at low levels of force had relatively long twitch contraction times (25-35 msec.) while units which were only recruited at high levels of force had relatively short twitch contraction times (15-25 msec.). In this respect the motor units of the monkey temporalis muscle followed the "size principal" proposed by Henneman (J. Neurophysiol. 28: 560, 1965), but exceptions to this principal were also seen. The most notable exception was that near threshold motor units often began firing late in a maintained force although they were not recruited at the same force earlier in the trial. (Supported by NIH grants NS08596, RR00166, and GM00260-16).

260 UNCERTAINTY OF RECRUITMENT ORDER WHEN TESTED WITH INTRACELLULAR TECHNIQUES H.P. Clamann and S.J. Goldberg. Depts. of Physiology and Anatomy, Medical College of Virginia-VCU, Richmond, Virginia, 23298.

The present experiments were designed to test the effects of glass microelectrode penetration on the recruitment order of spinal motoneurons in the cat. Animals were anesthetized with chloralose. The nerves to medial gastrocnemius (MG) and to lateral gastrocnemius and soleus (LG-S) were placed on electrodes for monophasic recording, and the L7 and S1 dorsal roots were cut and their proximal portions stimulated at one pulse every two seconds. Such stimulation produced monosynaptic reflexes whose size in either MG or LG-S was determined by measuring the integrated monophasic response. It is known that motoneurons are recruited in order of size in monosynaptic reflexes. Motoneurons of MG or LG-S were impaled with micropipettes. A cell was considered "healthy" if its resting membrane potential was stable and exceeded 50 mV. We were able to record from MG or LG-S motoneurons which were "healthy" and had conduction velocities greater than 80 m/sec, but which discharged reflexly in the absence of measurable MG or LG-S reflex response (critical firing level = 0). Cells which were not "healthy" regularly had critical firing levels of 0. It was possible to record shifts in the critical firing levels of some "healthy" cells; other such cells exhibited stable critical firing levels and appeared normal in their reflex responses. Cells with apparently normal CFL could only be recorded with small electrodes (impedance > 20 MΩ). (For results of intracellular recording see abstract by Goldberg and Clamann.) We conclude that impaling motoneurons may alter their functional properties without producing obvious changes in membrane potential or spike shape. (Supported by a grant from the A.D. Williams Foundation.)

261 EFFECTS ON MOTONEURON REFLEX DISCHARGE PRODUCED BY MICROELECTRODES OF DIFFERENT SIZES. S.J. Goldberg and H.P. Clamann. Depts. of Anatomy and Physiology, Medical College of Virginia-VCU, Richmond, Virginia 23298.

Intracellular records were made from either medial gastrocnemius or lateral gastrocnemius-soleus motoneurons in the cat spinal cord in response to muscle nerve and dorsal root stimulation. The recruitment characteristics of these motoneurons, as reflected by their critical firing levels (CFLs), were studied. Micropipettes filled with 1.6M potassium citrate were either advanced vertically through the dorsal root entry zone or at an angle of 65° to the cord starting medial to the dorsal roots. Both of these approaches produced varying degrees of spinal cord "dimpling" which varied among animals and electrodes. We were routinely able to penetrate and make stable intracellular recordings from motoneurons with either "large" micropipettes (1.0 to 1.5μm tip diameter and 1.0 to 5.0 MΩ impedance) or "small" finely drawn electrodes (20 to 70 MΩ) during the course of an experiment. Penetration of cells with "large" electrodes often caused an increase in the cells' susceptibility to reflex discharge, although membrane potentials exceeded 50 mV. The cells' CFLs were usually at or near zero, regardless of cell size as determined by conduction velocity. We hypothesize that the "large" electrodes may have depolarized these cells because this finding was consistent. Penetration of cells with "small" electrodes could cause depolarization or, more often, leave the cell normal with respect to reflex discharge and electrical criteria. We also observed cells with abnormally high CFLs using "small" electrodes. We hypothesize that spinal cord "dimpling" and/or alterations in afferent inputs by the approaching electrode could cause CFL shifts in either direction. Motoneurons with normal CFLs were usually electrically stable. Cells which discharged spontaneously upon penetration and/or had action potential durations >2 msec. always had CFLs at or near zero. (See abstract by Clamann and Goldberg for further details.) (Supported by a grant from the A.D. Williams Foundation.)

262 SPECIFICITY OF PROJECTION OF SINGLE TRICEPS IA AFFERENT FIBERS TO HOMONYMOUS AND HETERONYMOUS MOTONEURONS IN THE CAT. J. G. Scott* and L. M. Mendell. Duke Med. Ctr., Durham, N. C. 27710

We have investigated the averaged individual EPSP's evoked in identified triceps motoneurons (MG, LG and SOL) by the action of single identified Ia fibers from the triceps muscles. Many fibers projected to virtually all homonymous motoneurons studied; others, particularly LG made a less complete projection. The projection to heteronymous motoneurons was generally less extensive than to homonymous motoneurons. Analysis of variance indicated that EPSP amplitudes are significantly larger when 1) evoked in homonymous rather than heteronymous motoneurons, 2) evoked in a SOL motoneuron, and 3) evoked by an LG Ia afferent fiber. The larger EPSP's in SOL motoneurons probably reflect their higher input impedance. The larger EPSP's evoked by LG may reflect compensation for the smaller number of LG Ia fibers (35) compared to MG (60) and SOL (55). Comparison of pairs of EPSP's from different Ia afferents in the same motoneuron reveals that shapes of EPSP's are similar in homonymous and heteronymous motoneurons. Thus differences in EPSP amplitudes in homonymous and heteronymous motoneurons cannot be accounted for by segregation of Ia terminals on the motoneuron. The complete overlap of MG and SOL motoneuron pools suggests that specificity of Ia connections to them (and to LG) arises from inherent differences in their properties rather than their locations. However, there is also a tendency for MG and SOL afferents to make more efficacious connections with caudal motoneurons, and LG with rostral motoneurons. Supported by NS 34608, NS 08411 and GM 00929.

263 IA DISYNAPTIC PATHWAYS STUDIED BY SPIKE TRIGGERED AVERAGING OF SYNAPTIC NOISE. D. G. D. Watt*, E. K. Stauffer*, D. G. Stuart, A. Taylor* and R. M. Reinking*. Dept. Physiol., Coll. Med., Univ. Arizona, Tucson, Az. 85724.

This report describes the application of synaptic noise averaging techniques (Mendell & Henneman, J. Neurophysiol. 34: 171, 1971) to Ia excitatory and inhibitory pathways involving more than one synapse. In 38 cats, intracellular recordings were made from motoneurons of medial gastrocnemius (MG) and six synergists and antagonists. Isolated APs from a single MG Ia afferent were recorded from subdivided dorsal rootlets or by recording extracellularly from dorsal root ganglion cells. Synaptic noise was averaged while triggering from these spikes. Of 228 cells analyzed, depolarizing responses have been observed in 111. Of these, 86 were considered to be monosynaptic on the basis of latencies from cord entry ranging from 0.4 to 1.1 msec (mean amplitude ± S.D. = 54.1 ± 58.7 μV). In 14 cases, latencies were compatible with the presence of two or more synapses (1.2 to 2.4 msec, 12.9 ± 14.3 μV). In 11 cases, latencies were >2.4 msec and these responses (4.2 ± 2.1 μV) could have been tri- or polysynaptic. Hyperpolarizing responses were noted in 41 cells. In 20 cases, latencies from cord entry indicated the presence of two or more synapses (1.2 to 2.4 msec, 3.8 ± 2.2 μV). In 10 cases, latencies were >2.4 msec (2.6 ± 0.8 μV). These responses were judged to be IPSPs on the basis of the lack or sharp attenuation of such effects when recording extracellularly. Eleven cells showed earlier hyperpolarizations with latencies of 0.5 to 1.1 msec (5.5 ± 6.5 μV). If these early responses are true postsynaptic inhibitory effects, they would appear to confirm anatomical observations that individual primary afferent collaterals can directly innervate antagonistic motoneurones (Scheibel and Scheibel, Brain Res. 13: 417, 1969). The alternate explanation that they represent disfacilitation or intracellular recordings of excitatory fields attributable to monosynaptic effects of the single Ia afferent cannot be ruled out at this time, however. (USPHS grants NS 07888 and RR 05675).

264 SYNAPTIC EFFECTS OF SINGLE GROUP IB AND II MUSCLE AFFERENT FIBERS ONTO LUMBOSACRAL MOTONEURONS. E. K. Stauffer*, D. G. D. Watt*, D. G. Stuart, A. Taylor* and R. M. Reinking* (SPON: M. Wetzel) Department Physiology, College of Medicine, University of Arizona, Tucson, Arizona 85724.

This report describes extension of the afferent-triggered averaging technique to the study of central connections of group Ib and II muscle afferents. In 38 cats, single Ib or II afferents from medial gastrocnemius (MG) were isolated by subdividing dorsal rootlets or recording extracellularly from dorsal root ganglion cells. Synaptic noise from MG motoneurons and six of its synergists and antagonists was averaged while triggering from the afferent spikes. Ib effects were studied on 83 cells and were divided into: hyperpolarizing, 25; depolarizing, 18; no effect, 40. Hyperpolarizing effects were usually di- or polysynaptic (latencies from cord entry >1.1 msec) and recorded from MG or synergist motoneurons. Depolarizing effects were also predominantly di- or polysynaptic, but were usually seen in motoneurons of MG antagonists. Group II effects were studied on 66 cells and were divided into: hyperpolarizing, 3; depolarizing, 21; no effect, 42. Two of the hyperpolarizations were di- or polysynaptic (latencies >1.1 msec, mean amplitude 2.5 μV), in an indirect antagonist of MG; one was noted in an indirect synergist and its latency was 1.1 msec (1.1 μV). Depolarizing effects were predominantly monosynaptic (latencies <1.2 msec), larger (28.3 μV) and were seen in MG and direct synergists. Central connections of Ib afferents detected here agree with those previously reported. The group II excitatory connections confirm recent findings of Kirkwood & Sears (J. Physiol. 245: 64P, 1974) and are compatible with the view (Matthews, J. Physiol. 204: 365, 1969) that group II afferents may make a significant excitatory contribution to extensor stretch reflexes. These connections, and our preliminary finding of inhibition onto flexor motoneurons, suggests that activity in group II afferents does not necessarily produce the flexor reflex pattern (NS 07888 and RR 05675).

265 INTRACELLULAR POTENTIAL RECORDINGS FROM PHRENIC MOTONEURONS DURING SPONTANEOUS ORTHODROMIC ACTIVATION. C.L. Webber, Jr. and K. Pleschka.† Max-Planck-Institut für Physiologische und Klinische Forschung, D-6350 Bad Nauheim, West Germany.

Broken tip microelectrodes were used to intracellularly monitor membrane potentials of cat phrenic motoneurons, spontaneously driven by automatic respiratory "centers". Cells, identified by antidromic stimulation, exhibited complex membrane potentials of which three components were distinguishable: synaptic potentials (input); slow wave potentials (CRDP) action potentials (output). As a function of time, the spike frequency increased linearly to a peak just before the burst ended. Likewise, the slow wave depolarization paralleled the time course of frequency discharge so that a linear correlation existed between the two parameters; i.e. a mean slope of 5 cps increment in frequency for every millivolt of depolarization was found. This relationship could be explained by a similar mechanism driving each separately; e.g. increased temporal and spatial summations of EPSPs during the crescendo of input from medullary neurons during inspiration. To study the input-output coupling, membrane potential trajectories between spikes were examined. Phrenic interspike potentials, crudely resembling lumbosacral motoneurons rhythmically discharging during current stimulation, assumed a two component pattern: scoops (hyperpolarizations); ramps (EPSP summations). Initial interspike intervals were shortened by a shallowing out of the scoop until it disappeared. Then the ramp became steeper to maintain the increase in spike frequency. Increasing spike threshold throughout the train was approximately balanced by the slow wave depolarization.

266 TRACING SPINAL NEURONS PROJECTING TO THE VENTRAL HORN USING RETROGRADE TRANSPORT: POSSIBLE LAST-ORDER INTERNEURONS TO MEDIAL GASTROCNEMIUS MOTONEURONS. P.L. Strick, R.E. Burke, K. Kanda* and C.C. Kim*. Lab Neurophysiology, NIMH & Lab Neural Control, NINCDS, NIH, Bethesda, MD 20014.

Under aseptic conditions the L5 to S2 spinal segments were exposed in adult cats anesthetized with pentobarbital. Horseradish peroxidase type VI (HRP) was injected under pressure through glass micropipettes into the region of maximum antidromic field of medial gastrocnemius (MG) motoneurons, as recorded through the same pipette. After 24 hrs, animals were reanesthetized and perfused with 1% paraformaldehyde - 1.25% glutaraldehyde. The lumbosacral cord was removed and frozen cross-sections were processed to demonstrate HRP according to standard methods. In one cat selected from the series, injection of 0.03 μl of 5% HRP in saline into caudal L7 produced heavy extracellular deposition of HRP reaction product in a region of dorsolateral lamina 9 less than 0.5mm in dia, surrounded by an irregular region with appreciably less extracellular staining. Most of the labeled neurons (i.e., with HRP-positive granules indicative of retrograde transport) were found between mid-L6 to caudal L7, mainly in the 6 mms rostral to the injection site. Neurons in sections 1 mm on either side of the injection site were not included because of the possibility of direct labeling by diffusion. The highest concentrations of labeled cells were in ipsilateral laminae 5, 6 and dorsolateral 7; fewer were found in the rest of lamina 7 and in lamina 9. A small number occurred in L4 in dorsal lamina 7, up to 30mm rostral to the injection site. Contralateral labeled cells were rare. In another animal, a larger injection (0.06 μl) produced a larger deposition region and higher concentrations of labeled neurons, but with approximately the same laminar distribution. It is likely that at least some of the segmental interneurons identified by HRP uptake include the last-order interneurons that project to MG motoneurons.

267 THALAMO-CORTICAL PROJECTIONS OF THE VA AND VL IN THE RHESUS MONKEY. K. Kalil* (Spon: R.M. Benjamin). Department of Anatomy, University of Wisconsin, Madison, WI 53706

The thalamo-cortical projections of the nuclei ventralis anterior (VA) and ventralis lateralis (VL) were studied in the rhesus monkey with the autoradiographic methods for tracing neuronal pathways. Following injections of (^{3}H) proline into VA, labeled terminals were found in those regions of the premotor cortex (area 6) occupying the medial wall of the hemisphere and the dorsal bank of the cingulate sulcus. After injections confined to VLc (Olsziewski, 1952) heavy labeling was found in area 6 on the medial wall of the hemisphere and on the cortical surface dorsal to the arcuate sulcus. Injections of VLo and VPLo revealed a topographic projection to the motor cortex (area 4) such that medial regions of the VLo-VPLo project upon the arm and face areas and lateral regions project upon the leg area. Moreover, injections extending as far caudally as the VPLc revealed that a considerable extent of the VPL complex projects not to the postcentral sensory cortex but to the precentral motor cortex. The autoradiographic methods also demonstrated that the thalamic efferents to the motor cortex terminate massively in layer 3, moderately in layer 6, and sparsely in layer 1.

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268 DIRECT RELATIONSHIP OF THE DENTATE NUCLEUS WITH THE SPINAL CORD. Heinrich Bantli and James R. Bloedel. Depts. Neurosurg., and Physiol., Sch. Med., U. of Minn., Mpls., Minn., 55455.

Stimulation of the dentate nucleus was previously shown to affect the excitability of spinal neurons via pathways which do not involve the primary sensorimotor cortex. Experiments were performed in cats to determine whether one of these pathways would be a reticulospinal projection activated directly from the dentate nucleus via the descending limb of the brachium conjunctivum. A direct dentato-reticular projection was demonstrated by recording single unit responses to appropriate stimuli in the dentate nucleus and the reticular formation. On the assumption that these neurons may also receive a significant input from the spinal cord, responses to stimulation of four hindlimb nerves in five Rhesus monkeys were recorded from neurons in the ipsilateral dentate nucleus. Their location was subsequently verified by histological techniques. A change in firing patterns following stimulation of the dissected nerves was observed in 53 cells. In general the responses consisted of an initial increase followed by a marked decrease and a subsequent increase in impulse activity. The latencies of most responses were between 19-24 msec. Although some variation in the magnitude of the responses evoked by the different peripheral inputs were observed among the neurons studied, there was no consistent correlation between response magnitude and the particular peripheral nerve stimulated. These findings showing the presence of a significant spinal input to neurons in the dentate nucleus and the previous observation that the dentate nucleus can affect the excitability of spinal neurons without involving pathways via the primary sensorimotor cortex suggest that functional concepts based on the exclusive interaction of the dentate nucleus with the cerebral cortex and specific thalamic nuclei should be reexamined. This work was supported by NIH Grant NS-09447-05 and Contract NO1-NS-2332.

269 AN ELECTRON MICROSCOPIC STUDY OF RUBROSPINAL PROJECTIONS TO THE LUMBAR SPINAL CORD OF THE OPOSSUM. G. E. Goode. Dept. of Anat., Sch. Med., The Ohio State University, Columbus, Ohio, 43210.

The rubrospinal tract projects to the lateral and medial cell columns of lamina V-VII of the lumbar cord in the American opossum, Didelphis marsupialis virginiana (Martin and Dom, 1970). Neuron cell bodies within the lateral portions of these lamina measure 20-35 micra, whereas neurons more medially disposed measure 12-20 micra. Neurons within both groups have 5-7 primary, radially branching dendrites which measure 4-5 micra at their origin and taper to less than a micron. Following stereotaxic rubral lesions, or tractotomy at midbrain, or cervical levels, degenerating axons and presynaptic profiles were identified in the neuropil of the lumbar enlargement of fourteen opossum. Axons in the dorsolateral portion of the lateral funiculus were identified by their darkened axoplasm 4-9 days following the lesion. The majority of these axons measure two micra or less (including myelin); 25% have diameters between 4-12 micra. Degenerating axons (1-3 micra) were frequently encountered across the neuropil in the dorsal horn (lamina III-VI). Degenerating boutons were identified by the "dark reaction" at 2-4 days. They contacted distal dendrites (0.5-2.0 micra) in the lateral base (lamina IV-VI) and intermediate dendrites (2.0-3.5 micra) in the medial cell column. Some degenerating presynaptic profiles were present within synaptic complexes in more dorsal lamina of the cord. No axosomatic or "light reaction" contacts were observed. This attempt to identify synaptic end structures in the adult opossum is part of a project designed to study the development of long descending fiber projections to the lumbar cord in "fetal" pouch young.

270 PROPERTIES OF THE CORTICORUBRAL NEURON SYSTEM IN THE HAND AND WRIST AREA OF THE PRIMATE MOTOR CORTEX. D. R. Humphrey and R. R. Rietz*, Laboratory of Neurophysiology, Emory Univ. Sch. Med., Atlanta, GA., 30322.

Anatomical and pathway transection studies suggest that the cortico-rubrospinal system may play an important role both in the normal control of movement, and in mediating the wide range of voluntary motor activities that are retained by subhuman primates after pyramidotomy. It is not known at present, however, whether the corticorubral (CR) projection in the primate originates separately from the pyramidal tract (PT), or whether it is composed primarily of PT axon collaterals. Moreover, there is currently no direct evidence concerning the nature of the synaptic effects that are exerted by the primate motor cortex upon red nucleus (RN) cells.

To obtain data bearing on these questions, microelectrode techniques were used to examine the composition of the CR neuron system within the precentral wrist and hand area of the anesthetized monkey's motor cortex (rhesus monkeys, 30 mg/Kg Nembutal). Stimulating electrodes were placed within the RN and the medullary PT, allowing us to antidromically identify CR neurons and to determine the proportions that were PT as opposed to non-PT cells. By applying conditioning stimuli to the medullary pyramid and to the motor cortex, it was also possible to determine the general synaptic effects exerted separately by PT and by non-PT cortical cells upon rubrospinal tract (RST) neurons. Our test response consisted of the RST potential evoked by stimulation of interposito-rubral fibers, and recorded at the cervical cord. Extracellular recordings were obtained from a total of 325 CR neurons in 14 animals, and the conditioning-testing experiments were performed in an additional five. Our major results may be summarized as follows.

(1) The conduction velocity distribution for CRNs was bimodal, with peaks at 12 and 38 m/sec. A breakdown of the unit sample in terms of fast (cv > 25 m/sec) and slow cells, and PT versus non-PT neurons, is shown in Table I. It is clear from these data that the major cortico-rubral outflow from this region of the motor cortex is derived from a population of small extrapyramidal neurons.

Table I.

	PT	Non-PT
Fast	54	9
Slow	24	238

(2) The cells were concentrated in layers III-V of the cortex, intermingled with non-CR, PT cells. An average of 3 CR cells were isolated per electrode track, compared to an average of 7 PT cells. The CR neuron population is therefore a numerically significant one.

(3) In contrast to previous findings in the cat, where PT collateral inhibition is pronounced, stimulation of PT axons produced only a mild (15-30 %) increase in the excitability of RST cells, lasting 30-40 msec. Stimulation of the motor cortex produced a much larger excitability increase (50-120 %), apparently because of additional and stronger synaptic inputs from the population of non-PT CR cells. The cortical input was never sufficiently strong, however, to produce a synchronous discharge of RST neurons similar to that evoked by stimulation of interposito-rubral fibers. This finding, coupled with the observed pattern of cortically evoked field potentials, suggests that CR synapses are distributed principally over the peripheral dendrites of RN cells.

These findings suggest that the primate cortico-rubrospinal system is a descending motor pathway that is largely separate throughout its anatomical course from the PT system. Given plastic increases in the potency of CR synapses, a reasonably direct route is therefore available to the motor cortex for exerting a significant degree of control over spinal motorneurons following destruction of the pyramidal tracts. Neglecting possible anesthetic effects, however, our data suggest that, in the normal animal, the CR pathway may serve primarily to set the steady, background level of depolarization in RN neurons, thus affecting their responsiveness to more powerful synaptic inputs from the cerebellum. A study of the movement related behavior of CR cells is currently in progress. (Supported by NIH Grant NS-10183).

271 ORIGIN AND DISTRIBUTION OF NIGROTECTAL FIBERS IN THE CAT. Ann M. Graybiel and Thomas R. Sciascia*, Dept. of Psychology, Massachusetts Institute of Technology, Cambridge, Mass.

Following electrophoretic injections of tritiated amino acids into the substantia nigra of the cat, labelled fibers can be traced to the stratum griseum medium of the ipsilateral superior colliculus. Labelling over this layer is not uniform but instead, appears in both frontal and sagittal sections as a series of 300-500-μ wide patches or puffs alternating with more sparsely labelled zones. In cases so far analyzed, this puff-like pattern of afferent-fiber termination is most prominent in the caudal two-thirds of the superior colliculus, including the zone of representation of the temporal crescent, while at rostro-medial levels the projection appears sparse or diffuse.

In order to localize more precisely the origin of this projection system, horseradish peroxidase was injected into the superior colliculus in three cats. In each case, clusters of HRP-positive neurons appeared in the pars reticulata and pars lateralis of the substantia nigra. These observations suggest that, in the cat, a highly ordered nigro-tectal projection may serve to link the striatum with the "motor" layers of the superior colliculus. When taken together with recent evidence for an interrupted pattern of retinal-fiber termination within the stratum griseum superficiale (Graybiel '75, Hubel, LeVay and Wiesel '75), these findings further suggest that a vertical organization may characterize both superficial and deep layers of the superior colliculus.

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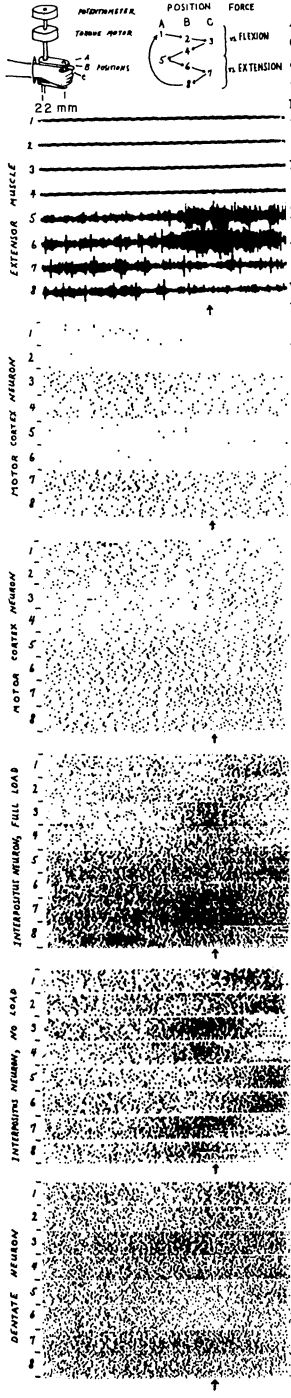
272 CODING DIFFERENCES IN ACTIVITY OF MUSCLES, MOTOR CORTEX, AND CEREBELLAR NUCLEI DURING A MORE COMPLEX MOTOR TASK. W.T. Thach. Departments of Physiology and Neurology, Yale Medical School, New Haven, Conn. 06510



A task was designed to dissociate pattern of muscle activity, force exerted by the muscles, angle at the wrist joint (position), and anticipation of the direction of the next movement (premovement directional set). A rhesus monkey moved a rod in a horizontal plane by flexing and extending the wrist. The wrist was stabilized and movements at elbow, shoulder, and trunk were minimized. A potentiometer monitored position and a torque motor applied force of 1 lb. against the hand. The monkey moved the rod 40° to each of 3 positions-A(flexor), B(middle), and C(extensor)-in a prescribed ABCBABCBAetc. sequence. There were no clues (at B) as to where the next movement should be and the monkey had to remember the sequence. The force changed from vs. flexion to vs. extension on alternate cycles (after return to A). To advance in the sequence, the monkey held within a 20° position window (correct holding signalled by a light) for a randomly varied 5-10 sec until another light signalled to move to the next position. If the monkey moved initially in the correct direction and to the next position without overshoot within about 400 msec it was rewarded with fruit juice. MUSCLES: EMG traces during each of the 8 experimental conditions are shown for a forearm extensor muscle. All traces are of 500 msec duration; onset of movement is at the arrow. Under flexor load, the muscle is silent. Under extensor load, the muscle was tonically active for holding in all positions; extensor movements were caused by increasing and flexor movements by decreasing extensor activity. An opposite profile was seen for forearm flexors. Similar but less intense profiles were seen for a few proximal muscles, but most muscles did not change activity in relation to the task. MOTOR CORTEX: Rasters of neural discharge (PTNs and nonPTNs) were made for each of the 8 experimental conditions. Of 48 neurons that showed differences in maintained discharge in relation to the experimental conditions, 22 had raster profiles resembling the first shown. Maintained discharge during the hold period was high prior to movements in one direction and low prior to movements in the opposite direction, independent of the pattern of muscle activity used to hold and move. Six neurons (possibly the PTNs of Evarts) had profiles like the second shown, more resembling those of muscle, and changed discharge frequency in relation to changes in the direction and magnitude of load. INTERPOSITUS: of 13 neurons showing differences in maintained discharge, 12 behaved as the one illustrated, having a more marked relation to the direction and magnitude of load than any neuron in motor cortex. But unlike muscle and load-related motor cortical neurons, in the unloaded state the burst associated with movement occurred for a movement opposite that direction for which holding against load had increased the maintained discharge. DENTATE: of 11 neurons with differences in maintained discharge, 8 were like the one illustrated, resembling the premovement directional set type of profile seen also in motor cortex. No dentate profile resembled muscle or interpositus profiles. No profiles for any of these four structures correlated with position of the wrist.

273 DISCHARGE OF INTERPOSITUS NEURONS IN RELATION TO A VOLUNTARY MOVEMENT. J. E. Burton and N. Onoda*. Div. of Neurosciences, City of Hope Natl. Med. Center, Duarte, CA 91010.

Cats were trained to flex and extend the elbow joint and prepared for chronic head fixation and access to the cerebellar nuclei. The discharge of single interpositus neurons, EMG of biceps and triceps muscles, and displacement of the forearm about the elbow joint were then recorded while the animals performed the task under various load conditions. Neurons were isolated, mainly within the anterior interpositus nucleus, whose behavior was uniform and related consistently to the movements. The discharge rate of these neurons increased in advance of the biceps activity responsible for the flexion and decreased in advance of the decrease in biceps activity responsible for the extension. Once the arm was in motion, the discharge rate changed in parallel with the velocity of the motion, being inversely related to velocity during flexion and directly related to this parameter during extension. It is suggested that this relationship was due to input to the cerebellum generated by rate-sensitive receptors in the moving limb. It was further observed that increases in biceps activity were always preceded by increases in discharge rate of the interpositus units and decreases in biceps activity by decreases in unit discharge rate both before and during the motion and regardless of the direction of the motion. Therefore, the discharge of the interpositus neurons may have been involved in regulating the motor output to the biceps throughout the performance of the task, with input data on the velocity of the motion playing a critical role while the movement was in progress. (Supported by NIH Research Grant No. NS 11798 from NINDS)

274 CEREBELLAR INFLUENCE ON SIMPLE REACTION TIME IN PRIMATES. J. Hore*, J. Meyer-Lohmann*+ and V. B. Brooks. Dept. Physiol., Univ. of Western Ontario, London N6A 5C1, Ontario, Canada.

Does the cerebellum participate in movement initiation through an influence on motor cortex discharge? Simple reaction time to a visual GO signal was investigated in 3 Cebus monkeys that were trained to make elbow flexions and extensions by moving a handle in a horizontal arc. Reaction time was measured from the GO signal to onset of EMG in biceps or triceps and to movement onset as detected by a velocity threshold. Mean reaction times were of the order of 200-250 msec to EMG onset and 250-300 msec to movement onset. Cooling to 7.5°C probe reference temperature through stereotaxically implanted probes adjacent to either the dentate or interpositus nucleus, increased mean reaction times by 50-100 msec as measured by onset of either EMG or movement. Raster plots of units recorded in motor cortex revealed that in general their normal relation to movement onset was preserved during cerebellar cooling. However cooling changed the overall discharge activity of some units while the discrete pattern of activity of others was lost. These results support the notion that the cerebellum plays a role in the initiation of movement through its influence on motor cortex.

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275 CONTROL OF OPERANTLY CONDITIONED FIRING PATTERNS OF SINGLE PRECENTRAL CORTICAL CELLS. Edward M. Schmidt, Martin J. Bak, Joan S. McIntosh and J. Stevenson Thomas. Laboratory of Neural Control, NINCDS, NIH, Bethesda MD. 20014.

Intracortical microelectrodes were chronically implanted in the precentral motor cortex of a monkey that had previously been trained to perform a wrist flexion-extension task to a specific target for a juice reward. Eight target lights were associated with eight separate wrist positions. The actual wrist position was indicated by a set of eight cursor lights. After electrode implantation, the position signal from the manipulandum associated with wrist position was replaced by a signal proportional to the firing rate of a single cell. The monkey was required to adjust the cell firing rate to reach a start target and, after this had been held for the required time, to readjust the firing rate to coincide with a second target. If the second target was reached and held within 5 seconds a liquid reward was delivered. The monkey usually made some movement that was associated with the cell firing. As he learned how to control the cell firing pattern the movement became more refined and at times almost imperceptable. Over a period of 99 days a total of 274 cell conditioning runs were conducted, each comprising approximately 25 trials. We estimated that 27 different cells were used for conditioning, with one cell being used for 9 days. Of the total number of runs, 80.3% had at least 50% successful trials. Not only was the monkey able to successfully modulate the firing rate of a cortical neuron between two levels on command, but was able to adjust the rate to a number of different specified levels.

276 ACTIVITY OF POSTCENTRAL CORTICAL NEURONS OF THE MONKEY DURING CONDITIONED MOVEMENTS OF A DEAFFERENTED LIMB. Bernard Bioulac* and Yves Lamarre. Dept. Physiol., Fac. Med., U. of Montreal, Montreal H3C 3T8, Quebec.

Some postcentral units change their pattern of firing before the onset of movement. It is not known if such early discharges are related to feedback from the periphery or if they occur in response to some central inputs such as postulated by the theory of corollary discharge. These two hypotheses were tested by recording the activity of single neurons in the postcentral gyrus of the monkeys before and after contralateral dorsal rhizotomy from C_2 to T_5. Animals were trained to make ballistic flexion or extension of the arm in response to sound stimuli, and were rewarded for short reaction-time movements of abrupt onset. The onset of movement was determined by recording arm muscle EMG and measuring elbow displacement. All data were collected and stored "on-line" with the help of a PDP-9 computer. As observed by others, a decrease of EMG activity in the antagonist prime movers of the elbow preceded the increase of activity in the agonist and this was still observed after deafferentation. Recordings from several hundred neurons indicate that nearly all postcentral units cease to discharge in association with the contralateral arm movement after deafferentation. Only two neurons (less than 1%) showed some change of their firing pattern at the onset of limb movement. These preliminary results lead to the conclusion that postcentral unit discharge associated with movement is mainly, if not exclusively, a result of peripheral feedback.

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277 RELATION OF ACTIVITY IN PRECENTRAL CORTICAL NEURONS TO FORCE AND RATE OF FORCE CHANGE DURING ISOMETRIC CONTRACTIONS OF FINGER MUSCLES. Allan M. Smith*, Marie-Claude Hepp* and Urs Wyss* (SPON: H. Jasper). Brain Research Institute, University of Zurich, CH 8029, Zurich, Switzerland.

Within the hand area of the primate motor cortex the activity of single neurons was recorded during performance of a maintained precision grip of the thumb and forefinger. Finger apposition forces were exerted against a strain guage under near isometric conditions. Task performance required the generation of a force ramp (dynamic phase) and maintaining a stable force for a 1 sec duration (static phase). From 221 recorded neurons, 51 activated cells were selected from the hand area identified by stimulation through the recording electrode. Most activated neurons discharged at higher frequencies during the dynamic phase. The discharge frequency of some "dynamic" cells could be related to both force and rate of force change but the discharge of certain other units could only be correlated with rate of force change. The change in discharge frequency of dynamic neurons generally occurred before the onset of EMG activity. Eight "static" neurons were more active during maintained force than during force change. The discharge frequency of only one of these cells showed a positive relation to force. The change in discharge frequency in these static neurons either coincided or occurred after the onset of EMG activity.

278 DISCHARGE OF RED NUCLEUS NEURONS IN RELATION TO A VOLUNTARY ELBOW FLEXION. N. Onoda* and J. E. Burton (SPON: R. McCaman). Div. of Neurosciences, City of Hope Natl. Med. Center, Duarte, CA 91010.

Cats were trained to flex the elbow joint for a food reward. The intentional motor output (EMG of biceps and triceps muscles) and the parameters of the motion were varied by changing the resistive force which the cat had to overcome to initiate the movement. Red nucleus neurons can be classified according to their behavior in relation to the task. The discharge rate of one type increased in advance of the intentional increase in biceps activity prior to the movement while the discharge of a second type decreased in parallel with the biceps activity. The discharge of the third type did not change prior to the onset of the motion. Once the arm was in motion, the discharge rates of all three types changed in parallel with the velocity of the motion. The activity of the first type decreased with increasing flexion velocity while the activity of the second and third types increased. These relationships were most likely due to an input generated by velocity-sensitive receptors in the moving limb and mediated by cerebellar activities since the velocity parameter was also represented in the discharge of interpositus neurons (Burton & Onoda, this meeting). It was further observed that a decrease in biceps activity during the movement was more closely related to the activity of the first and third types of red nucleus neuron while a burst of triceps activity during the movement was more closely related to the activity of the second type. The data are consistent with the idea that the participation of red nucleus activity in the regulation of the motor output to the biceps and triceps was based on inputs from the motor cortex related to intentional commands to the biceps and from the cerebellum related to the velocity of the motion. (Supported by NIH Research Grant No. NS 11798 from NINDS)

279 ANTIDROMIC RESPONSE EVOKED IN CEREBRAL CORTEX BY STIMULATION OF CEREBRAL PEDUNCLE. J. A. McMillan, M. D. Mann, and A. L. Towe. Dept. Physiol. & Biophys., Univ. of Wash. Sch. Med., Seattle, WA 98195.

Domestic cats were anesthetized with α-chloralose and immobilized with decamethonium bromide. Electrical responses to midbrain stimulation with concentric bipolar electrodes in stereotaxic plane A4 were recorded from pericruciate cortex. Two short-latency, surface-positive waves and a large positive-negative complex were observed; they correspond to the a, b and d waves recorded from the same tissue after ventral medullary stimulation (Jabbur & Towe, J. Physiol., 1961, 155:148-160), and hence will be referred to as such. Both the a and b waves remain positive through depth in the cortex; the d complex reverses polarity 0.2-0.4 mm below the pial surface. The a wave (0.3 msec latency) is best evoked by stimulation in the peduncle and is maximal in the neighborhood of the cruciate sulcus (field 4). The b wave (0.9-1.0 msec latency) and the d complex are best evoked by stimulation dorsal to the red nucleus and lateral to the central gray, and are maximal posterior to the postcruciate dimple (field 3b). Weak stimulation of the peduncle results in an a wave and a small, positive deflection similar to the β wave; weak stimulation dorsal to the red nucleus results in a b wave and d complex, without an a wave. The a wave responds to iterative stimulation in excess of 200/sec without a change in amplitude or configuration, whereas the b wave begins to alter at 50/sec and the d wave at lower iterative rates. Thus, the a and β waves appear to result from antidromic activation of cortico-peduncular (pyramidal tract) fibers, whereas the b and d waves result from activation of some other pathway (probably medial lemniscus). The results shown by Sasaki & Prelevic (Exp. Neurol., 1972, 36:319-335) could only be reproduced if jaw movement occurred, and most readily if the electrode had penetrated through the ventral surface of the brain stem; they could be abolished by additional paralyzing agent. (Supported by NS00396 & NS05136, USPHS)

280 INFLUENCE OF MOTOR UNIT RECRUITMENT ON TENDON ORGAN DISCHARGE. P.E. Crago, J.C. Houk, and W.Z. Rymer, The Johns Hopkins Univ. Medical School, Baltimore, Md. 21205 (Supported by NIH NS 06828 and NIH NS 57,240-02).

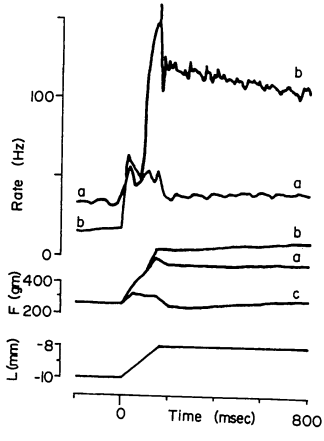
Golgi tendon organs lie in series with only a few muscle fibers, probably one or two from each of a few motor units. During normal muscular activity, the afferent discharge should be related to the tension in these particular muscle fibers (cf.[1]). Here we report evidence that tendon organs show a marked sensitivity to the recruitment of motor units that innervate the muscle fibers which lie in series with the receptor.

Cats were decerebrated intercollicularly, and the left hindlimb was denervated except for soleus. Tendon organ discharge was recorded from small filaments that had been teased from an intact L7 dorsal root. Receptors were identified by the usual criteria. The soleus was attached to a myograph mounted on a stretching device. A computer controlled muscle length, the A/D conversion of the myograph output, and the storage of interspike intervals.

When the muscle was held at a fixed length and its force was slowly modulated by a crossed extensor reflex, we observed abrupt and marked changes in discharge rate. We presume that these discrete changes occurred when a muscle fiber in series with the receptor was part of a freshly recruited or de-recruited motor unit, since tendon organs are known to respond vigorously to the contraction of single motor units. This observation in identified tendon organs supports Vallbo's [3] use of this criterion to identify tendon organs in human subjects.

We also studied the modulation of discharge rate that occurred during stretch reflexes. Two characteristic patterns are illustrated in the figure: a, a modest increase in rate that dropped near to the initial level at ramp plateau, and b, a prominent increase that began during stretch and was maintained during the plateau of the ramp. The pattern a did not correlate well with the net change in force produced by the stretch reflex (also labeled a). Force trace c shows an estimate of the non-reflex component of force, i.e., the mechanical response of those fibers that were active prior to stretch (cf.[2]). Pattern a correlates well with force trace c, which suggests that new fibers in series with the receptor were not recruited. The discrete frequency changes in response b implied that muscle fibers in series with the tendon organ were recruited at the beginning of stretch and additional ones during the course of the stretch, with maintained recruitment following the ramp. Rate modulation of already recruited muscle fibers may have augmented the responses, but presumably could not account for the abrupt changes in discharge rate.

In conclusion, the discharge rate of tendon organs may not reflect accurately the net muscular force because of their limited spatial sampling of muscle fiber activity. Further study is needed to determine whether muscle force may be derived from the population of tendon organs in a muscle.



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281 Factors of Importance for the Initiation of Flexion during Walking. S. Rossignol, S. Grillner and H. Forssberg[x], Dept. Physiol., University of Göteborg, Sweden

A central spinal network of interneurones can generate by itself the alternate activation of flexors and extensors during hindlimb walking movements. However, the activity in this network can also be influenced from the periphery. For instance a chronic spinal cat (spinalized at Th 12, 1-2 weeks after birth) can perform normal walking movements on the treadmill and change its speed of locomotion with the speed of the treadmill. The flexion phase changes little with speed but the extension phase varies dramatically as for the intact cat (EMG and movement, Grillner 1973, in Control of Posture and Locomotion, Plenum Press, New York 1973, 515-535). This adaptation to speed must be due to one or several types of peripheral signals providing essential timing cues for critical events of the step cycle such as flexion at any given speed. The following observation might be of importance in that context. If the hindlimbs of such a spinal cat are made to walk on the treadmill and the backward movement of one limb is prevented by holding the foot in the palm of the hand, the contralateral limb continues to walk while the hand-held leg exerts a continuous extensor EMG (see Forssberg et al. Brain Res. 1975, 85, 103-107) supporting the body but without whatsoever attempting to initiate flexion. If, however, the limb is slowly brought backwards at a sudden point a prompt flexion (EMG and movement) will occur and the animal will then continue to walk with that limb. These movements have been studied by 16 mm film (64 fr/s) and synchronized EMG records. The flexion is initiated at a hip joint angle similar to that associated with the onset of flexion during walking. The knee and ankle movements do not seem to have the same importance since changing the angle of these joints while the hip is kept stationary does not initiate flexion of the limb. Foot contact is not essential either since the same flexion movement can be induced by bringing the limb backwards holding only the sides of the foot. Thus the position of the hip joint appears to be of importance, but at present it is not clear if the afferents responsible are from hip muscles or from the joint itself or both. The initiation of limb flexion is, however, also influenced from the contralateral limb in such a way that the flexion tends to occur either in phase in the two limbs or 0.5 out of phase i.e. as in gallop and trot respectively. We therefore conclude that the hip joint angle and the phase of the step cycle of the contralateral limb are two factors of importance for determining the onset of flexion in the limbs. One can assume that in intact conditions, such incoming peripheral signals can retard or advance the flexion phase to meet with speed requirements differing from those centrally planned for a given speed.

282 EVIDENCE FOR MULTIPLE ENCODER SITES IN PRIMARY ENDINGS OF MAMMALIAN MUSCLE SPINDLES. Stevan Dawis* and R. E. Poppele. Lab. of Neurophysiol., Univ. of Minn., Minneapolis, 55455.

In vitro primary muscle spindle endings tend to lose their spontaneous discharge after several hours and respond only phasically to stretch. It can be shown that this change is not accompanied by any change in transduction properties and is therefore likely to be due to changes in the encoding mechanisms. Before the encoder behavior becomes completely phasic, spontaneous activity becomes very irregular. Multimodal interval histograms are observed in which a set of peaks have intervals at a, b, c...msec and their multiples (2a, 2b...). Another set of modes is found at combinations such as a+b, a+2b, 2a+b... . As many as five fundamental intervals (a - e) have been observed. Secondary endings also become phasic with time but no corresponding irregularities in firing pattern or multimodal histograms have been observed for these units. Treating a fresh preparation with 10^{-6} strophanthin K produces the same effect suggesting that it may result from a loss of metabolic pumping. The data suggest that primary endings have two or more encoder sites and that when firing becomes irregular, aborted spikes are produced which are capable of resetting nearby encoders but incapable of propagating along the afferent nerve. That this is not due to afferent nerve block is shown by the fact that a vibrational stimulus can restore the normal rhythmic activity.

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283 THE CONTRIBUTION OF STRETCH EVOKED REFLEXES TO THE FORCE PRODUCED BY JAW CLOSING MUSCLES DURING SINUSOIDAL STRETCHING IN ALERT MONKEYS. Donna Hoffman*, Guy M. Goodwin* and Erich S. Luschei* (SPON: O.A. Smith). Dept. Physiology & Biophysics and Regional Primate Research Ctr, Univ. of Washington, Seattle, Wash. 98195.

Monkeys have been trained to exert graded isometric biting forces across a pair of plates, one fixed, one positioned by a servo-controlled motor. The force and the EMG from the temporalis muscles have been recorded during sinusoidal movements imposed on the jaw at 3 to 50 Hz. Small amplitude movements (less than 300 μm peak-to-peak at the incisors) evoke approximately linear modulations of force and EMG activity averaged over a number of cycles. The responses can then be described by the amplitude and phase relative to the length input. The amplitude of the force response shows a maximum for frequencies of stretching between 5 and 15 Hz, the phase of the response leading and thus resisting the length change. At 15 Hz and beyond, the force response starts to "lag" the length change so that it assists rather than resists the movement from 15 to 20 Hz. Beyond 20 Hz the force modulation becomes phase advanced again. These observations can be understood by invoking a relatively high gain stretch reflex for frequencies between 5 and 20 Hz, the phase of which becomes progressively lagged by conduction delays and muscle properties. Larger amplitude movements appear to saturate the reflex component of force modulation so that the frictional components of muscle stiffness then largely determine the phase of the force response.

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284 MECHANISMS UNDERLYING ACHIEVEMENT OF FINAL HEAD POSITION. Andres Polit*, Emilio Bizzi, and Pietro Morasso*. Dept. Psychol., M.I.T., Cambridge, Mass. 02139.

In this series of experiments we investigated whether the neck proprioceptive apparatus (muscle, tendon, and joint receptors) is involved in the process whereby an orienting head movement comes accurately to a stop. To answer this question, we examined monkey head movements which are part of the coordinated eye-head response to a visual stimulus. Our basic preparation was a chronically labyrinthectomized monkey whose head movements were visually triggered but performed in complete darkness. Three monkeys were used. In the first series of experiments we unexpectedly increased head inertia at the beginning and throughout the movement with the aim of provoking a proprioceptive response in all types of neck receptors, and observed the outcome of this stimulation upon the head final position. Our results indicate that the unexpected proprioceptive signal originating from the moving neck failed to upset the process whereby a visually triggered movement, performed in the dark, comes accurately to a stop. In a second and complementary series of experiments, rather than stimulating the proprioceptive system, we interrupted the flow of afferent input by cutting cervical and upper thoracic dorsal roots and observed how the absence of proprioceptive feedback affected the achievement of final head position. Taken together the results obtained from both series of experiments indicate that the afferent activity originating in the course of centrally initiated movements does not determine the final position. The mechanism whereby head final position is reached and maintained is centrally preprogrammed. (Research supported by NIH Grant NS 09343 and NASA Grant NGR-22-009-798.)

285 MUSCLE RECEPTOR RESPONSES TO CONTRACTIONS OF SINGLE MOTOR UNITS. M. D. Binder*, J. S. Kroin*, E. K. Stauffer, D. G. Stuart and G. P. Moore. Depts. of Electrical Eng. and Biology, Univ. Southern California, Los Angeles, Calif. 90007 and Dept. of Physiology, Medical School, University of Arizona, Tucson, Arizona 85724.

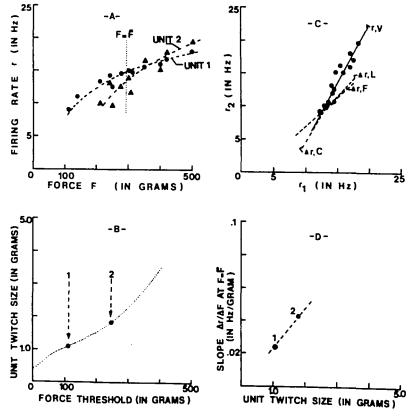
Crosscorrelation techniques have been developed which permit the detection and analysis of the response of group Ia, Ib, and II afferents to contractions of single motor units. We studied the response of receptors in the soleus and tibialis anterior of anesthetized cats when stimuli, delivered to subdivided ventral root filaments, were adjusted so that functionally only a single motor unit was active. The pattern of receptor responses was characterized by crosscorrelation (or post-stimulus time) histograms which plot the probability of receptor discharge as a function of time during a twitch contraction. In cases where the coupling between motor unit and receptor was strong, the histograms showed patterns which closely resembled the response of the same receptor to whole-muscle stimulation. In other cases involving small motor units or weak couplings, crosscorrelation was able to resolve responses not detectable by inspection of the raw spike-train data. The histograms enabled us to characterize the twitch-evoked response pattern of each receptor type (1) at different muscle lengths; (2) during random or periodic stimulation of the ventral root (which produce different average twitch responses); and, (3) in response to different motor units. Simultaneous recordings of spike trains from several afferents also permitted comparisons of the effect of one motor unit on different receptors. (Supported by USPHS grants NS 07888 and RR 05675).

286 FIRING RATES OF MOTOR UNITS DURING VOLUNTARY ISOMETRIC CONTRACTIONS. A. Willem Monster and H. Chan*, Temple University Health Sciences Center, Philadelphia, 19140.

The sizes and firing rates of motor units of the middle finger EDC muscle were recorded during isometric voluntary contractions. Units were recorded either individually, or simultaneously in pairs of two or more. Unit size estimates were derived by extracting the unit-synchronized (electro) myographic activity from the total muscle activity, using averaging (Monster & Chan, 1975). Firing rates of different units are plotted against total voluntary muscle force (A). The behavior of two units (1 and 2) is shown. Solid symbols mark firing rates in the steady state; the general trend of the symbols is approximated by the dashed lines.

Recruitment of motor units during isometric contraction is in accordance with the "size principle," that is, larger units are recruited at higher force levels (B). The dashed line in B shows the average relationship between unit sizes and force thresholds for 300 EDC units from 8 male subjects between 22 and 30 years of age. Solid symbols mark the sizes of the same two units as shown in A. The very systematic arrangement of unit sizes and firing rates provides a means of determining, by interpolation, the motor unit composition of the whole muscle (same ref. as above); more than 90% of the units is recruited at 500 grams.

It follows from A and B that larger units have higher thresholds but advance their firing rates more rapidly, once they are recruited. Such a finding is not entirely surprising in view of earlier findings on intracellular stimulation of motoneurons with injected currents (Kernell, 1966). The relative increase in the firing rates of the two units shown in A, with force, is consistent. This increase is illustrated by plotting r_1 vs r_2 (solid symbols in C); the solid line, marked r, V, was obtained from the solid symbols by linear regression. When unit sizes were plotted against the slopes of the dashed lines in A, at a given voluntary force level ($F=\overline{F}$), there resulted a monotonic relationship between unit sizes and slopes (dashed line in D). In other words, unit sizes are not only recruited (voluntarily) in accordance with the size principle, but unit sizes also specify average unit firing rates during steady isometric contraction. It is emphasized that this finding does not exclude the possibility of unit excitability gradients other than r, V for different types of inputs. For instance, firing rates of small units were found to be relatively more sensitive to small dynamic stretches (Δr, L in C) and to the rapid spontaneous variations (tremor) in voluntary force (Δr, F). The two units shown were relatively equally sensitive to volitional inputs (r, V) and to noxious cutaneous stimuli (Δr, C); the latter were induced by electrical stimulation at the wrist.



287 DYNAMIC INPUT-OUTPUT PROPERTIES OF RHESUS MONKEY FOREARM MUSCLES.

R. Rietz* and D. R. Humphrey (SPON: Y. Matsumoto). Lab. Neurophysiology, Emory Univ. Sch. Med., Atlanta, GA., 30322.

In any attempt to determine the functional significance of temporal correlations between central neuronal and muscular events, it becomes necessary to consider the dynamic input-output properties of muscle itself. The results of many studies have in fact suggested that central-to-peripheral motor control signals may undergo their most significant temporal changes as motor nerve impulses are transduced at the periphery into variations in muscle tension. Despite a general focus upon the hand and forearm muscles in motor control studies with primates, however, there are surprisingly few data available concerning their mechanical properties. In order to obtain such data, we have used standard physiological and control systems analysis techniques to study the tension generating properties of selected forearm muscles in the anesthetized rhesus monkey (30 mg/Kg Nembutal).

The muscles selected for study were the extensor digitorum communis (EDC), flexor carpi radialis (FCR) and extensor carpi radialis (ECR). The muscle origins were fixed by clamping a rod inserted through the olecranon process of the ulna, and the distal tendons were dissected free and attached individually to a myograph. Isometric muscle forces were generated by stimulating the muscle directly, or by stimulating the distal end of its cut nerve. Measurements were made of: (a) the passive length-tension (L-T) curve; (b) the active L-T curve; (c) the amplitude and time course of the muscle's twitch; (d) the tetanic tensions evoked by stepwise increases in stimulation frequency; and (e) the relative amplitude and apparent phase lag of the muscle's tension response to constant amplitude, sinusoidal changes in motor nerve or direct muscle stimulation frequency. Aside from a and b, all measurements were made with the muscle at its resting length (L_o).

A number of our experimental measurements are shown in Table 1, and our

TABLE 1

	EDC (N = 4)	ECR (N = 4)	FCR (N = 3)
Twitch tension (gm)	73 ± 14	93 ± 23	102 ± 26
Rise time (msec)	27 ± 0.8	30 ± 0.5	32 ± 0.6
Decay time (msec)	97 ± 2.6	124 ± 5	98 ± 6
Max. tetanic tension at L_o (gm)	309 ± 72	420 ± 53	327 ± 88
Transfer function (in radians/sec)	$\frac{(s+10.7)}{(s+6.3)(s+37.7)^2}$	$\frac{(s+21.4)}{(s+6.3)(s+56.6)^2}$	$\frac{(s+8.5)}{(s+6.3)(s+25.8)^2}$

major results may be summarized as follows. (1) The L-T curves were as expected from previous work, with peak active tensions generated in the length range L_o to L_o + 4 mm. (2) The twitch rise and decay times were generally similar for all muscles, and were comparable to those of fast muscles in cat hindlimb. (3) The curve relating tetanic tension to motor nerve stimulation frequency was sigmoidal, with its steeply rising, near-linear segment in the frequency range 12-30 pps. (4) When the motor nerve was stimulated with frequencies that were modulated sinusoidally over this range, the evoked tension output of the muscle was nearly sinusoidal, with a harmonic distortion < 15 % over the range of modulation frequencies used (0.2-10 Hz). This finding justified the use of standard frequency response methods in analyzing the input-output properties of each motor nerve - muscle preparation. Transfer functions which fit the frequency response data quite well are given in Table 1. Although the functions differ slightly from those reported previously for cat muscle, they reveal the same marked, low-pass filter properties of the muscle's contractile apparatus. These measurements should be of considerable value in future attempts to assess the relative contributions of central neuronal and muscular events to the overall dynamics of the primate's forearm control system. An example will be given which employs the data in precisely this way. (Supported by NIH Grant NS-10183).

288 RELEASE OF CENTRUM MEDIANUM FROM CAUDATE INHIBITION FOLLOWING LESIONS OF SUBSTANTIA NIGRA. M. Dalsass and G. Krauthamer. Dept. Anat., CMDNJ Rutgers Med. Schl., Piscataway, N.J. 08854.

Stimulation of a restricted area of the caudate nucleus (Cd) normally inhibits peripherally evoked polysensory responses in centrum medianum (CM). To determine whether the substantia nigra (SN) is a necessary link in this inhibition, five cats were studied one to three months after bilateral electrolytic destruction of SN. The histologically verified lesions destroyed most of SN with minimal damage to surrounding tissue. The cats were anesthetized with chloralose and immobilized with Flaxedil. Bipolar stimulating electrodes were placed in inhibitory regions of Cd and thalamic units were recorded extra and intracellularly with 3M K citrate filled micropipettes. Responses to peripheral stimulation were unchanged, consisting of an excitatory-inhibitory sequence. All units showed a high level of spontaneous activity; this contrasted sharply with the very low level seen in intact preparations. Conditioning stimulation of Cd was ineffective and failed to inhibit the unit responses to peripheral stimulation. The increase in spontaneous activity and the failure to inhibit peripheral responses indicate that SN is a necessary component in Cd modulation of CM responsivity.

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289 CHANGES IN RESPONSE TO DOPAMINERGIC AND CHOLINERGIC AGENTS AFTER LESIONS OF SUBSTANTIA NIGRA IN THE RAT. K.N. Gale* and A. Horita* (Spon: L.M. Halpern) Univ. of Washington, Seattle, WA 98195.

The effects of bilateral electrolytic lesions in the vicinity of dopamine (DA)-containing cell bodies in substantia nigra (SN) were investigated in rats trained in a swimming maze requiring choices based on spatial orientation. We reported previously (Fed.Proc. 34:768,1975) that the performance of normal rats in this task is impaired by apomorphine (APO)0.6-1.2 mg/kg and by d-amphetamine (AM) 3.0-6.0 mg/kg. Scopolamine alone (1.0 mg/kg) was without effect but potentiated APO and AM significantly. Rats with SN lesions (causing 30%-60% loss in striatal DA), exhibited 1) normal sensitivity to AM, but 2) decreased sensitivity to haloperidol (0.3-0.6 mg/kg), and 3) increased sensitivity to APO (0.13-0.50 mg/kg) when tested 1-2 months after surgery. The response to APO was attenuated by pretreatment with physostigmine (0.2 mg/kg). In addition, scopolamine alone in doses as low as 0.2 mg/kg was effective in disrupting performance of SN-lesioned rats. At the same time there was an apparent decrease in APO-induced stereotyped gnawing; this was associated with marked changes in the total pattern of the stereotyped behavior. Animals with lesions placed more medially did not exhibit these changes in pharmacological response. Our results are consistent with a concept of cholinergic-dopaminergic antagonism and demonstrate that SN lesions can cause disruption of cholinergic function. The enhanced effect of DA receptor stimulation and the reduced effect of DA antagonism observed after SN lesions may be either a consequence of, or a basis for, decreased activity in a cholinergic system.

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290 NIGRO-STRIATAL INHIBITION MEDIATED BY NON-DOPAMINERGIC AXON COLLATERALS. J.J. Miller, T.L. Richardson*, H. McLennan*. Dept. Physiology, Univ. British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Previous studies have shown that stimulation of the substantia nigra (SN) can produce excitation or inhibition of striatal neurones. Although it has been suggested that dopamine (DA) may be involved in these synaptic processes, the evidence continues to be controversial. In the present investigation extracellular unit activity was recorded from neurones in the caudate nuclei (Cd) of anaesthetized rats, using single and multi-barrel micropipettes. Stimulation of the SN evoked a rhythmic field potential ("burst") upon which unitary spikes of small amplitude were frequently superimposed. The bursts had onset latencies of 3-10 msec and durations of 5-15 msec. These excitatory responses are tentatively identified as due to inhibitory interneurones, for they were accompanied by inhibition of large amplitude spikes for periods of 50-300 msec. Both responses persisted following disruption of the nigro-striatal dopaminergic projection by intraventricular or intracerebral injection of 6-OHDA, and following systemic or iontophoretic application of the DA blocking agents haloperidol and α-flupenthixol. Electrolytic lesions of the SN 3-4 weeks prior to acute experiments caused a significant depletion of striatal DA but did not alter the burst discharge or the inhibition produced by stimulation of the internal capsule at the level of the hypothalamus. These experiments suggest that (1) neither the excitatory burst response nor the inhibitory response elicited by SN stimulation is mediated by DA and (2) stimulation of the SN orthodromically activates, via axon collaterals of the striato-nigral pathway, interneurones within the Cd which mediate the inhibitory influence on Cd target cells.

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291 DOPAMINE INDUCED DEPOLARIZATION IN THE CAUDATE NUCLEUS. J.D. Kocsis,* M. Sugimori,* S.T. Kitai. Morin Mem. Lab., Dept. Anat., Sch. Med., Wayne State Univ., Detroit, Mich.

Anatomical evidence has shown the presence of a direct dopaminergic pathway from the substantia nigra (SN) to the caudate nucleus (Cd). Subsequent physiological studies have suggested that this pathway may be inhibitory. However, recent electrophysiological analysis indicates that the initial responses of the caudate neurons to SN stimulation is excitatory. The present study was designed to test these alternative hypotheses by direct application of dopamine in the Cd to test its effects at the membrane level of cats anaesthetized with Nembutal. Intracellular recordings were obtained from one barrel (K-citrate) of a multi-barrelled parallel electrode. The other barrels were used for extracellular drug application and were staggered 20-40 microns from the recording electrode. Each of these barrels was filled with dopamine, chlorpromazine or NaCl. Drugs were applied electrophoretically with constant current (pulse width 2-100 msec, intensity 20-50 nA positive). The SN was stimulated monopolarly with insulated stainless steel wires. After impalement of a Cd neuron and monosynaptic SN induced EPSPs were recorded, drugs were applied to see their effects on the cell membrane. Dopamine induced depolarization of this membrane and chlorpromazine suppressed the SN elicited EPSP. Appropriate controls (e.g. injection of Na^+ or Cl^-, current neutralization, etc.) indicated that the drug effects were not artifactual. Some recorded neurons were identified by procion yellow dye injection. The results suggest that the dopamine effect on Cd neurons is excitatory and the intrinsic neurons appear to be the recipient cell of the nigral input.

(Supported by NIH Grant NS 00405, RR 5384 and Charles B. DeVlieg Foundation Fellowship).

292 DISTRIBUTION OF ACETYLCHOLINESTERASE (AChE)-CONTAINING NEURONS IN THE RAT SUBSTANTIA NIGRA: CYTOARCHITECTURAL INFORMATION AND EVIDENCE FOR CHOLINERGIC-DOPAMINERGIC INTERACTIONS. Konrad Talbot and Larry L. Butcher. Dept. Psychol., University of California, Los Angeles, 90024, U.S.A.

Using standard histochemical techniques for AChE it is often difficult to discern individual AChE-containing neurons and processes in the CNS. We have recently developed a light microscopic pharmaco-histochemical procedure for AChE (Butcher et. al., J. neural Trans., in press) which allows clear visualization of such neurons and processes throughout the CNS. Serial section analysis of the substantia nigra (SN) of rat brains prepared according to that technique reveals that a large proportion of nigral neurons, in the pars compacta (PC), pars lateralis (PL), and pars reticulata (PR), demonstrate significant AChE activity. These neurons range in cell body size from 8-40μm in the PR and primarily from 15-40μm in the PC and PL. Two broad categories of AChE neurons are noted. Type I cells are approximately 8-17μm in cell body size; round, oval, or, less frequently, fusiform in cell body shape; and stain most often lightly or moderately for the enzyme. Round or oval Type I cells often have no AChE-containing processes, although some may have one or two; fusiform Type I cells have two enzyme-containing processes. Type II cells are approximately 18-40μm in cell body size; round, oval, fusiform, or, less frequently, triangular in cell body shape with 0-1, 0-1, 2, or 3 AChE-containing processes, respectively, and stain moderately or intensely for the enzyme. PC AChE neurons are principally Type II cells which are densely packed, especially medially and at rostral levels, often fusiform or oval in cell body shape, and frequently intensely stained; many PL AChE neurons can be similarly described, although they are not as densely packed as medial or rostral PC AChE cells. In contrast to PC and PL, both Type I and II cells are common in the PR. AChE neurons in this latter area are generally more dispersed than, and their processes less prominent than those of, PC and PL neurons.

The caudal PR consists of a very heterogeneous collection of AChE neurons in terms of cell body size, shape, orientation, and number of enzyme-containing processes. At more rostral levels orientation of PR AChE cells is still often random, but such neurons are now often round, oval, or less often, fusiform Type I or II cells with 0-2 AChE-containing processes; the larger neurons tend to be located laterally and the smaller neurons medially. The ventromedial PR contains a prominent aggregation of moderately and intensely staining, closely spaced AChE neurons many of which are Type II cells. The central portion of the caudal PR dorsolateral to this aggregation possesses conspicuously few AChE neurons.

In transverse sections of the rostral PC the fusiform AChE cells and their enzyme-containing processes, particularly those located dorsolaterally, are oriented parallel to one another and to the medio-lateral axis of the SN. Many enzyme-containing processes, probably dendrites, of the more ventrally situated PC AChE neurons project into the PR; a number of these fibers sometimes form bundles of closely associated processes. Enzyme-containing processes of PL AChE cells extend into the lateral PR.

On the basis of the large number of intensely-stained AChE neurons and dopamine (DA) cells in the SN PC, it is likely that a large number of PC neurons contain both AChE and DA. This is supported by similarities between AChE and DA neurons in size and shape of cell bodies, as well as in the pattern of dendritic extensions into the PR. In addition, lesions of the nigrostriatal DA pathway result in retrograde degeneration of PC AChE cells and axonal accumulation of AChE caudal to the sites of fiber disruption. The presence of AChE in the dendrites of what appear to be DA neurons may serve to inactivate a cholinergic input to those neurons, suggesting that such dendrites could be sites of the cholinergic-dopaminergic interactions in the rat SN demonstrated pharmacologically by Javoy et. al. (1974) and behaviorally by Smelik and Ernst (1966).

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293 A GOLGI STUDY OF THE NEOSTRIATUM IN MONKEYS. Marian DiFiglia-Sekuler, Tauba Pasik and Pedro Pasik. Dept. of Neurol., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029.

Light microscopic examination of neostriatum from 7 Macaca mulatta prepared by the Golgi-Kopsch perfusion method reveals at least 6 neuronal types. Spiny I neurons are medium size (16-17 µm) with a high density of dendritic spines (16 per 10 µm) which appear first in the secondary dendrites. The axon extends well beyond the dendritic field, is directed towards the pallidum, and gives off many collaterals. Some of the latter are of the recurrent type and occasionally seem to contact a dendrite of the same cell. Spiny II neurons are either medium size (17-18 µm) or large and elongated in shape (40 X 10 µm), with long thick dendrites showing a relatively low density of spines (5 per 10 µm) which are present even in the proximal portions. The axon is similar to that of the previous type but with only few branching collaterals. Aspiny I neurons are smaller (14 µm) with varicose dendrites and a short thin axon exhibiting collateral processes which are also varicose. The aspiny II is a large neuron (34 µm) with many thick and thin varicose dendrites. The axon has not been identified. Aspiny III is a small cell (12 µm) with smooth dendrites and a short axon ramifying profusely within the dendritic field. A neurogliform neuron is small (11 µm) with many branching processes. Although the spiny I is the most frequently impregnated cell, some sections show a predominance of aspiny I neurons. According to these results, two types, the aspiny I and III can be classified with certainty as Golgi type II interneurons. Findings also suggest that spiny I and II neurons may contribute to the efferent systems. The nature of the aspiny II and neurogliform cells remains unclear. The fact that the most frequently impregnated cell varies among preparations cautions against using this type of quantitative data derived from Golgi material to interpret electrophysiologic findings. Aided by N.I.H. Grants # NS-11631 and F22-NS-01639.

294 ULTRASTRUCTURE OF MONKEY NEOSTRIATUM. Tauba Pasik, Pedro Pasik and Marian DiFiglia-Sekuler. Dept. of Neurology, Mount Sinai Sch. of Med., CUNY, New York, N.Y. 10029.

Electron microscopic examination of neostriatum from Macaca mulatta shows 5 neuronal types which correlate with those seen in Golgi material, i.e. spiny neurons I and II, and aspiny I, II and III (see Abstract by DiFiglia-Sekuler et al). The correlation is based on perikaryon size and shape, and dendritic characteristics such as the presence, location and density of spines or varicosities. At least 4 types of profiles are seen containing synaptic vesicles. The latter can be small round, large round, pleomorphic, or flat. Dense core vesicles can be present in all of these elements except those containing flat vesicles. Axosomatic synapses present on all neuronal types, are symmetric, the vesicles being either large round, or pleomorphic. Axodendritic synapses are made by terminals with pleomorphic vesicles which cover the varicose dendrites with symmetric contacts. All types of endings may synapse occasionally on the shaft of spiny dendrites. Profiles with large round, or pleomorphic vesicles contact the smooth dendrites of aspiny III neurons. Axospinous synapses involve the 4 axonic elements. Frequently 2 types and occasionally 3 articulate with a single spine. The most frequent presynaptic elements have small round, or pleomorphic vesicles and make asymmetric contacts. Those with large round, or flat vesicles make symmetric synapses. All of these contacts may be of the "en passage" type, and are seen articulating with several spines, or with spines and a dendritic shaft. Some of the presynaptic profiles with pleomorphic vesicles exhibit also cisterns and are occasionally postsynaptic as well (dendrodendritic synapses). They may represent presynaptic dendrites of interneurons (aspiny I or III) as found in other CNS structures. Experimental work in progress will hopefully elucidate the origin of the various profiles containing synaptic vesicles. Aided by N.I.H. Grants # NS-11631 and F2-NS-01639.

295 UNIT FIRING PATTERNS IN BASAL GANGLIA AND MOTOR CORTEX DURING MOVEMENT INITIATION. E.J. Neafsey,*SPON. N.A. Buchwald. Mental Retardation Research Ctr Dept. of Anat., Sch. Med., UCLA, Los Angeles, 90024.

This study was designed to compare firing patterns of basal ganglionic and cortical units during initiation of a lever press, specifically the lifting phase of this movement involving forelimb flexion. Single units were recorded extracellularly from the globus pallidus, entopeduncular nucleus and pericruciate cortex of cats during pressing for liquid food reward on a VI schedule. Cortical units were identified as PT or non-PT cells on the basis of response to pyramidal tract stimulation. EMG activity recorded from the brachialis, triceps, trapezius, and paraspinal muscles changed from the resting level 100-200 msec before the initiation of the lever press. This response (R) served as a trigger for averaging unit and EMG activity for 2 sec before and 0.5 sec after its occurrence. Two basic patterns of neural activity related to movement were shown. In the 1st, movement related units in all 3 structures displayed sustained anticipatory shifts in firing rates (80% increase, 20% decrease) beginning as much as 1 sec before R. Approximately two-thirds of basal ganglionic and one-third of cortical units showed this pattern. In the 2nd, firing rates of movement related units changed during or just before (<300 msec) R. No PT neuron changed its activity earlier than 500 msec before R. The units displaying an anticipatory pattern with a shift to an increased firing rate exhibited a gradual increase in this rate before R, often with a burst just before or during R. Units displaying the anticipatory pattern with a sustained shift to a decreased firing rate exhibited either gradual or sudden decreases in firing rate before R. Then the decrease either continued or gave way to a burst of firing. Preliminary results suggest that, despite considerable overlap in distribution of onsets, pallidal activity changes first, entopeduncular next, and pericruciate last. (Supported by USPHS MH-07097 and HD-05958).

296 EFFECTS OF FRONTAL CORTEX LESIONS ON SPONTANEOUS FIRING OF CAUDATE NEURONS. E. Garcia-Rill, M.S. Levine, C.D. Hull, N.A. Buchwald. Dept. Anat., Sch. Med., UCLA, Los Angeles, 90024.

This report is a continuation of experiments on the effects of brain lesions on the spontaneous firing patterns of basal ganglia neurons. Previous studies have shown that lesions in and around the nigrostriatal pathway, designed to deafferent the caudate nucleus partially, produce a significant slowing in the spontaneous firing rates of neurons in the caudate nucleus contralateral to the lesion. This effect is due to damage to caudatofugal fibers since unilateral caudate ablation produces a similar slowing. The present experiments describe the effects of unilateral and bilateral removal of frontal cortical areas on the spontaneous firing of caudate neurons in locally anesthetized, paralyzed cats. The spontaneous firing rates of caudate neurons in cats with bilateral frontal cortex lesions were significantly slower than those of caudate neurons in intact cats. Mean interspike intervals (ISIs) for caudate neurons were 5313 msec and 4154 msec for the left and right sides, respectively, in cats with bilateral frontal cortex lesions, compared to 1768 msec and 1544 msec for the left and right sides, respectively, in intact cats. Unilateral frontal cortex lesions produced a significant slowing in the spontaneous firing rates of ipsilateral caudate neurons only (Mean ISI = 5411 msec), while spontaneous firing rates of contralateral caudate neurons (Mean ISI = 1963 msec) were similar to values found in intact cats. These results are in agreement with electrophysiological data which show the initial response of caudate neurons to cortical stimulation is an EPSP and with histological data demonstrating a large input of corticofugal fibers to caudate neurons.
(Supported by USPHS grants MH-07097 and HD-05958).

297 MODIFICATION OF SINGLE UNIT RESPONSES IN THE CAT'S VISUAL CORTEX BY ELECTRICAL STIMULATION OF THE BASAL GANGLIA. Michael Fossel*, Maurice Ptito, Maryse C. Lassonde* and Karl H. Pribram. Depts. of Psychology and of Psychiatry and Behavioral Sciences, Stanford University, Stanford, California 94305, USA.

Previous studies have related the basal ganglia in the cat (Kadobayashi et al., Exp. Neurol. 33: 518, 1971) and in the monkey (Reitz and Pribram, Exp. Neurol. 25: 632, 1969; Buerger and Gross, J.C.C.P. 86(3): 440, 1974) to visual processing. The present study was undertaken to test directly whether visual receptive properties of cortical cells could be modified by electrical stimulation of these presumably "motor" structures.

The responses of single units in the visual cortex were recorded with extracellular Pt-Ir, glass-coated microelectrodes from paralyzed, unanaesthetized cats. Visual stimuli consisted of a moving line displayed on an oscilloscope by a computer (PDP-8) which also recorded the responses to the stimuli. The line moved in each of 36 directions in 10° increments and the directional sensitivity of all units was measured using a multiple histogram technique (Henry et al., Vis. Res. 13: 1771, 1973). The effect of bipolar electrical stimulation (I=.2 mA; d=.2 msec/c; F=2 Hz) of either the putamen or the caudate on the firing pattern of each unit was then measured by constructing response curves for each orientation.

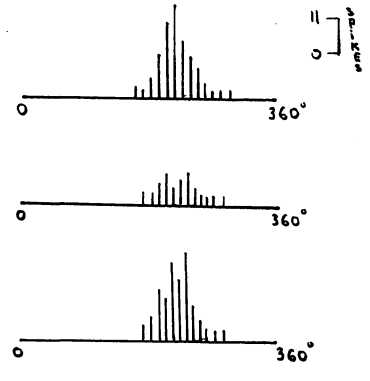

Fig 1.

Fifty-five (55) cells, most of them of the complex type (89%) were recorded. 90% of our sample were affected by electrical stimulation of the contralateral or ipsilateral putamen. Stimulation of the caudate nuclei also had an apparent effect on the cells' firing level, but this effect was variable, sometimes being inhibitory (42%) and other times excitatory (30%), so that no statistical reliability could be demonstrated for the effect. Figure 1 shows an example of a visual cortical complex cell recorded from the right cerebral hemisphere while the left eye is being stimulated. During visual stimulation only (Fig. 1A), the cell shows a maximum response at an orientation of 220° in the preferred direction. When the left (contralateral) putamen is electrically stimulated (Fig. 1B), there is a drastic drop in the cell's level of firing at this orientation. Figure 1C displays the control map obtained with a return to the pre-electrical stimulation level.

These data support the conception that the basal ganglia influence visual inputs, not only at the geniculate level (Kadobayashi et al., Exp. Neurol. 33: 518, 1971), but also in the striate cortex.

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298 DIFFERENTIAL EFFECTS OF BASAL GANGLIA AND CEREBELLAR LESIONS ON FUSIMOTOR ACTIVITY. D.M. McKeough* and S. Gilman. Dept. Neurol., Coll. P & S, Columbia Univ., New York, N.Y. 10032

In patients with CNS diseases producing limb rigidity, lesions of globus pallidus (GP) and ventrolateral nucleus of thalamus (VLN) have proven effective in reducing rigidity, though the mechanisms underlying the beneficial effects of these lesions remain largely unknown. In experimental animals, unilateral lesions of VLN decrease the responses to muscle extension of spindle primary afferents owing to a decline of fusimotor activity. The decreased responses of spindle afferents may relieve limb rigidity in humans by reducing local segmental facilitation of alpha motoneurons, both at rest and during muscle extension. The present experiments were undertaken to determine whether unilateral lesions of GP in experimental animals would also decrease the responses to muscle extension of spindle primary afferents. Recordings were made of the unitary responses of 41 single spindle primaries in the left medial gastrocnemius muscle of 11 cats anesthetized lightly with pentobarbital. In each experiment, serial recordings were made from 2-5 dorsal root filaments which had been dissected until the discharge of a single unit was recorded from each. These units were identified as the responses of muscle spindle primary afferents by standard techniques. A freezing probe was placed in the right globus pallidus and the tip temperature was decreased in 10^{o} steps from 0^{o} to -40^{o} C. Responses of each spindle afferent to progressive extension of the gastrocnemius muscle were recorded prior to probe insertion, immediately after insertion, and 5 minutes after the tip temperature had been lowered to each step. Finally, in order to abolish all residual fusimotor innervation to the spindles under scrutiny, the responses of each spindle afferent were recorded after de-efferentation by section of the left lumbosacral ventral roots.

The responses of 41 units were recorded during progressive cooling through -20^{o}, 40 units through -40^{o}, and 34 units through de-efferentation. Analysis of the resulting data revealed that cooling to -40^{o}C did not alter the responses of the spindle afferents, despite histologically verified destruction of the globus pallidus as well as portions of putamen, amygdala, and entopeduncular nucleus. The lesions did not encroach upon the internal capsule. Terminal de-efferentation markedly depressed the responses of the spindle afferents.

Data from the present experiments were compared with those obtained in previous experiments in which spindle afferent responses were studied following complete ablation of cerebellum, bilateral section of superior cerebellar peduncles, and ablation of VLN. Cerebellar ablation decreased spindle responses more severely than any of the other lesions. There was no difference between the effects of section of superior cerebellar peduncles and ablation of VLN, both of which depressed spindle responses essentially equally. Spindle responses in animals with ablation of globus pallidus were greater than those of any of the other groups.

Thus, interruption of a major cerebellar outflow by section of the superior cerebellar peduncles diminishes significantly spindle afferent responses through an effect on fusimotor discharge. Lesions of VLN have similar effects. However, interruption of a major outflow of basal ganglia does not affect spindle responses in the conditions of the present experiments. Accordingly, VLN lesions diminish spindle responsiveness by disrupting ascending cerebellar efferents to VLN and not basal ganglia projections to VLN. It is suggested that the major effects of basal ganglia lesions on muscle spindle control mechanisms are mediated by pathways other than ventrolateral nuclear projection to cerebral cortex, possibly through more direct descending routes.

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299 SHIFT OF BASAL GANGLION FOLLOWING STEREOTAXIC CANNULATION OF THE RAT SEPTUM. S. Roy Choudhury* (SPON: W. W. Kaelber). Dept. Anat. Coll. Med., Univ. Iowa, Iowa City, 52242.

The effects of septal cannulation on the displacement of basal ganglion form the basis of the present communication. In an experimental group of 20 male Wistar rats, in which cannula was chronically implanted unilaterally by stereotaxic procedures between the medial and lateral septal nuclei,considerable shift of the basal ganglion was always evident on the operated side. The displacement occurred along the long axis of the brain and took place either caudally or rostrally. Besides asymmetry in the relative sizes of the basal ganglia, no morphological differences were detected between the two sides. Studies of hydrolytic enzymes and of oxidoreductases for both aerobic and anaerobic oxidation also revealed no differences in activity. Control animals, in which the cannula was withdrawn after the septal lesion, manifested similar, but less pronounced, movements of the basal ganglion. Since these changes occur at some distance from the actual site of cannulation, it is evident that indirect forces, like changes in CSF pressure and introduction of air, are largely responsible for such intracerebral movements. These studies further demonstrate the extraordinary degree of plasticity in terms of mobility of the subcortical nuclear masses when subjected to indirect forces.

300 RETROGRADE PEROXIDASE TRANSPORT IN NIGRO-NEOSTRIATAL NEURONS AFTER INJECTION OF 6-HYDROXYDOPAMINE. A.M. Adinolfi and E. Garcia-Rill. Depts. of Anat. and Psychiat., Mental Retardation Research Center, Neuropsychiatric Institute, University of California, Los Angeles, 90024.

The effects of 6-hydroxydopamine (6-OHDA) on retrograde axonal transport of horseradish peroxidase (HRP) in nigro-neostriatal neurons of cats was studied. Stereotaxic injections of 6-OHDA (2 µg/µl in Merles solution) into substantia nigra produced selective degeneration of neurons, presumably dopaminergic, in zona compacta. After unilateral (left) 6-hydroxydopamine administration, hyperchromatic cells were seen by light and electron microscopy. Injections of Merles solution into the right substantia nigra did not produce comparable cellular destruction. Following pretreatment with 6-OHDA (up to 3 weeks postinjection), 0.5 - 1.0 µl of 33% horseradish peroxidase (HRP) in saline (Sigma, Types II and VI) was injected bilaterally into the caudate nuclei. Preliminary observations suggest that hyperchromatic nigro-neostriatal neurons continue to transport the peroxidase since there seems to be no significant reduction in HRP-positive cells in the left (6-OHDA pretreated) substantia nigra. (Supported by USPHS MH-07097, HD-05958).

301 ULTRASTRUCTURAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE IN THE CORPUS STRIATUM OF RODENTS. T. Hattori, V. K. Singh and P. L. McGeer. Kinsmen Laboratory, Dept. Psychiatry, U.B.C., Vancouver, Canada.

Choline acetyltransferase (CAT), the enzyme which synthesizes acetylcholine, has been localized at the electron microscopic level in the corpus striatum of rodents using a peroxidase-antiperoxidase labelling antibody technique. Antibodies produced in rabbits against a highly purified CAT from human neostriatal nuclei were utilized. The specific anti-CAT peroxidase product was highly localized within small round vesicles (250-450Å) which are evenly distributed in synaptic boutons. The outer membrane of mitochondria in the boutons also showed a positive reaction but the interior of the organelles did not. Highly positive vesicles are also distributed throughout small unmyelinated axons. The membrane of Golgi apparatus and endoplasmic reticulum is the major structure in the cell soma which contains the peroxidase reaction products. The major dendrites contain positive microtubules and the positivity diffusely expands into the cytoplasm of small dendritic spines. Some strongly positive spines received asymmetrical boutons terminaux or en passant which contained evenly distributed slightly pleomorphic vesicles. Since these ultrastructures have been shown to be characteristic of dopaminergic nerve endings[1] and the positive post-synaptic reaction is due to specific anti-CAT product, this suggests that dopaminergic nigro-striatal neurons make synaptic contacts with cholinergic interneurons in the corpus striatum as has been previously suggested by pharmacological studies[2].

[1] Hattori et al. (1973) Exp. Neurol. 41:599; McGeer et al. (1975) Brain Res. 86:475

[2] McGeer et al. (1974) Brain Res. 80:211

(Supported by the Medical Research Council of Canada)

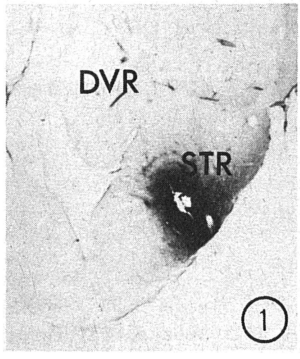
302 HORSERADISH PEROXIDASE STUDY OF THE STRIATAL AFFERENT CONNECTIONS IN THE TURTLE (Chrysemys picta). A. Parent and R. Boucher*, Lab. Neurobiol., Fac. Med., Laval Univ., Québec, Canada (G1K 7P4).

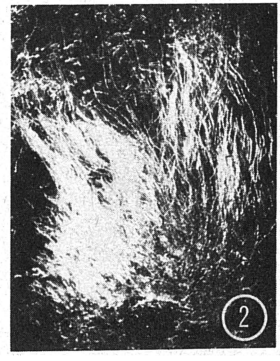
Retrograde axonal transport of horseradish peroxidase (HRP) has been used to demonstrate the cells of origin of some afferents to the basal striatum (STR) and the dorsal ventricular ridge (DVR) of the turtle (Chrysemys picta). HRP (30% solution, 0,1 to 0,3 µl) was injected unilaterally, either in the STR or the DVR of adult specimens, 72h. before sacrifice.

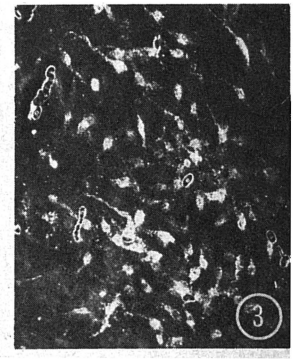
After selective STR injection, HRP-positive neuronal somata were visualized in the dorsal thalamus and midbrain tegmentum, ipsilaterally. At thalamic level, HRP-positive neurons were located around nucleus rotundus, that is within nucleus dorsomedialis anterior and, to a lesser degree, into nucleus dorsolateralis anterior and nucleus ventralis. At midbrain level, a large population of HRP-positive neurons were disclosed within the ventrolateral portion of the rostral tegmentum. These HRP neurons are strikingly similar to the catecholamine-containing cells disclosed in the same midbrain area of the turtle and which were also shown to project to the STR (Parent, A., J. Anat., 114: 379-387, 1973). Other HRP-positive cells that were found scattered throughout the lateral portion of the caudal midbrain tegmentum, can be related to the serotonin-containing neurons previously disclosed in the same midbrain region (Parent, A. and Poirier, L.J., J. Anat., 110: 81-89, 1971). In the vicinity of the STR injection site, HRP-positive axons coursing either towards the dorsal cortex, the DVR, or invading the lateral forebrain bundle (LFB), were also visualized. HRP-positive axons were found in both the dorsal and ventral components of the LFB. The HRP axons of the dorsal component were followed up to the HRP-positive cells of the dorsal thalamus where they appear to arise. The labeled axons of the ventral component of the LFB, in contrast, were traced down to the lateral border of the midbrain tegmentum where they appear to arborize.

On the other hand, after HRP injections into the medio-dorsal aspect of the DVR, a larger number of HRP-positive neurons were disclosed within all thalamic nuclei surrounding nucleus rotundus (see above), ipsilaterally. In addition, HRP cells were also found into nucleus rotundus itself and within nucleus reuniens. However, no labeled neurons could be identified caudally to the meso-diencephalic junction after DVR injection.
(Supported by the Medical Research Council of Canada)

Figures: FIG 1: Frontal section showing the site of a HRP injection within the STR of Chrysemys picta. x10 FIG 2: HRP-containing axons present within both components of the LFB of a turtle after STR injection. x40 FIG 3: HRP-positive neuronal somata of the rostral midbrain tegmentum after STR injection. x100







303 BEHAVIORAL EFFECTS FOLLOWING DISCRETE LESIONS OF PARS COMPACTA OF THE SUBSTANTIA NIGRA IN RATS. Gordon K. Hodge and Larry L. Butcher. Dept. of Psychology, UCLA, Los Angeles, California, 90024, U.S.A.

The extrapyramidal motor system, particularly the substantia nigra, appears involved in the pathology of Parkinson's disease, a malady affecting motor behavior. Lesions in the area of the nigra in rats have led to gross motor dysfunctions and to severe impairments of eating and drinking behavior (Ungerstedt, Acta Physiol Scand (Suppl) 367: 49, 1971; Fuxe et al., Int Rev Neurobiol 13: 93, 1970). But many of these lesions have been large, probably encompassing structures and systems other than the nigra.

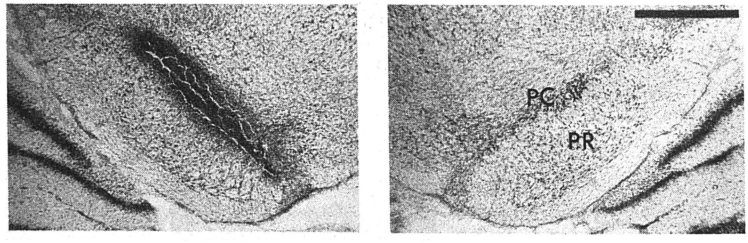
The difficulty in introducing lesions large enough to destroy significant portions of the nucleus while being discrete enough to avoid undue injury to other systems has limited the experimental analysis of the function of the substantia nigra. We have made both gross and relatively precise unilateral and bilateral radio frequency lesions in the nigra of rats. In addition, unilateral lesions were also placed in the median raphé, the globus pallidus, the medial forebrain bundle, and in the cerebellum and the dorsal tegmental bundle. Postmortem histologies have revealed some success in selectively destroying pars compacta (see figure).

Behavioral measures were recorded before and after surgery. Locomotor activity was recorded for all animals. Rotational behavior was measured for rats lesioned unilaterally; one week after the operations some groups received 5 mg/kg D-amphetamine or 1 mg/kg apomorphine. Animals receiving bilateral lesions were trained to position a lever in space between 15-20° from the horizontal for 1-2 seconds in order to receive a food pellet or to terminate grid shock; daily food and water intake were also monitored.

Contraversive turning was observed following both large and discrete unilateral lesions of the nigra, but all rats turned toward the side of discrete compacta lesions following amphetamine. After apomorphine, however, turning was seldom observed except in two rats; one turned ipsilaterally, the other, contralaterally, to the side of the lesion. Histological examinations showed that although these two lesions destroyed parts of compacta, they also involved the ventro-medial tegmentum through which other (e.g., serotonergic) fibers pass with those from compacta. Marked turning was observed after raphé lesions, but lesions of the medial forebrain bundle did not produce comparable turning. Lesions of either the globus pallidus or of the cerebellum and dorsal tegmental bundle resulted in some contraversive turning; amphetamine induced ipsilateral rotations.

Locomotor activity was increased following some bilateral lesions of pars compacta. However, hypoactivity was observed in the lever-positioning apparatus; e.g., inter-press-intervals were longer. In the food reward group, a few rats spent more time in consummatory activities such as chewing in excess of that normally seen. Some bilaterally lesioned animals displayed moderate aphagia and adipsia which lasted about 3-4 days after the operations; others, however, ate and drank normally.

Although the substantia nigra seems involved in the control of certain behaviors, the roles of other areas in the mediation of these behaviors may be of equal or of greater importance.



Unilateral lesion of pars compacta (PC) of the left substantia nigra; the right side was not lesioned. PR = pars reticulata. Scale = 1.0 mm.

This research was supported in part by USPHS grant NS-10928.

304 INTRACELLULAR RESPONSES OF CAUDATE OUTPUT NEURONS TO ORTHODROMIC STIMULI. D.R.G. Fuller,* N.A. Buchwald, C.D. Hull. Mental Retardation Research Ctr., Brain Research Institute, UCLA, Los Angeles, 90024.

This experiment was designed to compare aspects of the neurophysiology of striatal output cells with those of striatal interneurons. Intracellular recordings were obtained from 161 neurons in the head of the cat caudate nucleus. Ten of these neurons (a proportion similar to that estimated by morphological studies) were identified as output cells on the basis of antidromic responsiveness to stimulation of the entopeduncular nucleus or the substantia nigra. The responses of these output neurons to orthodromic activation by stimulation of cortical and subcortical sites projecting to the caudate nucleus was recorded. PSPs in output cells were characterized by large initial excitatory potentials which were sometimes followed by small hyperpolarizing potentials. Most of these small IPSPs were only apparent after several responses had been averaged. In contrast, caudate interneurons generally respond to the same caudatopetal stimuli with an EPSP-IPSP sequence in which the IPSP is quite prominent. The fact that initial excitation occurs in output neurons as well as in interneurons is consistent with our hypothesis that nearly all afferents to the caudate nucleus are excitatory and suggests that caudatopetal fibers may project directly onto output neurons. A second aspect of our hypothesis is that stimulus evoked inhibition of caudate interneurons is generated intrinsically. Since output neurons show relatively little inhibition following an initial excitation, it may be that they are more polarized than are interneurons, a supposition supported by the fact that depolarization by current injection often reveals the presence of small IPSPs following the initial excitation. (Supported by USPHS MH-07097 and HD-05958).

305 SOME CHANGES IN MOTOR BEHAVIOR PRODUCED BY SURGICAL LESIONS OF THE STRIATUM IN THE MONKEY. Samuel L. Liles. Dept. Physiol., Louisiana State Univ. Medical Center, New Orleans, 70119.

There is abundant anatomical evidence that the projection from the cerebral cortex to the striatum (caudate and putamen) is topographically organized. As a preliminary approach to examining the functional significance of these anatomical mosaics the present study has sought to determine the effects on motor behavior of selective lesions placed in the striatum. During aseptic surgery the region of the striatum to be destroyed is accurately defined by a brief mapping experiment to determine the distribution of evoked gross or focal potentials to stimulation of a specific area of the cerebral cortex. Striatal lesions are then made via temperature-controlled thermocoagulation. Only unilateral lesions have been made so far. The data in which histological analyses are available indicate that lesions restricted to the dorsal part of the putamen and dorsolateral caudate (the area of the striatum from which evoked electrical potentials are recordable to stimulation of the medial part of the premotor cortical area) result in dystonic posturing of the contralateral hand (hyperextension of fingers, flexion of wrist) but pronounced abnormal movements have not been seen. Lesions in the rostroventral area of the striatum (projection target of the orbitofrontal cortex) cause marked general hyperactivity and visual compulsion reactions described by Denny-Brown (eg., "leaping automatism"). The data provide tentative support for the hypothesis that the topographic features of the corticostriatal projection are of functional significance. (Supported by NIH Grant NS-08907.)

306 ABNORMAL FIRING PATTERNS IN THE GLOBUS PALLIDUS OF MONKEYS WITH DOPAMINE DEPLETING LESIONS. Michel Filion and Louis Larochelle. Lab. Neurobiol., Fac of Med., Laval Univ., Quebec, QUE., Canada G1K 7P4.

During quiet waking, neurons of the internal division of the globus pallidus (GPi) of intact monkeys (macaca mulatta) typically fire at high tonic rates in irregular but continuous patterns. Following brain stem lesions bilaterally involving the nigrostriatal and other monoaminergic and non-monoaminergic pathways monkeys have displayed akinesia, rigidity and tremor, the three most characteristic signs of parkinsonism. Under these conditions many GPi neurons continuously fire in short rhythmic bursts at frequencies around 14/s. The number of rhythmic units is higher in the GPi ipsilateral to the most important nigral cell loss and therefrom ipsilateral to the most important striatal dopamine depletion (which has been shown to be linearly correlated with the amount of ipsilateral nigral cell loss). The involvement of other monoaminergic systems has however not been investigated in relation with pallidal rhythmic activity. A striatal dopamine defect is characteristic of the parkinsonian syndrome; our data bring evidence of a related dysfunction at the outflow of the striopallidal system. This pallidal dysfunction may be responsible for either one, two or all three signs of parkinsonism shown by monkeys with brain stem lesions.

(Supported by the Medical Research Council of Canada).

307 ELECTRON MICROSCOPIC STUDIES OF p-CHLORAMPHETAMINE-INDUCED DEGENERATION OF STRIATAL SYNAPSES. E. G. McGeer, T. Hattori and P. L. McGeer. Kinsmen Laboratory of Neurological Research, Dept. Psychiatry, Univ. of B. C., Vancouver, Canada.

p-Chloroamphetamine (p-ClA) in low doses (5-10 mg/kg i.p.) has been reported to cause destruction of serotonergic neurons as indicated by decreases in serotonin levels and by pathological cell changes visible at the light microscopic level.[1] At higher doses (20 mg/kg i.p.) the effect is less specific since destruction of dopaminergic cell bodies and decreases in catecholamine levels also occur. An electron microscopic search for degenerating synapses in the rat striatum two days after various intraperitoneal doses of p-chloroamphetamine was carried out. With low doses (5-7 mg/kg) of p-ClA very few degenerating synapses could be found but they all appeared to be of a very small, symmetrical type; with larger doses (10-20 mg/kg) larger, asymmetrical synapses were also seen to degenerate. The latter degeneration is the type seen after 6-OHDA treatment and has been ascribed to dopaminergic nerve endings. The former type would appear, therefore, to be associated with serotonergic nerve endings. Measurements of tyrosine and tryptophan hydroxylases were used as an index of the drug's effects on dopaminergic and serotonergic nerve endings in the striatum; striatal levels of GAD and choline acetylase were not affected at even 20 mg/kg.

[1] J. A. Harvey et al. Science 187, 841-843 (1975).

Supported by a grant from the M.R.C. of Canada.

308 EVIDENCE THAT DOPAMINE NEURONS IN THE PARS COMPACTA OF THE RAT SUBSTANTIA NIGRA CONTAIN ACETYLCHOLINESTERASE (AChE) — RETROGRADE DEGENERATION OF NIGRAL AChE-CONTAINING NEURONAL SOMATA AFTER LESIONS ALONG THE TRAJECTORY OF THE NIGROSTRIATAL PATHWAY. Larry L. Butcher and Konrad Talbot. Dept. Psychology, University of California, Los Angeles, CA, 90024, U.S.A.

In previous papers we argued that dopamine-containing neuronal perikarya in the pars compacta of the rat substantia nigra also contained AChE. First, there was considerable morphological similarity in cell body shape, size, and topographical pattern of processes between somata containing dopamine, as demonstrated by fluorescence histochemistry, and those possessing AChE, as demonstrated by a pharmaco-histochemical regimen used routinely by us over the last 3 years (Butcher et al., 1974a, Proc. West. Pharm. Soc.; 1975b, J. neural Trans.). Second, the proportion of pars compacta cells possessing dopamine (≈ 90%; from data in Gulley and Wood, 1971, Tissue and Cell) and the proportion containing AChE (at least 90%; Butcher et al., 1975b, above ref.) was too great to permit the simultaneous existence of independent, non-overlapping sets of neurons. Third, radio-frequency ablations in the ventromedial tegmental area of the mesencephalon, through which course the axons of the nigrostriatal dopamine pathway, produced retrograde degeneration of AChE-containing neuronal somata in the substantia nigra, pars compacta (Butcher et al., 1975a, above ref.). Similarly placed lesions in the monkey result in loss of pars compacta neurons correlated with reductions in neostriatal dopamine levels (Poirier and Sourkes, 1965, Brain).

In this report we demonstrate that retrograde degeneration of AChE-containing cell bodies in the rat substantia nigra, pars compacta, occurs after radio-frequency lesions at additional loci along the trajectory of the nigrostriatal dopamine pathway — the medial forebrain bundle and the globus pallidus. Lesions in either of these loci produce retrograde degeneration of pars compacta dopamine neurons and reduced levels of neostriatal dopamine (Andén et al., 1966, Acta physiol. scand.). We have also found that large lesions of the terminal projection area of the nigrostriatal pathway, the caudate-putamen nucleus, results in retrograde degeneration of pars compacta AChE-containing neuronal somata; retrograde degeneration also occurs in dopamine neurons in pars compacta after neostriatal lesions (Andén et al., 1966, Acta physiol. scand.).

Recently, Fonnum et al. (1974, Brain Res.) measured nigral AChE in the cat after lesions in the caudate nucleus and other telencephalic nuclei and concluded that the "reduction in AChE...reflects an unspecific localization of the enzyme in the substantia nigra (p. 91)." Our results agree with this conjecture in that nigral AChE in pars compacta is localized, at least in major part, within dopaminergic, and hence non-cholinergic, neurons. Indeed, substantia nigra lesions in the rat do not lead to biochemically detectable reductions in neostriatal acetylcholine levels (Butcher and Butcher, 1974, Brain Res.).

Many investigators consider AChE merely as a marker for acetylcholine-containing neurons rather than as a physiologically significant substance in its own right. However, Fonnum et al. (1974, above ref.) note that the "abnormally high ratio (1000) between AChE and ChAc activities in the substantia nigra makes AChE an unreliable marker for cholinergic fibers in this region (p. 90)." In view of this consideration, the function of AChE within dopamine-containing neurons in the substantia nigra might be explained according to the cholinergic-adrenergic link hypothesis of Koelle (1963, Handbuch exp. Pharmak.) and Burn and Rand (1964, Ann. Rev. Pharm.). Such an hypothesis does not explain, however, the presence of AChE in the dendrites of pars compacta cells extending far into pars reticulata of the substantia nigra. We propose that the dendritic localization of the enzyme may serve as a locus of inactivation of acetylcholine released from cholinergic neurons afferent to nigral dopaminergic cells.

This research was supported in part by USPHS grant NS-10928.

309 INPUT TO THE INFERIOR OLIVE OF THE CAT. A COMPARISON OF SPINO-OLIVARY PROJECTIONS WITH THOSE FROM THE DORSAL COLUMN NUCLEI, LATERAL CERVICAL NUCLEUS AND CEREBELLUM. Ian G. Worden* and Karen J. Berkley (SPON: Karen K. Glendenning). Dept. Psychol., Fla. St. Univ., Tallahassee, Fla., 32306.

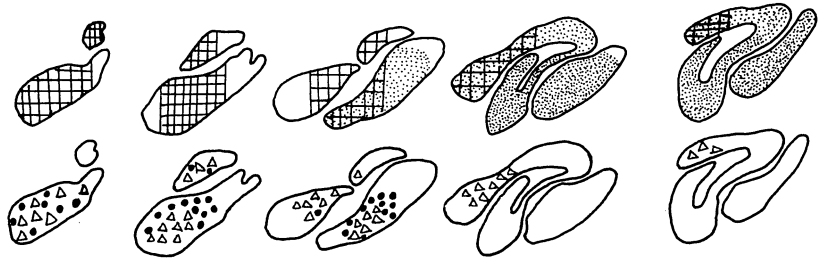
A differential labeling strategy was used to study the projections of spino-olivary tract, dorsal column nuclei (DCN), lateral cervical nucleus (LCN) and cerebellum (CB) to the inferior olive in 10 cats. In each cat, the projection of one source of afferent fibers to the inferior olive was labeled using autoradiographic tracing methods while the projections of another source was labeled using degeneration tracing methods. Using this strategy, the convergence or divergence of terminals from the various afferent sources could be studied directly in the same cat.

With some exceptions, it was generally found that, whereas there was limited overlap between CB terminations and those of the spino-olivary tract, DCN or LCN, there was considerable overlap between the spino-olivary and either the DCN or LCN terminals.

Medial accessory portion of the inferior olive (MAO): The spino-olivary terminations in the caudal portions of MAO converge on the same regions as those from either DCN or LCN. There is very little convergence, however, between DCN and LCN terminations, except in the most caudal regions. There also appears to be very little convergence between these projections and those of the cerebellum. The CB projects predominantly to the rostral portions of MAO.

Dorsal accessory portion of the inferior olive (DAO): As in MAO, the spino-olivary terminations in the ventrolateral portions of DAO converge on the same regions as those from DCN. Only a very few fibers from LCN, however, appear to project to DAO. Both the spino-olivary and DCN terminations overlap with those from the cerebellum. The CB projections, however, are more widespread and less dense within DAO. Furthermore, of the four pathways studied, only the CB appears to send fibers to the principle division of the inferior olive.

These projections are summarized in the schematic diagrams of the coronal sections through the inferior olive shown below. The upper drawings show projections from the spino-olivary tract (###) and the cerebellum (▒). The lower drawings show projections from DCN (△△) and LCN (•••).



From left to right, the diagrams are of successive rostrad sections approximately 720μ apart.

(Supported by PHS grants NS 11892 and NS 02992.)

310 THEORETICAL ASSOCIATION OF CLIMBING FIBER ACTIVITY WITH LONG TIME-COURSE, NON-PLASTIC CHANGES IN THE SPATIAL DISTRIBUTION OF CEREBELLAR OUTFLOW. C. C. Boylls* (SPON: W. J. Roberts). Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, Oregon 97210.

Much difficulty has been encountered in correlating non-vestibular, cerebellar climbing fiber activity with immediately observable motor consequences. A mathematical model of cat anterior lobe cortex and associated cerebellar and reticular nuclei has been used to demonstrate a possible relationship between climbing fiber inputs and long time-course alterations in the spatial distribution of cerebellar neuronal activity. Specifically, cerebellar nuclear areas receiving from a single active Oscarsson strip in the cortex are shown slowly to become relatively more excited than areas receiving from mediolaterally adjacent strips; in fact, the latter regions are actively suppressed. Such spatial activity changes persist for hundreds of milliseconds, a phenomenon attributable not to plasticity, but to the effects of reticulocerebellar "reverberation" as described by Tsukahara (Brain Res. 40: 67, 1972). By virtue of anterior lobe projection patterns, any climbing fiber induced alterations in cerebellar spatial activation patterns will also redistribute excitation in the brainstem targets of the anterior lobe which give rise to descending spinal pathways. Thus, musculature influenced by these brainstem centers could be biased in characteristic ways for prolonged periods by volleys in specific climbing fiber strips. This suggests that the olivocerebellar system might not operate in "movement-time" at all, but may instead act to adjust the spatial structure of anterior lobe outflow over much broader intervals, perhaps in accordance with slowly changing patterns of spinal reflex utilization and/or to provide short-term adaptation of motor output to changing external conditions.

311 AN INTRACELLULAR ANALYSIS OF THE RELATIONSHIP BETWEEN PRECEREBELLAR NUCLEI AND THE NUCLEUS INTERPOSITUS ANTERIOR OF THE CAT. R.A. McCrea, R.J. Preston, * S.T. Kitai. Morin Memorial Laboratory, Dept. Anatomy, School of Medicine, Wayne State University, Detroit, Mich.

Anatomical studies indicate that in addition to inhibitory input from the Purkinje cells of the cerebellar cortex, the nucleus interpositus anterior (NIA) receives afferents from the lateral reticular nucleus (LRN) and the inferior olive (IO). NIA efferents are in turn monosynaptically excitatory to the red nucleus, the nucleus reticularis tegmenti pontis (NRTP) and the IO. In the present study the nature of the input to NIA from the precerebellar nuclei was examined in barbiturate and decerebrate cats. Bipolar stimulating electrodes were placed in the contralateral IO and NRTP, ipsilateral LRN, and three ipsilateral cerebellar peduncles. Antidromic spikes were observed following stimulation of the brachium conjunctivum (latency 0.5 msec) and often following stimulation of NRTP (latency 0.8 msec). Stimulation of LRN and IO elicited responses which consisted of short latency, monosynaptic EPSPs (2.0 and 3.7 msec, respectively) followed by IPSPs and a late depolarization. The late depolarization was often quite powerful and prolonged, and appeared to be due to disinhibition of Purkinje cells. Monosynaptic EPSPs were rare with NRTP stimulation, although polysynaptic EPSPs were occasionally observed. It appears that there are excitatory loops between the deep cerebellar nuclei and certain precerebellar nuclei. Precerebellar nuclear excitation of IP may provide a background depolarization upon which the inhibitory and disinhibitory output of the cerebellar cortex can be expressed.

(Supported by USPHS Grant NS 00405 and RR 5384).

312 SPINAL CONNECTIONS TO THE VESTIBULOCEREBELLUM. Dietrich W.F. Schwarz, and A. Craig Milne*. Lab. of Otoneurology, Depts. Otolaryngology and Physiology, University of Toronto.

Neurones of the nodular and uvular vermis were recorded extra-cellularly in cats under N_2O analgesia after surgical halothane anaesthesia was discontinued. Responses to electrical stimuli of mixed limb nerves, neck muscle nerves, both vestibular nerves and light flashes were monitored as stimulus time histograms. Somatosensory receptive fields (RF) were mapped manually. More than 300 units could be completely investigated of which only few could be identified as Purkinje cells. Most units responded to vestibular stimuli with a similar response pattern for each side with characteristically shorter latencies for the ipsilateral side or identical latencies for midline units. Vestibular climbing fiber responses were rare and variable. Somatosensory responses of vestibular units were less frequent than within the vestibular nuclei. These cells are concentrated in narrow zones within the uvula and less frequently the nodulus. Two categories were recognized: one group of neurones specialized for deep neck afferents including muscle afferents and a second group receiving deep afferent input from larger RFs. No influence of skin receptors was seen. Comparison of latencies indicates that transmission via the vestibulocerebellum can account for only part of the somatosensory influence on vestibular nuclei cells.

313 A THEORETICAL APPROACH TO MECHANISMS OF ELECTROLOCATION IN FISH. Walter Heiligenberg, SIO-UCSD , La Jolla, Cal. 92037

Weakly electric fish generate electric fields by continually firing their electric organ. Objects which differ in conductivity from the surrounding water distort electric fields and can therefore be detected by arrays of electroreceptors on the animal's body surface. At large distance an electric fish's field approaches the field of an electric dipole. However, due to the geometry of the fish's body, its near field differs greatly from a dipole field.

Previous theoretical estimates of the range of electrolocation were based on the assumption of a dipole field. However, behavioral experiments have demonstrated that objects are detected only within the animal's near field which cannot be approximated by a dipole model. Since no suitable analytical model is available to describe the near field of an electric fish, fields are simulated numerically on a digital computer. The electric fish is considered an isopotential anterior body and a nonconducting tail filament. The anterior body is surrounded by skin of higher resistivity than that of fresh water. During the electric organ discharge the anterior body becomes positive relative to the end of the tail. The electric fields obtained by this simulation strongly resemble measured fields of electric fish. Distortions of such fields by various objects can be calculated and the corresponding electric "images" of objects on the animal's body surface can be expressed in terms of changes in local potential as a function of object size and location.

Results of these calculations are in good agreement with behavioral and electrophysiological data. The significance of the electric fish's body geometry, its relative skin resistivity and receptor densities are discussed.

314 SIMPLE AND COMPLEX SPIKE GENERATION IN A COMPUTER MODEL OF CEREBELLAR PURKINJE CELLS. A. Pellionisz and R. Llinás. Div. Neurobiology, Univ. of Iowa, Oakdale, Ia. 52319.

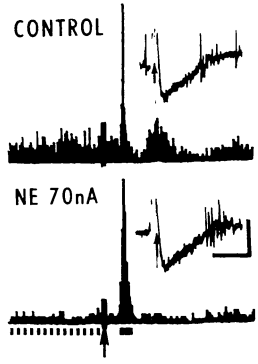
A software Purkinje cell model has been developed utilizing a PDP-15 (DEC) computer. The model is based on current morphological and physiological data from frog Purkinje cells. It allows detailed analysis of the passive cable properties, spike generation activity in the axon, soma and dendrites, and overall integrative properties of this neuron. The model comprises 31 dendritic branches, 15 branch bifurcation areas, a soma, an axonal initial segment, 7 nodes and 7 myelinated segments. Each of these 62 compartments includes morphological parameters such as the physical dimensions of each segment as well as physiological parameters such as Hodgkin and Huxley equations (after Frankenhaeuser and Huxley, J. Physiol., 1964). The equations were solved with an integration time varying between 200 nsec and 25 μsec, depending on the rate of change of potentials with respect to time. The Hodgkin-Huxley parameters were successively approximated to provide a proper antidromic invasion as well as a climbing fiber evoked EPSP and climbing fiber spike bursts, as known from intracellular recording. Different configurations of parallel fiber orthodromic input were also studied. The analysis suggests that the climbing fiber evoked spike burst is triggered by repetitive firing in the initial segment, supported by the firing of active regions in the dendritic tree. The model also postulates a slightly higher sodium permeability for the initial segment (125%) than that in the node, and a considerably lower potassium permeability for the dendritic tree than for the soma and axon. This model exemplifies the class of Purkinje cell utilized in the development of a large simulation of electrical activity of the frog cerebellar cortex. (Supported by PHS research grant NS-09916 from NINDS)

315 BRACHIO-RUBRAL SYNAPTIC SYSTEM IN THE BABOON. Ronald D. Huffman, Univ. of Texas Health Science Center at San Antonio, Texas 78284.

Eight barbiturate anesthetized female baboons (8-15 kg) were employed to investigate the brachium conjunctivum (BC) synaptic input to the midbrain tegmentum. Stainless steel microelectrodes were employed to record single and multineuronal responses from mesencephalic neurons following stimulation of the hilium of the BC. Topographic recordings demonstrated that most of the BC-evoked activity was restricted to red nucleus (RN); however, some BC-evoked activity was recorded from neurons located in the surrounding reticular formation. The synchronous activity of the BC volley resulted in excitatory activation of both magnocellular (RNm) and parvicellular (RNp) red nucleus neurons. The BC-RN response consisted of an initial positive-negative potential, the (b) response, of short latency (0.56 msec for RNm; 0.64 msec for RNp) representing the activity of synchronous impulses in BC terminals and fibers of passage. The (b) response was followed after a synaptic delay of approximately 0.84 msec by a prominent negative potential, the (r) response. The (r) response (1.80 msec for RNm; 1.82 msec for RNp) is assumed to be the result of monosynaptic excitation, generation, and propagation of impulses in rubral neurons. RNp neurons occasionally responded to a single BC volley with repetitive discharges. The BC-RN synaptic system was found to be a secure synaptic system and could transmit activity at high rates of stimulation without failure. The responsiveness of the BC-RN synaptic began to decrease 5 msec after a single or repetitive transmission and was reduced to about 50% of normal at 35 msec. This may represent a disfacilitation of RN neurons produced by electrical stimulation of climbing fibers in the region of the hilium of the BC. Activation of climbing fibers by stimulation of the inferior olivary nucleus has been reported to result in hyperpolarization of RN neurons; this hyperpolarization has a latency of 4 msec and a duration of 20-50 msec (Toyama et al., Exp. Brain Res. 4:292, 1968).

316 INTERACTION OF NOREPINEPHRINE WITH CEREBELLAR NEURONAL CIRCUITRY. Barry J. Hoffer*, Robert Freedman*, Donald Puro*, and Donald J. Woodward. Lab. of Neuropharmacology, NIMH, IRP, SMRN, St. Elizabeth's Hospital, Washington, D. C. 20032, and Department of Physiology, Univ. of Rochester, Rochester, New York, 14642.

Although the adrenergic input from the nucleus locus coeruleus to the cerebellar cortex previously has been shown to inhibit spontaneous activity of the Purkinje (P) cell by release of noreprinephrine (NE), the interaction of NE with cerebellar neuronal circuitry has not yet been determined. NE was applied to P cells by microiontophoresis during simple or complex spike excitation evoked by stimulation of climbing fiber and mossy fiber afferents or during inhibition elicited by basket and stellate cells via "off beam" parallel fiber stimulation. NE reduced P cell spontaneous activity to a greater extent than the response probability of mossy or climbing fiber input. Typically, simple and complex spikes could be reliably elicited even when spontaneous activity was almost abolished by adrenergic inhibition. In many cases, the number of full-sized action potentials in the complex spike was increased. This differential effect could be readily quantitated by construction of poststimulus time histograms before, during and after NE.



The above figure shows an example of a complex spike excitation produced by electrical stimulation (arrows) of the cerebral cortex (200 sweeps of 3 shocks each at 2 sec. intervals). Iontophoresis of 70 nanoamps of NE reduced spontaneous activity (dotted line) from 25.8 to 7.9 spikes per second. Evoked complex spike excitation (solid line), on the other hand, increased from 0.88 spikes/stimulus to 1.18 spikes/stimulus. This increase in the number of full size action potentials in the complex spike is also shown in the extracellular specimen records. Calibrations are 0.5 mV, 10 msec. for specimen records and 5 counts/address, 30 msec. for histograms. Off-beam inhibition was potentiated by NE. This augmentation was seen even with low doses of NE, which only minimally depressed spontaneous activity, and often outlasted by many minutes the duration of NE application. NE thus enhances the P cell's responses to both excitatory and inhibitory afferents, relative to the level of background spontaneous activity. These data suggest that adrenergic input may act to facilitate the transfer of information in the cerebellar cortex. (Supported in part by NIH NS11030 and NSF GB43301).

317 CEREBRAL AND PERIPHERAL INPUTS TO INTERPOSITUS NEURONS IN PRIMATES. Gary I. Allen, Rolando Marini* and Wolfram Schultz*. Lab. of Neurobiology, Dept. of Physiology, State University of New York at Buffalo, N.Y. 14226.

The intermediate zone of the cerebellum, because of its outflow through the interpositus nucleus to the rubrospinal and corticospinal systems, is thought to participate in the execution of skilled limb movements. The goal of the present experiment was to study the inputs from cerebral cortex and peripheral nerves to the intermediate zone to further assess its role in movement. Single interpositus neurons were recorded in cebus monkeys under nitrous oxide anesthesia. The response characteristics and the projection pattern of inputs from motor, somatosensory, supplementary motor and premotor cerebral areas, and peripheral nerves were analyzed. In contrast to dentate neurons in the same species, interpositus neurons receive a stronger input from the primary motor and somatosensory areas (93%) and nerves (57%) and a weaker input from area 6 (supplementary motor and premotor). 55% of the neurons receive inputs somatotopically restricted to cerebral and nerve inputs representing either the forelimb or the hindlimb, while 40% are mixed. By comparison with the cat, twice as many interpositus neurons are related to a single limb, which is consistent with the increase in independent movement of the limbs in the monkey. The neurons in the anterior interpositus nucleus are predominantly hindlimb specific, while those in the posterior nucleus are primarily mixed or forelimb specific. These observations are consistent with the hypothesis that the intermediate zone of the cerebellum integrates signals from the motor cortex and sensory information describing limb position and velocity to up-date a limb movement during its course.

318 Interaction of cerebellar, somatosensory, and thalamocortical evoked potentials in the monkey. John J. Hablitz, Department of Neurophysiology, The Methodist Hospital and Baylor College of Medicine, Houston, Texas.

Single pulse shocks delivered to the cerebellum (CB) of chronically prepared monkeys evoked responses in pre- and post-central cortical areas. The interaction of CB responses with potentials evoked by stimulation of peroneal nerve, n. ventralis lateralis or cerebral cortex (DCR) was studied by presenting a conditioning shock to the CB followed by stimulation of the above 3 areas after delays of 0, 10, 60, 100 and 200 msec. Eight trials of each condition were averaged on a PDP-12 computer. Significant interaction appeared to occur between the cerebellar response and all the other responses. The VL-evoked response and the DCR were fully recovered by 60 msec. Somatosensory (SS) evoked potentials (EP) were not fully recovered at 200 msec but were returned to approximately 80% of control values. Since the interaction with VL and DCR responses only appeared at short interstimulus intervals, it was suspected that this might be an occlusive phenomenon resulting from simple algebraic summation. To test for this, averaged baseline recordings of CB EP's were digitally subtracted from the paired stimulation averages. The result was a VL or DCR response similar to the baseline record, indicating a lack of physiological interaction. This was not true of SS responses. SS EP's were also inhibited by continuous CB stimulation at rates (200 Hz) which caused the CB EP to fail.

These results do not resolve the question of where in the nervous system the cerebellum exerts its effects on sensory processes, but the lack of interaction with thalamocortical responses is suggestive of a mediation by cerebellar bulbar rather than cerebellar-cerebral pathways.

319 GANGLIOSIDE COMPOSITION OF THE GRANULOPRIVAL POSTNATAL MOUSE CEREBELLUM. Margaret Jones and Warren Taylor*. Department of Pathology, Michigan State University, East Lansing, Michigan 48824.

Compared to other parts of the central nervous system, the cerebellum of some species, including the human, has a high proportion of trisialoganglioside. This region of the brain is also rich in synapses which differentiate postnatally in many species. Since gangliosides are highly concentrated in synaptic endings, it was hypothesized that changes in ganglioside composition might parallel cerebellar postnatal synaptogenesis. The present study using granuloprival and normal cerebellums, was initiated to test this hypothesis and to determine if trisialoganglioside or one of the lower homologs increased concomitantly with postnatal differentiation of granule cells and granule cell-Purkinje cell synaptic contacts (GCSC). The latter differentiate after the first week of life.

Swiss Albino mice were sacrificed at 0, 5, 15, 20 and 25 days postnatal following injections with 0.05 ul methylazoxymethanol (MAM) or saline/gm of body weight on the first day of life. The body, whole brain and cerebellum weights were determined. Selected samples were studied histologically. Cerebellar homogenates were extracted, the gangliosides were separated by thin-layer chromatography and individually quantified (except for presumed tetrasialoganglioside). As in previous experiments, following MAM treatment, cerebellar hypoplasia resulted from destruction of differentiating cells and subsequent granule cell deletion (Jones, Mickelsen & Yang. Prog. Neuropath. 1973). Cerebellar ganglioside NANA was reduced in treated animals.

On the first day of life; the molar percentages of gangliosides were. G_{M1} 27%; G_{D1a} 28%; G_{D1b} 21% and G_T 24%. The presence of trisialoganglioside in significant quantities at this time indicated that its presence was not dependent upon morphologic differentiation of granule cells and GCSC. On days 5, 15, 20 and 25, values in control and treated animals did not differ significantly and were quite similar at all periods studied. Average molar percentages of gangliosides were: G_{M1} 38%; G_{D1a} 22%; G_{D1b} 20% and G_T 20%. The increase in G_{M1} was the predominant compositional change with development. Moreover, monosialoganglioside rather than trisialoganglioside predominated in the Swiss Albino mouse cerebellum. Whether any of these compounds has a significant role in synaptogenesis remains to be determined, but it is clear that their presence does not depend on morphologic differentiation of the granule cell or its contacts.

320 BRAIN STEM AFFERENTS TO THE DENTATE NUCLEUS AS DETERMINED WITH HORSERADISH PEROXIDASE. Alvin J. Beitz. Dept. of Anatomy, University of Minnesota, Minneapolis, Minnesota, 55455

The inferior olive and the pontine nuclei provide two important sources of imputs to the dentate nucleus of the cerebellum. The projections from these precerebellar nuclei in the brainstem to the dentate were studied in adult cats, utilizing the technique of retrograde transport of horseradish peroxidase (HRP) as described by LaVail (Brain Res., 58:470, 1973). Approximately 0.1 ul of HRP (35% in distilled water) was stereotaxically injected into the right dentate nucleus. Following a 1-3 day survival period, the brain was fixed and serial frozen sections from the appropriate regions were prepared for histochemistry. In the left inferior olivary complex a large number of neurons in the rostral portion of the medial accessory olive were found to contain small brown refractile granules characteristic of HRP positivity. Additional peroxidase activity was identified in the ventral lateral outgrowth and in the ventral lamella of the principal olive. A bilateral projection to the dentate was found from the pontine nuclei. Positive cells were identified in the medial and lateral divisions of the pontine gray with a slight predominance of cells on the left side. In addition, some peroxidase activity was identified bilaterally in the tegmental reticular nucleus of the pons. These data suggest that the dentate nucleus receives a significant input from the medial accessory olive and an important bilateral projection from the pontine gray.

321 SYNAPTIC ORGANIZATION OF THE CEREBELLO-OLIVARY FEEDBACK LOOP. J.S. King and J.A. Andrezik*. Dept. Anat., The Ohio State University, Columbus, Ohio, 43210.

Previous fine structural accounts of the inferior olivary nucleus include description of a synaptic complex (King and Andrezik, Anat. Rec., 181: 394, 1975) and the distribution of different categories of presynaptic profiles on dendritic shafts (Bowman and King, J.C.N., 148: 491, 1973). The terminal distribution of cerebello-olivary fibers seen after amino acid placements varies with their site of origin within individual cerebellar nuclei (Martin and Henkel, abstract this meeting). Thus each of the olivary subnuclei were examined with the electron microscope subsequent to surgical interruption of the cerebello-olivary system of axons. The majority (84%) of degenerating axon terminals (1-1.5μ) within the principal nucleus are presynaptic to spiny appendages which constitute the central core of the synaptic complex. The remaining terminals (16%) contact the shafts of distal dendrites. Within the dorsal accessory nucleus the post synaptic locus for cerebello-olivary axons is similar to that expressed for the principal nucleus. The rostral portion of the medial accessory nucleus also conforms to this pattern. In contrast, degenerating axon terminals in the caudal region of the medial accessory nucleus are somewhat larger (2.0μ) and primarily contact dendritic shafts (75%). Irrespective of the area sampled these presynaptic terminals contain round clear synaptic vesicles and display Gray's type I active sites. The above difference in post synaptic distribution of this system of axons is reflected by nuclear origin as the fastigial nucleus projects to the caudal medial accessory nucleus. The dentate-interpositus nuclei likely influence synaptic interactions within the complex synaptic islands throughout the rostral one-half of the nucleus. Supported by USPHS Grant NS-08798.

322 CEREBELLO-OLIVARY FIBERS: AN ANALYSIS OF THEIR ORIGIN, COURSE AND DISTRIBUTION USING HORSERADISH PEROXIDASE, AUTORADIOGRAPHIC AND DEGENERATION TECHNIQUES. George F. Martin and Craig K. Henkel*. Dept. Anat., Coll. Med., Ohio State University, Columbus, Ohio, 43210.

Although degeneration techniques suggest that cerebello-olivary fibers are limited in their origin and distribution, horseradish peroxidase and autoradiographic experiments make it clear that they arise within each cerebellar nucleus and project to most, if not all, areas of the contralateral inferior olive. The autoradiographic material shows that cerebello-olivary fibers are highly ordered, although it is clear that a single cerebellar nucleus does not project to only one subdivision of the olivary complex. The interpositus nuclei project in an organized fashion to the dorsal accessory, medial accessory and principal nuclei. The available material suggests that fibers from the dorsal part of the dentate relay particularly heavily to the lateral bend and adjacent ventral lamella of the principal nucleus, as well as to certain areas of the medial and perhaps dorsal accessory nuclei. In contrast, axons from ventral dentate neurons appear to favor the dorsal lamella of the principal nucleus. Although fibers from both the interpositus and dentate nuclei project to portions of the medial accessory nucleus, those which distribute to its caudal end arise from the fastigial complex. Olivary fibers from both the interpositus and dentate nuclei traverse the brachium conjunctivum descendens, whereas those from fastigial neurons take a different route. Experiments utilizing horseradish peroxidase as a tracer suggest that cerebello-olivary fibers from both the interpositus anterior and dentate nuclei take origin from a population of generally small neurons. Supported by USPHS Grants NS-07410 and NS-08798.

323 RESPONSE OF FLOCCULUS PURKINJE CELLS TO INTERACTIONS OF SMOOTH PURSUIT EYE MOVEMENTS AND NATURAL VESTIBULAR ROTATION. Stephen G. Lisberger* and Albert F. Fuchs (SPON: H.D. Patton). Dept. of Physiology and Biophysics and Regional Primate Research Center, Univ. of Washington, Seattle, Washington 98195.

The previous abstract (Fuchs and Lisberger) shows that flocculus Purkinje cells (P-cells) are more deeply modulated with $\pm 10°$ sinusoidal smooth pursuit eye movements in the absence of head movements than with $\pm 10°$ sinusoidal head rotation in the absence of eye movement and that P-cell firing rate increases with ipsilateral eye and head velocity. Since normal tracking conditions involve the coordination of smooth eye and head movements, monkeys were trained to track a moving target while undergoing sinusoidal horizontal vestibular rotation. By controlling target position with the signal monitoring head position, it was possible to drive the target in or out of phase with head rotation and at velocities equal to or twice head velocity. Except for saturation effects in some cells, P-cell firing frequency during all these conditions can be accurately predicted by the linear addition of the same cell's responses to eye velocity and head velocity alone. The greater sensitivity to smooth eye velocity predominates and P-cells reach maximum firing frequency near maximum ipsilateral eye velocity, independent of the direction of the concurrent head movement. For example, when the rotating monkey fixates an earth-fixed target (eye velocity equal and opposite to head velocity) P-cell discharge is in phase with ipsilateral eye velocity but 180° out of phase with the same cell's response to head velocity alone. Since flocculus P-cells inhibit the interneuron in the vestibulo-ocular reflex (VOR) pathway, this out of phase discharge would improve the response of the interneuron during vestibular stimulation, thus aiding the VOR in keeping the eyes fixed on the target.

324 RESPONSE OF FLOCCULUS PURKINJE CELLS DURING SMOOTH PURSUIT EYE MOVEMENTS. Albert F. Fuchs and Stephen G. Lisberger* Dept. of Physiology and Biophysics and Regional Primate Research Center, Univ. of Washington, Seattle, Washington, 98195

Our earlier report (Brain Res. 69(1974), 347-353) showed that flocculus Purkinje cells (P-cells) are modulated by passive head rotation only when the monkey uses a visual fixation point rotating with him to suppress the vestibulo-ocular reflex (VOR). The present study shows that the same P-cells are also modulated during smooth pursuit eye movements. Eye movements and extracellular single unit responses were recorded from alert monkeys trained to track a smoothly moving target. During ±10° sinusoidal smooth eye movements at frequencies from 0.3 to 1.4 Hz, P-cell simple spike discharge is deeply modulated, reaching maximum firing rates as high as 250/sec near maximum eye velocity. During constant eye velocity ramps (range -50 to 50°/sec) P-cells discharge at a steady rate which increases linearly with ipsilateral eye velocity. All smooth pursuit P-cells which were tested during suppression of the VOR increase their discharge rate during ipsilateral head movements, but are more deeply modulated during ±10° smooth pursuit eye movements than by ±10° head rotation. Burst-tonic eye movement fibers believed to be mossy fibers were also recorded in the flocculus. In contrast to the P-cells, these fibers respond during sinusoidal smooth pursuit eye movements with a phase shift intermediate to eye position and eye velocity indicating a relationship to both eye position and eye velocity. No fibers have been found whose discharge was related solely to eye velocity. In fact, flocculus P-cells are the only known cell within the oculomotor system whose discharge is related to eye velocity without an eye position component.

325 SINGLE UNIT DISCHARGES IN THE PRIMATE FLOCCULUS DURING VISUAL TRACKING. F. A. Miles and J. H. Fuller. Lab. Neurophysiology, NIMH, Bethesda, Md. 20014.

Single units were recorded in the flocculus of alert Rhesus monkeys trained on a visual fixation task. When monkeys tracked targets undergoing sinusoidal excursions in the horizontal plane, most Purkinje cell simple spike discharges modulated in phase with target/eye velocity, usually increasing their rates during the ipsilateral movements; only minor, mostly transient, changes in firing accompanied saccadic eye movements. Simultaneous oscillation of the whole animal about the vertical, either in or out of phase with the pursuit target, did not disrupt tracking and revealed that the track-related discharges could be readily dissociated from eye movements but not from target movements. We conclude from this that the Purkinje cell simple spike discharge specifically encodes track target velocity. Presumed mossy fiber afferents discharged variously in relation to visual, vestibular and oculomotor inputs, the latter with no apparent special concern for tracking. The visual units often showed a strong preference for ipsilateral target movement, were very responsive in the region of the fovea, and discharged in response to slippage of the pursuit target's retinal image during tracking. We conclude that mossy fiber inputs potentially provide all of the information required for the Purkinje cell to synthesize a true neuronal facsimile of track target velocity. We suggest that the Purkinje cell output is in essence an output of the smooth pursuit sub-system which supplies oculomotor centers with the velocity commands to support tracking.

326 VISUAL POSTURAL CONTROL MECHANISMS IN FISH. D.L. Meyer (SPON: T.H. Bullock). Department of Neurosciences, Sch. Med. and Neurobiology Unit, UCSD and Neurobiology Unit, University of Goettingen, Germany.

Behavioural studies of visual influences on postural control (p.c.) in fish revealed that a) the dorsal light response (d.l.r.) is temperature dependent, b) the up-side-down catfish displays a ventral light response when light enters the tank from the side, and c) in addition to the d.l.r. a second mechanism of visually guided p.c. exists which enables certain fish to keep their ventral side towards a vertical wall when swimming close to it. Brain lesions were inflicted in fish to study their effects on the d.l.r. Forebrain and cerebellum lesions did not significantly interfere with this behaviour, whereas tectal and tegmental lesions did. After tectal lesions the animals do not show a d.l.r. to the side contralateral to the lesion, swim slightly tilted if illumination is from above, and appear to be almost normal in darkness. After tegmental lesions disorders are present under illumination and in darkness. These results are interpreted as showing that the pathway which mediates visually guided postural control mechanisms runs through the optic tectum and that the visual-vestibular integration performed by the fish is related to structures in the rostral tegmentum. In single unit studies cells have been found in the rostral tegmentum that are driven by visual and vestibular stimulation. Most of the cells recorded received excitatory input from one eye and the labyrinth contralateral to it.

327 PATHWAYS FOR NECK AND SECOND-ORDER LABYRINTH PROJECTIONS TO THE CAT FLOCCULUS. V.J. Wilson, M. Maeda, J.I. Franck* and H. Shimazu*. Rockefeller University, New York, N.Y. 10021.

We have studied, in 5 decerebrate cats and 2 respirated with a 4:1 N_2O-O_2 mixture, inputs to cells in the vestibular nucleus complex that project to the flocculus by the mossy fiber route.

Neurons were located by antidromic bipolar stimulation through metal electrodes placed in the rostral flocculus with the guidance of vestibular-and neck-evoked field potentials. Antidromic thresholds ranged from 5 to 260 μA; 2/3 were lower than 50 μA; latency was 0.4 to 1.1 msec. Once cells were identified, their responses to stimulation of the following structures were studied extracellularly: ipsi- and contralateral labyrinth; ipsi- and contralateral C2 dorsal root ganglion; ipsi- and contralateral C2 and C3 dorsal rami; forelimb nerves. In some experiments we stimulated the area of neck joint receptors, particularly between C1 and C2, but also between C2 and C3. The location of many cells was marked by ejection of Fast Green and the marks, as well as lesions made at the floccular stimulating locations, were recovered in frozen sections.

27 neurons that projected to the rostral flocculus were activated by stimulation of the ipsilateral C2 ganglion. In the area where these cells were located stimulation of the ganglion evoked field potentials with the positive peak at 0.7-0.9 msec. Neurons usually responded with 2-3 action potentials with the first at 1.3-2.0 msec, 0.5-1.1 msec later than the positive peak. This short latency demonstrates monosynaptic activation by neck afferents. In two experiments we identified the afferents as coming from neck joint receptors. Stimulation of the joint area fired cells at thresholds which rose sharply when an incision was made around the stimulating area. The firing observed after the incision was undoubtedly due to stimulus spread to the C2 ganglion. Neurons driven by joint receptor or C2 ganglion stimulation were never activated by any of the other available inputs. Many of these cells were located in a restricted area, on the border or of just lateral to the caudal half of the descending vestibular nucleus. This region appears to correspond to the group x of Brodal and Pompeiano (1957). Some cells were in the external cuneate nucleus, and one was in the descending nucleus.

A second group of 24 cells fired antidromically from the flocculus was activated, usually monosynaptically, by stimulation of the ipsilateral labyrinth. In most tested cases (11/13) these neurons were inhibited by stimulation of the contralateral labyrinth. They were not fired by stimulation of any neck or forelimb nerves. We usually searched for labyrinth-driven neurons in the descending vestibular nucleus and found them at various rostro-caudal levels, but mainly caudally. One cell was in the medial vestibular nucleus.

Neck and labyrinthine inputs that reach the flocculus at short latency by the mossy fiber route do not converge at the level of the vestibular nuclei, but instead are relayed to the same area of the flocculus by two different groups of cells. The pathway from joint afferents via group x and nearby areas is presumably responsible for the mossy fiber field potentials that stimulation of neck afferents evokes in the flocculus (Wilson, Maeda and Franck, 1975). The nature of the labyrinthine activity relayed by vestibular nucleus neurons is not known, but this activity is modulated in the brainstem, at least by commissural vestibular inhibition.

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Wilson, V.J., Maeda, M., and Franck, J.I. Brain Res. 89, 133-138, 1975

328 DISCHARGES OF INTERPOSITUS NEURONS DURING SEQUENTIAL MOVEMENTS. Lee T. Robertson and Robert J. Grimm. Neurological Sciences Institute of Good Samaritan Hospital and Medical Center, Portland, Oregon, 97209.

The interpositus (IP) nucleus of the cerebellum responds to input from ascending spinal pathways and effects motor output either by the rubrospinal tract or via VL-motor cortex. Neurophysiological studies show that IP neurons discharge in conjunction with simple flexion or extension of the forelimb in cats and monkeys. The purpose of the present study is to examine activity of IP neurons during the performance of a sequential task that requires multiple forelimb flexions and extensions. Extracellular recordings of IP neurons were made in squirrel monkeys as they precisely touched three buttons in a left to right sequence. In addition, by changing the position of the buttons in relation to the monkey, various limb trajectories were required in order to execute the task. The majority of neurons gave a high frequency burst of activity that were strongly correlated with either flexions or extensions of the forearm. About half of these neurons increased in activity during forearm flexion and decreased during its extension, whereas the activity of the other half increased during extension and decreased in conjunction with flexion. Changes in the trajectory of the limb resulted in alterations of neuronal activity that appeared to accompany changes in the velocity of the movements. A number of neurons were also identified that displayed a tonic pattern of discharge during the sequential movement. These cells usually showed an increase in firing frequency that was coincident with the start of the forearm movement. The neuronal activity was maintained throughout most of the performance but was independent of either flexion or extension of the forearm. The pattern of activity of these neurons was not affected by various limb trajectories.

329 ACTIVITY IN CAT CEREBELLAR PURKINJE CELLS EVOKED BY ACTIVE MOVEMENT. Donald S. Rushmer and Gary K. Augter*. Neurological Sciences Institute of Good Samaritan Hospital & Medical Center, Portland, Oregon 97210.

Activity of cerebellar Purkinje cells in the forepaw climbing fiber projection area of lobule V of the cat pars intermedia was recorded during locomotion. A variable speed treadmill supported the animal throughout the step cycle with the force of each limb monitored by a separately instrumented force plate beneath the treadmill belt. Extracellular unit activity was sampled by a computer and correlated with onset of stance phase, maximum force of stance and onset of swing phase of the ipsilateral forelimb. Climbing fiber and single spike responses were differentiated separately. Climbing fiber responses (CFRs) were found to be correlated primarily to onsets of stance and swing phase during the step cycle in a parasaggitally oriented "strip" of Purkinje cells in lobule V. The relation of CFRs evoked by descending inputs and those evoked by peripheral stimulation during locomotion was also examined.

330 ROLE OF THE INFERIOR OLIVE IN VESTIBULAR COMPENSATION. R. Llinás, K. Walton*, D. E. Hillman and C. Sotelo*. Div. Neurobiology, Univ. of Iowa, Oakdale, Iowa 52319, and Laboratoire de Neuromorphologie (INSERM U-106), 123, bd. de Port Royal, 75014 Paris.

The abnormal motor behavior which follows hemilabyrinthectomy is compensated, in the otherwise intact rat, after a period of 24 hrs. Weeks or months following compensation, cerebellectomy produces reversion to vestibular motor abnormalities. This decompensation is maintained unmodified for months after cerebellectomy. Exactly the same syndrome (i.e. reversion of vestibular abnormalities) occurs after bilateral chemical inferior olive (IO) lesion. This lesion was induced with an i.p. injection of 3-acetyl-pyridine (75 mg/kg) followed by harmaline (15 mg/kg) two hours and niacinamide (200 mg/kg) four and a half hours later. This mixture of drugs generates a selective IO lesion with complete damage of the olivo-cerebellar system. It is concluded that since vestibular compensation disappears following IO lesion, in the presence of an otherwise normal cerebellum, the olivo-cerebellar system is required in generating such compensation.

Experiments in which the cerebellar cortex was removed, but the olivo-nuclear projection was left intact, did not show reversal of vestibular compensation, strongly implying that the important pathway for vestibular compensation is the olivo-cerebellar nuclear system. Compensation probably occurs through the olivary projections to fastigial and Deiters' nucleus, these in turn projecting to brain stem and spinal centers. The olivo-cortical input, although probably quite important, does not seem to be as central in the compensation of vestibular damage. It is our conclusion, therefore, that the loss of vestibular compensation by cerebellectomy results mainly from damage to the olivo-nuclear pathway rather than to the cerebellar cortex. (Supported by PHS grant NS-09916 from NINDS)

331 DISTRIBUTION OF OLIVO-CEREBELLAR FIBERS IN SAGITTAL BANDS IN THE CAT. Jacques Courville. Dept. of Physiol., Univ. of Montreal, Montreal H3C 3J7.

The origin of the climbing fibers from the inferior olive can be directly demonstrated by injecting L-leucine or L-proline in the latter nucleus and by tracing the labelled axons in sections prepared for radioautography. After small injections in the olive, a patchy distribution over a number of lobules can be observed. In most of these sites, the grain deposits in the molecular layer appear as a series of sagittally distributed bands, alternating with empty spaces where fibers are not labelled. In order to determine whether these unlabelled climbing fibers also originate from the olive, or from other brain stem nuclei, triple injections in the olive were made in a series of cats. Each injection consisted of 0.2 µl (40-50 µCi) of radioactive amino-acid. When the deposit of the label in the olive was extensive, a continuous distribution of silver grains could be observed over a number of regions of the cerebellar cortex while the sagittal band pattern persisted in a few places. This is interpreted to result from a labelling of all the olivary cells whose axons converge onto the cortical areas where uninterrupted deposits were found. The persistance of the band pattern is found in regions of the cortex which receive contributions partly from labelled cells and partly from unlabelled ones. It suggests therefore that most, and perhaps all, of the climbing fibers originate in the inferior olive. Armstrong, Harvey and Schild ('74) have shown that the different regions of the olive project to two or three regions of the cerebellar cortex by collateral branching of the climbing fibers. The present results show that in any given region of the cerebellar cortex, there is an interdigitation of climbing fibers originating from different parts of the olive.

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332 AFFERENT AND EFFERENT CONNECTIONS OF THE CEREBELLUM IN A REPTILE, IGUANA IGUANA. Robert E. Foster. Dept. of Anatomy and Psychology, Duke University Medical Center, Durham, North Carolina 27710.

Afferent and efferent connections of the cerebellar cortex and the deep cerebellar nuclei were analyzed in 20 specimens of Iguana iguana using the Fink-Heimer technique for demonstrating anterograde degeneration. Following transections of the VIIIth nerve, hemisection of the thoracic spinal cord and small electrolytic lesions in the vestibular nuclei, coarse degenerating fibers were traced into the granule cell layer of the cerebellar cortex. The spinocerebellar fibers appeared to terminate more ventrally in the granular layer than did either the VIIIth nerve or vestibular nuclei fibers.

Several small electrolytic lesions confined to the dorsal two-thirds of the cerebellar cortex were used to delineate its efferent connections. Following a unilateral lesion of the cerebellar cortex, terminal degeneration was found in the ipsilateral medial and lateral deep cerebellar nuclei and in the ipsilateral vestibular nuclei. A few degenerating fibers could be traced across the midline and into the contralateral vestibular nuclei. The efferent connections of the deep cerebellar nuclei were traced following small unilateral lesions of the medial and lateral deep cerebellar nuclei. Efferent fibers from these lesions were followed into the IIIrd, IVth and, to a much lesser extent, the VIth cranial nerve nuclei. Efferent fibers were also traced to the spinal cord, the lateral brainstem reticular formation and to an area of the rostroventral midbrain that contains large cells which resemble those in the mammalian red nucleus. Efferents from the deep cerebellar nuclei to the vestibular nuclei could not be determined in these cases since the lesions also damaged the cerebellar cortex. Efferent connections were not seen from either the cerebellar cortex or the deep cerebellar nuclei to the dorsal thalamus. (Supported by NIH Grant NS-09623 to W. C. Hall.)

333 ANATOMIC AND PHYSIOLOGIC OBSERVATIONS CONCERNING AUDITORY AND VISUAL PROJECTIONS TO THE CAT CEREBELLUM. S. Shinnar*, R. Maciewicz* and R.J. Shofer. Dept. of Neurosci., Albert Einstein Coll. of Med., Bx.,N.Y.10461

Recent findings have indicated the importance of the dorsolateral pontine nuclei in the transmission of auditory and visual information to the cerebellum. With the aid of retrograde horseradish peroxidase (HRP) labeling techniques and with electrophysiologic methods, present studies aim at further description of brain stem projections to the vermis of lobules VI and VII and their responses to auditory and visual stimuli. Following cerebellar cortical injections of HRP, numerous labeled cells were found in the medial accessory inferior olive, the central and pericentral divisions of the tegmental reticular nucleus, and the medial and lateral pontine nuclei. In restricted regions of the inferior olive and dorsolateral pons, almost every cell contained label. Scattered labeled cells occurred in the prepositus hypoglossi and raphé nuclei, and a few isolated cells were found in other brain stem sites.

To determine if these pathways could be involved in the relay of telenceptive information to cerebellar cortex, focal potentials in the pontine brain stem were recorded. Preliminary findings indicate that short latency click-evoked brain stem responses occur prior to the onset of evoked activities in cerebral- or cerebellar surface recordings. At such brain stem locations bipolar stimulation elicits short latency (less than 2 msec) spike-like cerebellar potentials that follow stimulus repetition rates in excess of 100/sec. Histological reconstruction of the electrode tracts show that the activities described can be recorded in regions of the dorsolateral pons shown by the HRP experiments to project to the cerebellar cortex. Although these findings do not preclude other telenceptive inputs to the cerebellum, they are evidence that the dorsolateral pons plays an important role in relaying auditory information to the cerebellar cortex.

334 POSSIBLE ROLE OF THE CLIMBING FIBERS IN THE REGULATION OF cGMP CONTENT IN CEREBELLAR CORTEX. Giovanni Biggio, Alessandro Guidotti and Erminio Costa. Laboratory of Preclin. Pharmacol., NIMH, Saint Elizabeths Hospital, Washington, D. C. 20032
(SPON: R. J. Wyatt)

In rats 3-acetylpyridine (3AP) produces degeneration of the inferior olivary complex and of the cerebellar climbing fibers (Desclin, Brain Res. 77, 365, 1974). Since harmaline produces tremor and increases the cGMP content of cerebellar cortex presumably by activating the olivo-cerebellar pathway we used harmaline and 3AP as tools to elucidate the role of the climbing fibers in the regulation of cGMP in cerebellar cortex. Harmaline (28 μmoles/kg i.v.) produced tremor with a rythm of 10-12/sec and increased the cerebellar cGMP content (about 4 fold in 15 minutes) without increasing the cGMP content of the deep cerebellar nuclei. This finding excludes a direct effect of either harmaline or its metabolites on the synthesis and metabolism of cGMP and suggest that the action of harmaline is indirect. The time course of the tremor and that of the increase in cerebellar cortex cGMP content were identical. In rats treated with 3AP (0.81 μmoles/kg i.p.) 72 hours before, the increase of cerebellar cGMP and the tremor elicited by harmaline were completely abolished. In contrast the increase of cGMP in cerebellar cortex and cerebellar nuclei induced by isoniazid (2.8 mmoles/kg s.c.) remained unabated by 3AP pretreatment. Moreover Oxotremorine (7.2 μmoles/kg i.p.) produced tremor in control and 3AP treated rats, and failed to increase cerebellar cGMP content at the time of the peak of the tremorigenic response. These results suggest that the climbing fibers, release a transmitter that activates cGMP formation in the dendrites of Purkinje cells.

335 STRUCTURE OF THE PURKINJE CELL MEMBRANE IN STAGGERER AND WEAVER MUTANT MICE. D.M.D. Landis and T.S. Reese. NINCDS, NIH, Bethesda, Md. 20014.

The cerebellar cortex of developing (15 and 30 days postnatal) and mature weaver and staggerer mutant mice was examined with the freeze-fracture technique in order to see whether structural abnormalities of the Purkinje cell membrane accompany abnormal synaptic development. In weaver few, if any, parallel fiber synapses on Purkinje spines develop. However, the Purkinje cell dendritic arbors acquire myriad spines which, in thin-sections, appear to have typical postsynaptic specializations but no presynaptic elements. In freeze-fracture replicas, these spines had aggregates of particles arrayed on the external half of the spine membrane. These aggregates were smaller but otherwise indistinguishable from the aggregates that characterize the postsynaptic membrane at normal synaptic specializations. The spines fronted astrocytes, recognized by characteristic arrays of assemblies. In staggerer, mature parallel fiber synapses on Purkinje spines fail to develop and the stunted Purkinje dendritic arbors lack spines. No abnormalities in the membranes of these dendrites were found; in particular the distribution of postsynaptic particle arrays attributed to climbing fiber synapses was normal and there were no aberrant arrays of particles that could be attributed to aborted parallel fiber synapses. Membrane structure at other classes of synapse in the cerebellar cortex appeared to be normal in both mutants and there was no evidence of abnormal astrocytic membrane structure. Thus, in weaver, postsynaptic membrane specializations on spines can develop without functional synaptic contact and, in staggerer, no postsynaptic membrane specializations are acquired in the absence of spine formation. These findings suggest that the postsynaptic specialization at parallel fiber synapses can develop in the absence of synaptic terminals although the presence of a spine may be necessary to develop or maintain this specialization.

336 THE TOPOGRAPHICAL ORGANIZATION OF THE CEREBELLO-OLIVARY PATHWAY IN THE CAT. D.L. TOLBERT, M.G. MURPHY, P.A. YOUNG, L.C. MASSOPUST. Department of Anatomy, Saint Louis University, St. Louis, Mo. 63104

A previous autoradiographic study (Graybiel et al., Brain Res. 58:205-211, 1973) has suggested a well organized connection between the deep cerebellar nuclei and the contralateral inferior olivary nucleus in the cat. The topographic nature of this cerebello-olivary pathway can be defined even more precisely following discrete injections of tritiated leucine into the individual cerebellar nuclei. A leucine placement in the dorsomedial part of the dentate nucleus (DN) resulted in silver grains which were localized over the dorsal lamina of the contralateral principle olivary nucleus (PON). Following an injection into the ventral half of the DN silver grains were present over the lateral bend of the PON. They also extended over the lateral aspect of the ventral lamina of the PON through its more rostral levels. Leucine placements into the ventral aspect of the posterior interpositus nucleus resulted in silver grains heavily concentrated over the lateral half of the medial accessory olive from the level of the caudal end of the ventral lamina of the PON rostrally through intermediate levels of the dorsomedial cell column. Conversely, anterior interpositus injections resulted in label localized over regions of the dorsal accessory olive and portions of the dorsal cap and ventrolateral outgrowth of Kooy. These findings indicate that the major subdivisions of the inferior olivary complex receive a highly organized input directly from dentate and interpositus cerebellar nuclei.

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337 DESCENDING THALMO-CEREBELLAR CONNECTIONS IN PIGEON AND CATFISH. H.J. Karten & T.E. Finger*. Depts. Psychiatry & Anat. Sci., SUNY, Stony Brook, NY 11794

Direct projections from optic tectum to cerebellum were first reported in pigeons in 1888. Using anterograde and retrograde methods, we were unable to confirm this finding. Following thalamic lesions in pigeon, a cerebellopetal tract was found identical in course to the tract reported in 1888. This tract, brachium conjunctivum cerebellopetales (BCP), descends ipsilaterally, ventromedial to the tectum, terminating as mossy fibers predominantly contralaterally in cerebellar posterior lobe (Folia VIb-IXa). Injection of HRP into these folia results in retrograde labelling of nucleus spiriformis medialis (SpM). SpM receives major afferents via descending tracts that resemble components of mammalian pyramidial tracts.

The teleost "tecto-cerebellar tract" is generally recognized to arise from cells near the posterior commissure. Following lesion of this area in catfish, tractus mesencephali-cerebellaris anterior (MCa) courses through the dorsal mesencephalon to enter cerebellum. After partial decussation within cerebellum, MCa terminates bilaterally amongst granule cells and in lamina interposita between granule and Purkinje cell layers. HRP injections in catfish cerebellum label cells in Brickner's nucleus mesencephalicus dorsalis (nMD). nMD receives no direct retinal, tectal or commissural afferents and, in these respects is similar to SpM. Thus, SpM in birds and nMD in fish are comparable on the basis of position and efferents as suggested by Craigie and Brickner.

Existence of thalamo-cerebellar systems in birds and fish indicates its likely presence in other non-mammalian vertebrates. Relationships between this system and the cortico-ponto-cerebellar system will be discussed.

338 ACCESSORY OPTIC NUCLEAR PROJECTIONS TO THE FLOCCULO-NODULAR LOBE OF THE CEREBELLUM; A POSSIBLE CHANNEL FOR EYE-NECK CONTROL SYSTEMS. S.E. Brauth* & H.J. Karten. Depts. of Psychiatry & Anat. Sci., SUNY, Stony Brook, NY 11794. (SPON: T. Parks).

The nucleus of the basal optic route (nBOR) is a readily identifiable structure in all vertebrates with prominent visual systems. It receives a separate and distinct fascicle of fibers which are segregated from the optic nerve at the level of the optic chiasm. This nucleus is also known as the nucleus ectomammilaris in birds, the nucleus opticus lateralis tegmenti in reptiles and anamniotes, and the medial accessory optic nucleus or nucleus transversi pedunculi of Bochanek in mammals. The axons of this tract are substantially larger than any other axons in the optic nerve or tract itself, and are estimated to be approximately 10,000 in number. Nevertheless, the efferent connections of nBOR have not been determined.

In the present study small injections of horseradish peroxidase were injected into the auricular portion of the flocculo-nodular lobe of pigeons under direct visual control. Results show retrograde transport of the enzyme to the ipsilateral nucleus ectomammalaris. Injections of the nodular portion of the cerebellum result in transport of HRP bilaterally to the nucleus ectomammalaris. The authors suggest these findings may provide evidence that the accessory optic system participates in programmed eye movement control in avian forms.

339 SOURCES OF MULTIVALUEDNESS IN THE POSITION-AFFERENT RATES RELATION IN THE UTRICLE. O.Macadar* and G.E. Wolfe*. (SPON: J.P.Segundo). Dept. Anat., Sch. Med.,UCLA. Los Angeles, CA, 90024.

Identification of sensory coding requires investigation of replicability using stimulus repetition. First order utricular afferents do show replicability, but only within broad limits, e.g. have different rates in different stations at a same position. Spike activity was recorded from first order afferents, while the isolated Rhinobates head was brought repeatedly to, and held for around 80 sec at, a central close to normal position "C", reached from either side up "A" or down "B" through step-like transitions. Stationarity at the central position "C" was evaluated using a Kendal correlation procedure i. within-stations by observing the discharge during the last portion of each station, and ii.across-stations,by observing rates during successive stations. Only cells within-station stationary for over 20 sec were studied. None of the units were across-station stationary, exhibiting spontaneous, stimulus independent, rate variations, of amplitude similar to tilt effects, and with fast or slow time courses. "Fast" variations commenced abruptly, decayed like tilt-effects and could occur in simultaneously observed cells, in which case they had the same (opposite) sense when the cells responded to tilt in the same (opposite) way. Stations with such variations were disregarded. "Slow" variations involved several stations; it was not clear whether they represent natural behavior or deterioration. The rates at "C", evaluated with a Wilcoxon rank test, were significantly higher after arrival from a low rate position (B) than what they would have been, had the arrival been from a high rate position (A)(OB>CB in fig 3): therefore, the transients contributed to the multivaluedness of the "tonic" response. Non-stationarity, regardless of its genesis, cannot be ignored in the evaluation of experiments testing utricular function. (Supported by NIH and NSF).

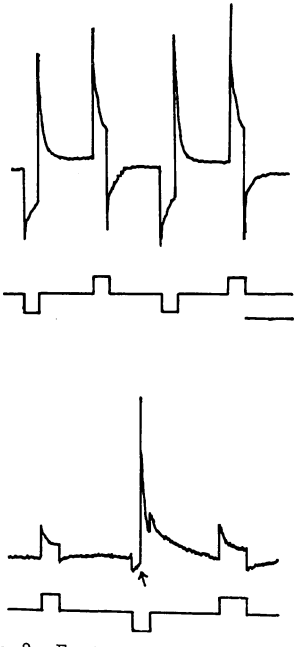

fig 2: Fast spontaneous variation beginning at arrow.

fig 1: Response to tilts, while cell is stationary. In all figures upper record: firing rate (i.p.s.) as function of ongoing time; lower record: position; time bar: 80 sec.

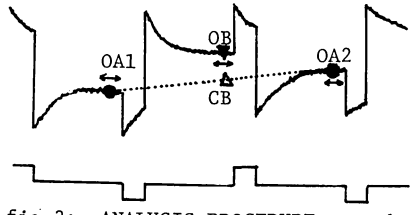

fig 3: ANALYSIS PROCEDURE: each observed value (OA or OB) is the rate averaged over the stationary period indicated by double arrow. CB is the rate calculated by interpolation between OA1 and OA2.

340 HAIR CELL ORIENTATION PATTERNS IN GOLDFISH OTOLITH ORGANS. Christopher Platt. Dept. Elec. Eng. Comp. Sci., U. California, Berkeley, Cal. 94720.

Hair cells of the vertebrate vestibular sensory epithelia show a structural polarization, in having a single kinocilium on one side of their apical ciliary bundle, that correlates with physiological directional sensitivity (Flock, J. Cell Biol. 22:413, 1964). Study of goldfish otolith organs using scanning electron microscopy, by which the orientation of each cell bundle in an entire intact macula can be plotted with confidence, shows some striking differences from observations of elasmobranch and other teleost species (cf. Lowenstein, in Fish Physiology, Vol. V (Hoar & Randall, eds.) pp.207-240, 1971), and amphibia (Lewis & Li, Brain Res. 83: 35, 1975). The horizontal utricle shows the conventional teleost pattern, each bundle with its kinocilium facing towards a transverse curving line of opposition in the anterior macula. The macula neglecta consists of two small patches posterior to the utricle; the medial patch has all kinocilia facing posteriorly, the lateral patch anteriorly, unlike the radial outward pattern in the single macula neglecta of the ray. The vertical saccule is conventional in having kinocilia facing away from a longitudinal line in the central macula, but a remarkable narrow loop in this line swings up almost to the dorsal edge of the anterior macula. This loop occurs consistently in the same area in both left and right sides of fish ranging in size from 40-55 mm long. The vertical lagena has kinocilia facing toward a bent longitudinal dividing line, unlike the frog's with kinocilia facing away, and unlike the ray's patchy opposing clusters. The bidirectional opposition lines in all maculae extend smoothly across regions of different cell types, across regions covered by otoliths and just otolith membrane, and the location of bends or loops does not correlate obviously with pattern or texture of the otolith surfaces. A functional role for the specificity of these patterns remains unknown.

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341 FIELD POTENTIALS RECORDED FOLLOWING BILATERAL LABYRINTHINE STIMULATION IN THE PIGEON. James C. Schadt and Charles D. Barnes. Dept. Life Sciences, Indiana State Univ., Terre Haute, Ind., 47807.

Field potentials were recorded in the vestibular nuclear complex of the pigeon following bilateral stimulation of the labyrinth. Bipolar stimulating electrodes constructed from two pieces of 50 μ wire insulated with enamel except at the tip were implanted in all three ampullae and the utricle bilaterally. Stimuli consisted of constant current rectangular pulses 100 μsec in duration at a rate of one per second. Field potentials were recorded via bipolar electrode pairs separated 0.5 mm at the tip inserted sterotaxically into the vestibular nuclei. Ipsilateral and contralateral responses usually showed the typical P, N_1, and N_2 deflections although the P wave was sometimes difficult to detect. Both ipsilateral and contralateral stimulation produced P, N_1, N_2 waves at similar latencies. The difference in latencies between ipsi- and contralateral stimulation was usually less than .15 msec. Occlusion was not evident with bilateral stimulation of the same structure, and simultaneous stimuli produced a summed potential. These results show that in pigeons information from both labyrinths reaches the nuclei at about the same time unlike the situation in other vertebrates examined thus far. The lack of occlusion and presence of summation may indicate little convergence between ipsilateral and contralateral information in the vestibular nuclei. Supported in part by PHS Grant NS 11284.

342 MULTI-MODAL RESPONSES OF CELLS IN THE MEDIAL GENICULATE BODY OF THE CAT. L. Abraham*, P. Blum and S. Gilman. Dept. of Neurol., Columbia Univ., College of Physicians and Surgeons, New York, N.Y. 10032

Investigations of the rostral vestibular system in the cat have shown short latency evoked responses to vestibular stimulation in the medial geniculate body (MGB) of the thalamus. In this study, investigations of the responses of single cells in the MGB were undertaken. Of particular importance were: 1) whether the MGB could be considered a primary relay area for the ascending vestibular pathway, and 2) the degree and organization of convergence of sensory modalities including vestibular, auditory, muscle, joint, and cutaneous. 60% of cells examined in MGB did not respond to the sensory stimuli used in this study. Analysis of the responsive cells showed a difference between the magnocellular and principal nuclei of MGB. Vestibular nerve stimulation excited approximately 30% of magnocellular MGB cells but none of the principal MGB cell No units were found with short (less than 5 msec) latency to vestibular nerve stimulation. This is not consistent with MGB as a primary relay area for vestibular activity; it is likely this relay area is located in some other portion of the thalamus. In other respects the two areas of MGB were similar. Auditory responses were found in 75% of the units. A few units responded to muscle, joint or cutaneous stimulation. About 30% of the responding units were activated by more than one of the stimuli used. Thus, some cells of MGB may function to integrate vestibular, auditory, and other sensory inputs in the cat.
(Supported by USPHS grant NS 11307)

343 RECIPROCAL SYNAPSES IN THE CRISTA AMPULLARIS OF THE BULLFROG.

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Reciprocal synapses consist of two synaptic complexes of opposite polarity in which one neuronal process is both the pre- and the post-synaptic element to another neuronal branch. This type of synapse has been found in the central nervous system (1), the olfactory bulb (2), and in the retina (3), where they appear to serve in dendro-dendritic interactions. More recently reciprocal synapses were reported in the cardiac ganglia between cholinergic axons and adrenergic interneurons (4,5).

Reciprocal synapses have been observed in the crista of the bullfrog where they are located between an afferent dendrite and the receptor cell. One of the two synaptic components of the reciprocal complex consisted of a characteristic afferent synapse having a synaptic sphere with its halo of vesicles and plasma membrane specializations (6); the second component resembled an efferent synapse and was located 1-2.5 μm from the first component. Most often the second contact zone was characterized by an accumulation of clear vesicles within the dendrite and a small membranous cisterna within the receptor hair cell. Other configurations of the second component included: an abundance of vesicles in the dendrite, and an apparent increased membrane osmiophilia , but no membranous cisterna; or only a few vesicles within the dendrite, and a well formed intrareceptor membranous cisterna. These reciprocal synapses have been observed in both single and in serial sections.

Several functional interpretations are suggested in which the second contact component of the reciprocal synapse is presumed to alter the hair cell's membrane potential opposite to that associated with the afferent synapse. One interpretation, based upon reports of antidromic activity in lateral line organs (7,8), suggests that the reciprocal synapse may be the structural component responsible for interaction between distant hair cells since the close proximity of the two reciprocal components and temporal arguments do not favor simultaneous reciprocal interplay either within a single hair cell or between adjacent hair cells. The presence of hair cells within the crista which are grouped as a functional cluster has been suggested (9), and would require that their interaction be defined by the dendritic arborization. In order to enhance the effectiveness of the group, in case of dendritic overlap, a mechanism of fringe discrimination would be reasonable in which cells at the periphery of the cluster, which might share dendrites from two separate groups, would be effectively prevented from contributing to the neural activity generated by the primary group in response to a stimulus. The resultant border enhancement could be based upon antidromic neural activity by means of the reciprocal synapses.

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344 MODELS OF SOURCE MECHANISMS IN THE VESTIBULAR NEUROEPITHELIUM. Jack P. Landolt and Manning J. Correia. DCIEM, Downsview (Toronto), Canada, and Dept. Otolaryng. UTMB, Galveston, Texas.

The neuroepithelium, consisting of the sensory endorgans in the vestibular apparatus, forms a complex matrix of hair cells, synaptic elements, and nerve fibers. Although there are basically two types of hair cells -- the types I and II hair cells (Wersall, '56) -- other intermediary forms are known to exist. Large, medium, and thin nerve fibers make synaptic contact with the hair cells, either as bouton endings or else in the form of nerve calyces. There is strong evidence that both excitatory and inhibitory effects are involved in the transmission of neural information. Such a complex arrangement must have a bearing on the impulse-generating mechanism of the vestibular neuroepithelium. To this end, single unit, spontaneous activity was recorded from cell bodies in Scarpa's ganglion that innervated the anterior semicircular canal of the pigeon. A point process theoretic approach was used to analyze these data. The results indicate that units, with activity having highly-skewed distributions, may be described by a renewal process having probability density functions that are representative of the first passage times of Brownian motion processes. Units having negligible or even negative skewnesses tend to be of a non-renewal nature, and, furthermore, may be described as a periodic point process in which each event is displaced randomly by time "jitter".

345 DUAL INPUT DESCRIBING FUNCTION ANALYSIS OF SPONTANEOUSLY INACTIVE FIRST-ORDER AFFERENTS INNERVATING THE ISOLATED GUITARFISH SEMICIRCULAR CANAL. Dennis P. O'Leary and Conrad Wall III* (SPON: Charles G. Lineberry). Dept. Otolaryngol., Univ. of Pittsburgh Sch. Med., Pittsburgh, Pa. 15213.

First-order afferent neurons innervating the semicircular canal are described usually as spontaneously active and responding linearly to rotational accelerations. But we describe a sub-population of horizontal canal afferents that is spontaneously inactive (SI), and exhibits nonlinear response properties. Spike train responses from cell pairs, one spontaneously active and one inactive, to a pseudorandom white noise rotational acceleration input were obtained simultaneously and separated via amplitude discrimination. The SI units would respond only during a restricted portion of each white noise input period. However, the superposition of a high frequency sinusoidal "dither" signal along with a pseudorandom white noise input resulted in systematic recruitment of new units from the SI population that were not evoked by either "dither" or white noise delivered individually. Theoretical analysis, using dual input describing functions, has shown that such a dual signal input will linearize a threshold nonlinearity. The existence and response properties of this new sub-population have important implications concerning threshold nonlinearities for both models of the receptor transduction process and also the coding of head acceleration information transmitted to higher vestibular centers.

346 FORCE-RESPONSE RELATIONS FOR PERIPHERAL OTOLITH NEURONS IN BARBITURATE-ANESTHETIZED SQUIRREL MONKEY. Jay M. Goldberg and César Fernández*, Univ. of Chicago, Chicago, Ill., 60637.

The response to centrifugal force in the range of ± 4.92 g was studied in regularly discharging otolith neurons. The afferent's polarization vector was aligned with the force vector. Force-response relations are sigmoid shaped, displaying both excitatory and inhibitory saturations. Excitatory saturation rates varied from 100-350 spikes/sec. Most units exhibited a residual discharge of 5-30 spikes/sec, even in the presence of intense inhibitory forces. The presumed physiological range (± 1 g) is represented in the lower (concave upward) portion of the relation, the zero-force point being biased 0.5-3.0 g below the inflection point. The bias has two consequences for responses in the physiological range: (1) the sensitivity around the zero-force point (s_o) is only some 40-80% of the sensitivity around the inflection point (s_{max}); (2) there is a response asymmetry, the inhibitory response to a 1-g force being some 40-80% of the corresponding excitatory response. The entire force-response relation can be described in terms of three variables: a vertical-gain factor (X_1), a horizontal translation or bias (X_2), and a horizontal-gain factor (X_3). Results are consistent with the interpretation that X_1 reflects a transduction gain, X_2 a receptor bias, and X_3 a mechanical gain. The resting discharge (d_o) is jointly determined by X_1 and X_2; the sensitivity (s_o) by all three variables; and the response asymmetry, expressed on a percentage basis, by X_2 and X_3. The positive relation between d_o and s_o, previously reported, reflects the fact that both of these discharge characteristics are determined by the same factors, more particularly, by X_1 and X_2. (Supported by NASA and NIH)

347 ANOMALOUS RECTIFICATION IN APLYSIA STATOCYST RECEPTOR CELLS. Michael L. Wiederhold. Neurobiology Department, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20014.

The ciliated mechanoreceptor cells of the Aplysia statocyst respond to the physiological stimulus of tilting by membrane depolarization which, if of sufficient amplitude, can generate action potentials in these primary receptor cells. The receptor potential is accompanied by increased fluctuations in membrane potential. These are interpreted as being due to increased collisions between the receptor-cell cilia and the statoconia, which come into contact with a receptor cell when the preparation is tilted such that that cell comes to lie near the bottom of the cyst. The depolarizations are due to a conductance increase, primarily to sodium ions. More extensive examination of the membrane properties has revealed marked anomalous rectification in these cells, the slope resistance in some cases decreasing by a factor of three as the receptor cell is hyperpolarized. This rectification causes the potential fluctuations to decrease in amplitude as a cell is hyperpolarized; whereas, with a linear or normally-rectifying membrane, one would expect an increase in the fluctuations since they presumably represent increases in the conductance of an ionic system with an equilibrium potential more positive than the resting potential. Anomalous rectification in such a system enhances the sharpness of threshold for the receptors. When they are at large negative membrane potentials, fluctuations in the potential are essentially "shorted out" and only as a cell is substantially depolarized do the fluctuations reach sufficient amplitude to initiate action potentials.

348 THE INFLUENCE OF THE OTOLITHS ON SEMICIRCULAR CANAL INDUCED NYSTAGMUS. Maria Regina Coccia and David L. Clark.*
Dept. of Anat., Coll. of Med., OSU, Columbus, Ohio, 43210.

Semicircular canal induced nystagmus has been shown to be modified by concomitant or previous exposure to linear acceleration. It has been argued that this effect of linear acceleration on semicircular canal induced nystagmus is due to a direct effect of linear acceleration on the semicircular canal neuroepithelium. It has also been argued that this is due to a cross-coupling effect between the otoliths and the semicircular canals.

A recessive mutant of the house mouse, Mus musculus, tilted head (th), produces mice which lack otolith crystals. The inner ear neuroepithelium is otherwise normal. This strain of mice was used to further explore the cross-coupling hypothesis.

Mice homozygous for th, (otolith deficient, OD, mice) and mice heterozygous for th, (otolith intact, OI, mice), were exposed to chronic 2G centrifugation at 22 rpm for 30 days. To control for potential Coriolis stimulation of the semicircular canals, and concomitant habituation of postrotatory nystagmus, a second group of OD and OI mice was exposed to rotation at 22 rpm for 30 days. Cages were located over the center axis of rotation and only the 1G environment was experienced. A third, 1G Control group, consisting of OD and OI mice, was examined to control against rotation and hypergravity.

Horizontal semicircular canal function was determined at 1G, using the cupulogram technique. A cupulogram was constructed using post-rotatory nystagmus duration values following impulsive deceleration stimuli from a series of graded angular velocities.

Results from the 1G Control group indicate that the congenital absence of otoliths has an effect on semicircular canal function.

Results from the Rotation group indicate that the congenital absence of otolith crystals has no effect on changes seen in habituation of post-rotatory nystagmus.

Results from the 2G group indicate that OD mice are not affected by exposure to hypergravity to the extent that the OI mice are affected under these same conditions. Data from this experiment provides conclusive evidence that exposure to chronic hypergravity has a significant effect on semicircular canal function, and that this effect is mediated by the otolith organ. These data further substantiate the contention that the neuroepithelium of the semicircular canal is not directly influenced by the force of gravity.

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349 SYNAPTIC LINKAGE OF THE RETICULO-OCULAR PATHWAY IN CAT.

C.R.S. Kaneko, A.Steinacker*, B. Cohen, R. Maciewicz*and S. M. Highstein.
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Previously it was asserted that pontine reticular formation projects monosynaptically to the oculomotor and abducens nuclei. We now report a monosynaptic connection to abducens interneurons. In addition, cells of origin of the projection to the ocular nuclei have been identified by their antidromic responses to stimulation within the oculomotor and abducens nuclei. Neurons were localized by measurement of the depth of penetration from the floor of the IVth ventricle and subsequent identification of the recording electrode tracks. The majority of neurons were located within the rostral pole of the Nucleus reticularis pontis caudalis (N.r.p.c.) and the tail of the N.r.p. oralis (N.r.p.o). Neurons were classified according to their antidromic and orthodromic response patterns. There was a class of cells which gave antidromic responses exclusively to stimulation of the IIIrd or VIth nuclei or both. 24% of the cells responded antidromically to stimulation of the IIIrd or VIth nucleus and orthodromically to stimulation of one of the other nuclei. Many cells were not antidromically identified but received PSPs subsequent to IIIrd or VIth nucleus stimulation. Horseradish peroxidase was iontophoresed into the VIth nucleus in order to confirm the location of reticular neurons projecting to VIth nucleus. Preliminary evidence demonstrates that the distribution of labeled reticular neurons is consistent with the physiological findings. This morphophysiological evidence supports the hypothesis that N.r.p.c. and N.r.p.o cells project monosynaptically to motoneurons in the IIIrd nucleus and to motoneurons and interneurons within the abducens nucleus.

350 INTERNEURONS IN THE OCULOMOTOR NUCLEUS THAT PROJECT TO THE ABDUCENS NUCLEUS IN THE CAT. R.J. Maciewicz*, C.R.S. Kaneko, S.M. Highstein, and R. Baker: Albert Einstein College of Medicine, Bronx, New York 10461.

Short latency postsynaptic potentials in abducens motoneurons and interneurons are evoked by stimulation in the region of the oculomotor complex. To determine candidates for these inputs we employed the technique of retrograde transport of horseradish peroxidase (HRP) in order to label neurons afferent to the abducens nucleus. In addition to labeling cells in nuclei known to project to the abducens nucleus, HRP-positive cells were found within and around the oculomotor nucleus. These cells are 16-20μm and most common in the caudal 2/3 of the nucleus. They are found both ipsilaterally and contralaterally in approximately equal numbers, many of the cells appearing to form dorsal and lateral bands that cap the nucleus. This distribution of cells is not restricted to any of the regions of the oculomotor nucleus subserving discrete eye muscles. These morphological findings were verified using extracellular unit recording techniques. A population of cells were found that could not be antidromically activated by IIIrd nerve stimulation, but were antidromically activated by stimulation of the abducens nuclei. In agreement with the morphological findings, these cells were located in and around the caudal 2/3 of the oculomotor nucleus. In conclusion, we have morphophysiologically demonstrated a population of interneurons within and around the oculomotor nucleus which project to the abducens nucleus.

351 RESPONSE CHARACTERISTICS OF RETICULAR FORMATION NEURONS TO VESTIBULAR STIMULATION AND/OR EYE MOVEMENTS. J. Kimm* and A.F. Fuchs (SPON: D. Sutton) Depts. of Otolaryngology and Physiology & Biophysics, Regional Primate Res. Ctr., Univ. of Washington, Seattle, Wa. 98195.

Information regarding vestibular stimulation can reach the oculomotor nuclei monosynaptically from the vestibular nuclei or by a multisynaptic pathway involving the reticular formation (RF). The reticular pathway is thought to contain neural networks which can provide additional neural processing for vestibular ocular mechanisms. To examine this hypothesis, neuronal activity of RF cells and eye movements were recorded from an alert monkey subjected to sinusoidal rotation (vestibular stimulation). The neural activity of a large majority of the cells responded only to the velocity component of oscillation. Another group of the neurons exhibited a firing rate proportional to eye position and upon which was superimposed a firing rate proportional to head velocity. Other cells exhibited a burst tonic pattern characteristic of oculomotor neurons which were related to eye movements per se. Therefore, while eliciting the vestibular ocular reflex during sinusoidal rotation, a majority of units in the reticular formation exhibited periodic modulations in firing which lagged head acceleration by 60 to 100°, similar to results obtained from vestibular nucleus neurons. However, in comparison to vestibular nucleus cells a higher proportion of RF neurons have phase lags outside of the range of 60-100°.

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352 BRAIN STEM AND VESTIBULAR NEURON BEHAVIOR DURING EYE MOVEMENTS EVOKED BY CEREBELLAR STIMULATION. Michael Hassul*, Barbara Cogdell* and Joseph Kimm* (SPON: R. B. Pinter). Dept. Otolaryngol. and Physiol. and Biophysics, Univ. of Wash., Seattle, 98195.

Earlier work has demonstrated that cells lying within the vestibular nucleus (VN) and reticular formation (RF) have activity related to vestibular stimulation and/or horizontal eye movements. The present study was performed in an attempt to demonstrate cerebellar (cb) vestibulo-ocular interactions. We recorded single unit activity in the brain stem of the alert, trained monkey in response to vestibular and visual input in order to characterize the unit. The cb was electrically stimulated in areas which produced short latency horizontal eye movements while simultaneously recording from the brain stem neurons.

Three classes of eye movement related cells were observed (tonic, pausers, and bursters). The firing rate of the tonic units changed in accordance with the new eye position elicited by the cb stimulation. The units which paused with normal eye movements also paused for cb evoked eye movements. The bursters were unaffected by cb stimulation even though an evoked eye movement was elicited. Cells which were modulated only by vestibular stimulation were also differentially affected by the cb stimulation.

353 MESENCEPHALIC RETICULAR NEURONS RELATED TO VISUAL AND VESTIBULAR EVOKED EYE MOVEMENTS. W. Michael King*, Michel Magnin*, and Albert F. Fuchs. (SPON: M.E. Anderson). Dept. of Physiology and Biophysics and Regional Primate Research Center, Univ. of Washington, Seattle, Washington 98195

Lesion and single unit evidence suggest that the role of the mesencephalic reticular formation (MRF) in vertical eye movements is comparable to that of the pontine reticular formation in horizontal eye movements. To test this hypothesis we recorded from single MRF neurons in monkeys trained to perform a visual tracking task and subjected to adequate vestibular stimulation in the vertical plane.

In addition to the previously reported populations of burst and burst-tonic neurons, our new behavioral paradigm revealed a third population of vertical eye movement related cells. The discharge of these cells was deeply modulated during visual or vestibular evoked smooth eye movements and showed a strong dependence on eye velocity as well as eye position. However, when the monkey fixated a stationary visual target, the neurons discharged with irregular interspike intervals at a rate related only on the average to vertical eye position. This position sensitivity was even less apparent during spontaneous eye movements unrelated to the visual tracking task.

We also analyzed the firing patterns of the burst-tonic neurons during visual or vestibular evoked smooth eye movements. These cells discharged with eye velocity as well as eye position but in a fashion similar to oculomotoneurons. Like motoneurons, their discharge was unmodulated during head movement in the absence of compensatory eye movements.

Thus the presence of neurons in the MRF related to all phases of voluntary and vestibular eye movements supports its role as a major premotor center for vertical eye movements.

354 PERCEPTION, EYE MOVEMENTS AND NEURON ACTIVITY DURING SEQUENTIAL PHOTIC STIMULATION. M.F. Decostre-Voisin*, M. Meulders and C. Veraart*. Lab. Neurophysiol., Univ. Louvain, 1200 Brussels, Belgium.

Humans and cats were submitted to static stimulation realized by light spots presented at different spatial and temporal intervals. Humans were invited to describe their perception and ocular movements were recorded. Neuron activity of pulvinar-lateralis posterior complex of paralyzed cats was tested in the same stimulus situation.

The following observations were made : 1) Stimulus is described by human subjects as motion even when intervals are large enough to be detected as a discontinuity of stimulation ; 2) mean eye pursuit velocity increases when stimulus pseudo velocity (i.e.: spatial intervals between spots/temporal intervals) increases ; 3) for any constant stimulus pseudo velocity, mean velocity of pursuit eye movements increases when spatial and temporal intervals between spots decrease ; 4) in the same way, for any constant stimulus pseudo velocity, a comparable increasing of mean frequency discharge of thalamic neurons is observed when spatio-temporal intervals between spots decrease.

355 MODELING OF NEURAL ACTIVITY IN THE PONTINE RETICULAR FORMATION (PRF) RESPONSIBLE FOR GENERATION OF RAPID EYE MOVEMENTS. T. Raphan,* B. Cohen and R. Mekel.* Mt. Sinai Sch. of Med., CUNY, New York, N.Y. 10029

The PRF contains neuron classes which appear to be responsible for driving the eyes to perform saccades and quick phases of nystagmus. We modeled the saccadic generator and related it to the behavior of burst and pause units in the PRF using a state theoretic approach. In the established model, the firing frequencies of particular unit types were considered to be the state variables of the system. If all the state variables can be found and the coefficients that determine its dynamic response can be identified, then theoretically all oculomotor system behavior could be described. Accepting the hypothesis that the saccadic generator is a state-determined system, realization theory was utilized to gain insight into the organizational structure of the PRF. Conceptually, a "controllable" form realization seems to be the correct one for the saccadic generator as its states are controlled by the input and then coordinated centrally to determine the output. A model which is physically more likely assumes that all integrations are nonideal. This leads to a general system matrix which can be used to explain various aspects of oculomotor function. In the model the subsystem generating the state variables within the PRF (long and medium lead burst units) is the mechanism which controls the neurons which are driving the motor nucleus directly (short lead burst units). These neurons also input to a neural integrator to provide a pulse-step necessary to produce saccades and quick phases. The pause units could act as a switch to enable or disable the saccadic generator.

The controlling part of the model behaves essentially as a "relaxation oscillator" (Van der Pol, 1926; Stern, 1965). When a pulse is applied to the saccadic generator, there is a slow buildup of activity in the state variables. When a critical threshold level is reached, the dynamic characteristics of the model change. This forces the saccadic generator back to its equilibrium position. In terms of the physiology, the saccadic generator is outputing to the motor nucleus during this interval. When a step is applied, a stable condition can never be achieved, since as soon as the feedback drives the generator below some threshold, a slow buildup of activity will resume. This leads to the periodic quick phases which are similar to those observed during induced nystagmus.

Coefficients that determine the dynamic response of the saccadic system were identified by comparing unit activity with the state variables of the model. A parameter adaptive technique was used to adapt the model parameters so that the error between unit data and model was reduced to a minimum. The adaptive algorithms were derived using Liapunov's Direct Method in order to insure convergence of the derived algorithms.

The model has focused on the dynamic response of neuron classes to various stimuli and how they are organized centrally to drive the eyes in the saccadic mode. In the realization, functional relationships were used, which the CNS actually performs, e.g. multiplication, addition, integration, threshold sensing and switching. The PRF is generally conceived as a multiloop system with positive and negative feedback. The integrators in the PRF need not have long time constants in order to achieve the desired dynamic responses. It can be shown that if particular feedback elements are altered and if appropriate slow phase information is introduced into the neural integrator, the model becomes unstable and spontaneous oscillation may result. This may be analogous to spontaneous nystagmus which occurs after PRF lesions. Thus a theoretical basis has been established to understand the neuronal organization of the reticular formation and how it might produce rapid eye movements.

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356 Eye movements and frontal eye fields: electrical stimulation and unit recording in the awake cat. G. Mandl and D. Guitton, Aviation Medical Research Unit, McGill University, Montreal, Canada.

The experiments described were undertaken to answer the following questions: 1. How are unit discharges in the frontal eye fields (FEF, Area 8) related to (a) eye movements, (b) changes in the neck muscle EMG and (c) electrical shocks applied to neck muscles? 2. What are the characteristics of (a) eye movements, and (b) neck muscle EMG responses, elicited by electrical stimulation of those FEF regions from which unit records had been obtained? Methods. With the animal's head rigidly attached to a stereotaxic frame by means of implanted head brackets, eye movements were measured using a modified version of the scleral coil method described by Fuchs & Robinson[1]. Stimulating currents to the FEF never exceeded 300 microamperes. Unit activity in the FEF was recorded extracellularly with metal microelectrodes. FEF unit recording. Marked changes in the spontaneous discharge rate of some (6%) FEF units were closely related to spontaneous or vestibularly driven eye movements. Such unit responses were frequently associated with a given direction of eye movements; no consistent relation to eye position or velocity was found. In some cases, the correlation of unit responses with eye movements was enhanced when the animal was made to visually follow a predictably moving target. Single shocks to the biventer cervices neck muscle elicited burst responses in a number of units, after latencies of 12-20 milliseconds. FEF stimulation. Eye movements, elicited after latencies of some 60 milliseconds by stimulation of the right FEF, were similar to normal voluntary saccades: amplitudes were in the range of 1-15 degrees, and for amplitudes of 2-8 degrees, velocities ranged from 100 to 270 degrees/sec. The direction of evoked saccadic eye movements depended on (a) the site of stimulation, and/or (b) the initial position of the eye in the orbit. Some sites at which unit discharges had previously been recorded gave rise, when electrically stimulated, to evoked saccades whose direction was opposite to that previously favoured by increased unit discharges. Saccadic responses to FEF stimulation were significantly reduced while the animal was made to visually follow a predictably moving target. Frequently, bursts of EMG activation in the biventer cervices muscle preceded evoked eye movements by some 20 milliseconds (i.e. 40 msec. latency), while in some cases such EMG activation was evoked from the lateral FEF regions without accompanying eye movements. These results, while emphasizing the sensitivity of FEF units to "set" (attention), are in support of some current evidence suggesting a role for the FEF in the coordination of eye-head movement.

[1]Fuchs, A.F. & Robinson, D.A. (1966). J. appl. Physiol. 21, 1068-1070.
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357 A NEW VIEW OF VISUAL-OCULOMOTOR INTEGRATION IN MONKEY SUPERIOR COLLICULUS. C.W. Mohler and R.H. Wurtz, Lab of Neurobiology, NIMH, Bethesda, Md. 20014

Cells in the most superficial layers of monkey superior colliculus respond to visual stimuli while the cells in intermediate layers discharge before saccadic eye movements. The general view of collicular organization is that there is a chain of processing beginning in the superficial visual cells and progressing toward an output from the deeper eye movement related cells. Our experiments cast doubt on this view as it applies to the eye movement related cells. In two monkeys we studied 70 cells which discharged before saccades. By systematically advancing the electrode we correlated the time by which the cell discharge preceded the eye movement with depth within the colliculus. On 25 of 29 penetrations, the discharge of the first cell encountered preceded the saccade by a shorter time than the next cell below. The most dorsal cells usually led the start of the eye movement (EOG) by about 50-60 msec while the deeper cells led the eye movement by about 100-125 msec. On the remaining four penetrations the time of onset of the cell discharge differed by less than 10 msec and the cells were within one half mm of each other . Because the most superficial eye movement related cells responded later in time with respect to the saccade, the superficial cells cannot provide the only input to the deeper eye movement cells. If there is sequential processing the colliculus, the direction of eye movement related processing must be upward through the intermediate layers and might converge with the descending visual information. At the border between visual and eye movement cells, we recorded 11 cells which were different from cells previously reported. These cells discharged before a visually triggered saccade but did not respond before spontaneous saccades of equal amplitude. We suggest that these cells may be combining descending visual and ascending eye movement information and be one output of superior colliculus.

358 CHARACTERISTICS OF CELLS IN MONKEY SUPERIOR COLLICULUS WHICH DIFFERENTIATE BETWEEN EXTERNAL AND SELF-INDUCED STIMULUS MOVEMENT. David Lee Robinson and Robert H. Wurtz. Neurobiology Dept., AFRRI, and Laboratory of Neurobiology, NIMH, Bethesda, Md. 20014.

While monkeys fixated their gaze on a spot of light on a tangent screen in front of them, we studied the response of cells in the superficial layers of the superior colliculus to rapid stimulus movement. In four monkeys we found that 64% of 139 cells responded to externally generated movements up to 900°/sec - a velocity comparable to the peak velocity occurring in a 20° saccadic eye movement. Of these cells, 66% did not respond to similar stimulus movements when such movements were generated by 20° saccadic eye movements. The majority of these colliculus cells responded to externally generated stimulus movements in any direction. Nine of eleven cells did not respond to stimulus movements generated by eye movements in any direction tested; the remaining two cells showed only slight directionality. Within a range of 2-20° from the fovea the proportion of cells responding to rapid stimulus movement increased with increasing distance from the fovea. The proportion of cells which differentiated between the two types of stimulus movement did not vary with retinal eccentricity. Cells which differentiated continued to do so when the experiments were conducted against a darkened background, suggesting that the movement of the background during a saccade does not account for the lack of response to the stimulus during an eye movement. Cells which differentiated also showed a suppression of spontaneous firing in association with eye movements in total darkness, indicating that they receive an extraretinal signal. We conclude that these colliculus cells provide a sensitive signal about external stimulus movement uncontaminated by self-induced stimulus movement.

359 PURSUIT EYE MOVEMENTS GENERATED DURING APPARENT MOTION. Laurence R. Young and William Chu* Man-Vehicle Lab.,Aero-Astro Dept. MIT, Cambridge, MA 02139

It has often been noted that smooth pursuit eye movements can be generated in the absence of any retinal slip velocity, as in the case of tracking an after-image[1-3], and aided by tracking one's own hand[4]. The perceptual feedback hypothesis[1] was advanced to account for such behavior on the basis that smooth eye movements were generated to move the eye at a velocity proportional to the perceived velocity of a target relative to the head, and that this perceived velocity could be generated by vestibular inputs, efferent copy during after-image viewing, or even by an imagined target driven by active hand movement. In the current experiments, we examined the relationship between apparent motion of a series of horizontally spaced dots (phi phenomenon) and the following eye movements. Although it is known that the phi phenomenon does not require eye movement[5], and that smooth pursuit can be generated by discrete target steps[6], it remained undetermined whether the stimulus conditions which produced continuous apparent motion would also generate pursuit tracking.

The stimulus consisted of eight spots on a CRT, covering a visual angle of 12 deg, which were illuminated sequentially with various periods and duty cycles. For each of 7 subjects, a given "velocity", between 6 and 12 deg/sec was chosen, and the duty cycle varied to present more or less phi[7]. For conditions which did not produce phi, 50% more saccades per sweep were generated on the average then when phi was present. Similarly, for duty cycles which produced phi occasionally, the eye tracking showed more pursuit and fewer saccades during times when phi was reported.

These results support the perceptual feedback hypothesis by showing the tendency for pursuit eye movements to follow apparent smooth motion in the absence of continuous retinal slip.

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4. Steinbach, M.J. and Held, R. "Eye tracking of observer-generated target movements" Science 161:187-188, 1968.
5. Kohlers, P.A. Aspects of Motion Perception Pergamon Press, Oxford, pp.172-174, 1972.
6. Westheimer, G. and Conover, D.W. "Smooth eye movements in the absence of a moving visual stimulus" J Exper Psychol 47:283-284,1954.
7. Related experiments for phi with multiple targets and a fixed duty cycle are being performed independently by Drs. Muelders and Decostre in Brussels, with similar results.

360 NON-VISUAL TARGET AND FEEDBACK INFORMATION IN THE CONTROL OF SMOOTH EYE MOVEMENTS. Kim R. Jones*, M. A. Berkley and D. Whittington*. Dept. Psych., Florida State Univ., Talla., Florida 32306.

A person visually tracking a moving target has available two sources of information: target position and tracking error (eye position feedback). Under ordinary viewing conditions, these two sources are not separable. To estimate their relative contributions, we provided target position or eye position information independently, using non-visual signals. We were interested in determining 1) whether non-visual "targets" could elicit smooth eye movements without eye position feedback; 2) whether non-visual eye position feedback could support smooth eye movements without target position information; and 3) the relative importance of these two inputs. Electro-oculogram (EOG) recordings were taken from human subjects who were instructed to make smooth tracking eye movements in total darkness under two conditions. In the first, the subject was given target position information via proprioceptive, tactile, or auditory signals. In the second, these signals were used to indicate eye position but not target position. The subject's ability to generate smooth tracking movements under both conditions was measured qualitatively, by inspection of the eye movement records, and quantitatively, using harmonic analysis of the EOG recordings. These analyses revealed that smooth tracking eye movements were possible using non-visual target information only but were not possible using only eye position feedback information (Supported by NSF BMS72-02103 to M.A.B.)

361 CENTRAL PROJECTIONS OF DORSAL NECK MUSCLES. B. Dubrovsky and H. Barbas*. Neurophysiol. Labs., Allan Mem. Inst., McGill University, Montreal.

The influence of neck structures on the extraocular musculature has been clearly established but the physiological significance of these bi-directional relationships is still a controversial issue (Easton, T. A., Exp. Neurol., 31: 419, 1972). Superficial neck muscles extend through th cervical column and functionally link the head with the upper girdle. Th deep posterior neck muscles - recti and obliques - have, on the other han a connectivity restricted exclusively to the head and upper two cervical vertebrae. The different fiber arrangement observed in the area - parallel fibered and pinnate muscles - indicates adaptations to the mechanical strain imposed during the evolutionary process by the increased development of the size of the head with the cephalization of telereceptors (Alexander, McNeil, R., Animal Mechanics, 1968; Gans, C., Biomechanics, 1974). Of relevance in this context is the fact that the nuchal musculature and extraocular muscles derive from the same myotomes, (Hildebrand, M., Analysis of Vertebrate Structure, 1974). These embryological, evolutionary and anatomophysiological considerations prompted us to study individually the central projections of superficial and deep dorsal neck muscles. Cats were anaesthetized with chloralose, paralyzed with Flaxedil and maintained under artificial respiration. The nerves of the biventer cervicis and splenius, both superficial muscles, as well as the nerves of two deep muscles, the rectus capitis dorsalis major and the obliquus capitis caudalis, were prepared for stimulation. The nerves were kept moist in a bath of warm paraffin oil. Afferent volleys were recorded from second and third dorsal rootlets to determine threshold stimuli. Superficial and deep neck muscle afferents were found to arrive at the post-cruciate dimple. These results confirm Landgren's et al (Acta physiol. scand., 74: 340, 1968) data reporting splenial muscle projections to this area, and extend these findings to the other superficial and deep neck muscles. The cortical potential reversed at about 1.5 mm below the surface and disappeared approximately 3 mm below the surface. Also, further to the findings of neck muscle projections to lobes V, VI and VII of the cerebellum (Berthoz, A., and Llinas, R., Exp. Brain Res., 20: 385, 1974; Dubrovsky, B., Proc. Int. Cong. Physiol., XI: 182, 1974), we now report the activation by low threshold afferents of brain areas corresponding with the site for induction of oculomotor responses in the cat (FEF) (Schlag, J., and Schlag-Rey, M., Brain Res., 22: 1, 1973). Penetrations with tungsten microelectrodes were concentrated mainly in the presylvian zone of the FEF, that is, 1-2 mm posterior the cruciate sulcus, 4-5 mm from the midline and 7-10 mm ventrally. The recording positions were verified histologically. Over 50 cells were studied, of which 26 responded to stimulation of the contralateral superficial neck muscles, 11 responded to stimulation of the ipsilateral superficial, and 12 to the contralateral deep muscles. Unit responses were obtained as early as 5 msec for the contralateral superficial muscle with threshold stimulation. Ipsilateral responses had later latencies (average of 12 msec) and higher thresholds. No convergence of cell activity between stimulation of superficial and deep muscles was found in the sample studied. Complete sectioning of the dorsal columns abolished the responses to threshold contralateral stimulation, but with increased intensity of stimulation, a potential with longer latency could still be obtained. The results are interpreted in the context of previous behavioral findings which revealed deficits in visual tracking during a sequential motor act following sectioning of the dorsal columns (Dubrovsky, B., and Garcia-Rill, E., Exp. Brain Res., 18: 165, 1973). Based on the present data we believe that proprioceptive stimuli from neck muscles coursing through the dorsal columns play an important role in proper coordination of eye movements in complex motor acts which require movement of the head in relation to the entire body while the body is in motion.

362 THE ROLE OF THE MLF IN EYE MOVEMENTS: FUNCTIONAL PHYSIOLOGY UNDERLYING ANTERIOR INTERNUCLEAR OPHTHALMOPLEGIA. L.C. Evinger*, W.M. King*, S.G. Lisberger*, A.F. Fuchs, R. Baker. (SPON: T.T. Kennedy), Dept. of Physiol. and Biophysics, Regional Primate Research Center, Univ. of Wash., Seattle, WA 98195 and Dept. of Physiol. and Biophysics, Univ. of Iowa, Iowa City, IA

On monkeys trained to a visual tracking task, the effects on horizontal and vertical eye movements of a bilateral lesion of the medial longitudinal fasiculus (MLF) between abducens and trochlear nuclei was compared with single fibre recordings in the MLF. In the horizontal plane MLF lesioned monkeys exhibited a paresis of all adducting movements except convergence, suggesting the lesion functionally denervated the medial rectus cell group. Surprisingly, however, there was almost no change in the phase of the vestibulo-ocular-reflex (VOR). In accord with the functional denervation hypothesis, one of the two fibre types recorded in the MLF had a burst-tonic discharge with the same characteristics as an ipsilateral medial rectus motoneuron. The tonic firing rate increased with medial eye rotations, the burst began prior to adducting saccades, and sinusoidal modulations of the firing rate preceded sinusoidal smooth eye movements by phase leads characteristic of motoneurons. In the vertical direction the lesion did not affect saccades, but the eye always drifted back toward the horizontal meridian from eccentric vertical eye positions, so that repeated saccadic corrections for the drift produced a fixation nystagmus. The lesion also completely abolished the vertical VOR. Deficits in vertical eye movements produced by the lesion were consistent with a second fibre type which paused for saccades and had vertical eye position sensitivity. When the monkey's head was sinusoidally tilted in the vertical plane, the fibres' firing were modulated in relation to head velocity even when the monkey suppressed the VOR. These results suggest the deficits seen in anterior internuclear ophthalmoplegia can be completely explained by the loss of the two fibre populations found in the MLF.

363 OCULOMOTOR COMPONENTS OF PREFRONTAL ELECTROCORTICAL POTENTIALS IN MONKEYS. John H. Robinson*, Steven C. Rosen*, and John S. Stamm. Psych. Dept., SUNY at Stony Brook, New York, 11794.

In order to assess whether cortical potentials evoked in monkeys' dorsolateral prefrontal cortex by visual stimuli reflect sensory and/or oculomotor functions, three monkeys with chronically implanted nonpolarizable electrodes in prefrontal, precentral, and occipital cortex, and subcutaneously across the eyes, were trained on a reaction time task for liquid reward. The task required that the monkey fixate upon a centrally located conditioning light and then rapidly foveate upon one of six test lights. These were perimetrically presented in random order at 20,40, or 60 degrees to the left or right of center. A fruit juice reward was dispensed if the monkey pressed a lever during a 0.5 sec dimming of both the conditioning and test stimuli. Computer averages of cortical evoked potentials (EPs) for forty presentations of each of the test lights revealed that the left prefrontal EPs had largest amplitudes to the extreme right light and the converse for right prefrontal EPs. By contrast, the largest occipital EPs were observed with the centrally presented stimuli. The precentral recordings showed no changes in EPs with different light locations. Averaged EOG data revealed appropriate saccadic eye movements with latencies of 240-340 msec, that coincided with the time course of later components of the EPs. Since, the amplitude of components of the prefrontal EPs, both preceding and following eye movements, were related to the amplitude of contralateral saccades, it is concluded that prefrontal EPs reflect predominantly oculomotor, rather than sensory, functions of prefrontal cortex. (Supported by NSF Grant GB-35735X).

364 EYE MOVEMENT RELATED NEURONS IN THE PREPOSITUS HYPOGLOSSI NUCLEUS.

R. Baker, M. Gresty* and A. Berthoz*. Div. Neurobiology, Univ. of Iowa, Iowa City, Ia USA; Laboratoire de Physiologie du Travail, 41 Rue Gay-Lussac, Paris (France).

Many neurons in the prepositus hypoglossi nucleus are ortho- and antidromically activated by electrical stimulation of the oculomotor complex. To determine whether these neurons have an oculomotor role their activities during eye movements evoked by natural visual and vestibular stimuli were recorded in alert cats. In six experiments 77 cells clearly related to either horizontal (ipsi, 34%; contra, 9%) or vertical (up, 21%; down, 12%) eye movement were isolated. Such prepositus neurons could be divided conveniently into two classes. Neurons in the first group (50%) produced a burst of activity preceding saccades (0-20 msec) in a preferred direction and had a steady firing rate linearly related to eye position. This population showed a continuum of velocity-position responsivities and nearly all had relatively high threshold position sensitivity. The activities of the second group were correlated only with changes in eye position; however, the neurons were distinctly inhibited either during saccades or other high velocity movements in the off (and sometimes on) direction. A third profile of activity (24% of total) was found in neurons which exhibited a burst, or pause, (both bi- and directional types) during saccades and an invariant tonic activity in the absence of eye movement. Often all three of the cell types could be recorded in close proximity to each other within 'glomerular-like' clusters. In addition, the activities of all neurons were modulated during both voluntary and vestibular evoked eye movement. These results provide evidence that prepositus neurons may play a significant role in the generation of both vertical and horizontal saccades, fixation and vestibular or visually guided pursuit eye movement.

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365 CORTICAL AND THALAMIC SOMATOSENSORY EVOKED RESPONSES IN RHESUS MONKEYS RECEIVING LITHIUM. George R. Heninger. Dept. Psychiat. Sch. Med., Yale, New Haven, Conn. 06519.

Increased amplitudes of early components of the scalp recorded somatosensory evoked response have been observed prior to and during the time that behavioral changes occur in patients receiving lithium treatment (Heninger, G.R., EEG, 27:670, 1969). In order to elucidate the neural basis of these findings, comparable studies have been conducted in rhesus monkeys given lithium in doses within the range of those used in humans. Averaged evoked responses to percutaneous median nerve stimulation have been recorded from scalp, dura, subcortical, and thalamic (VPL) electrodes. Lithium produces an increased amplitude of the early positive potential (9-20 ms) recorded over a wide area from scalp and dura. Bipolar cortical electrodes (base on dura, tip 3 mm subcortical) located in somatosensory, motor, and frontal cortex demonstrated polarity reversal of potentials peaking at 12, 17 and 22 ms respectively. Lithium produced increased amplitudes of these early potentials even though the amplitudes of late potentials were not markedly altered. Lithium effects were most obvious during the second half of the early potentials and effects were most evident in frontal, motor and somatosensory cortex respectively. There were no consistent changes in primary potentials recorded from VPL. Changes occurred at lithium ion concentrations between 1.0-1.7 and 0.3-0.7 meq/L for serum and CSF respectively. Similar to human studies, changes occurred only after 5-7 days of lithium treatment and they persisted 5-10 days after lithium was discontinued. The data suggest that processes involving sensory input to frontal cortex are relatively more sensitive to lithium than primary sensory areas and that lithium effects on subcortical primary somatosensory paths is minimal. The importance of these findings relative to the neuropsychological and symptomatic changes observed in patients receiving lithium treatment can be demonstrated.

366 PHARMACOLOGIC ASPECTS OF THE LOCOMOTOR STIMULATION PRODUCED BY PHENCYCLIDINE IN THE RAT. M. Kanner, H. Y. Meltzer, and J. M. Davis, Dept. of Psychiatry, Univ. of Chgo. Pritzker Schl. of Med., Chicago, Ill. 60637 and the Ill. State Psych. Inst.

Phencyclidine (PCP) is a potent psychotomimetic agent in man whose neuropharmacologic actions are still unclear. Some of its effects may be mediated by effects on catecholamines although serotonergic and anticholinergic actions have been noted. Phencyclidine produces increased locomotor activity in mice which can be augmented by pretreatment with iproniazid (Chen et al. J.P.E.T. 127, 241, 1959). Phencyclidine also stimulates locomotor activity in rats and we have studied this phenomenon on Animex activity meters. We have found an increasing stimulatory effect in doses of 2.5-25 mg/kg, i.p. although doses greater than 7.5 mg/kg produce significant ataxia which interfere with the expression of locomotion. A dose of 5 mg/kg, i.p. PCP produced increased motor activity over saline injection from 243 to 5601 counts in 1 hr (n=8, $p<.05$). Pretreatment with AMPT (250 mg/kg, i.p.) decreased PCP-elicited activity by 43% ($p<.05$) in 1 hr. Iproniazid (100 mg/kg, i.p.) increased PCP-elicited activity by 61%. Pretreatment with phenoxybenzamine (10 mg/kg, i.p.) decreased PCP-elicited activity by 85% ($p<.05$) while pretreatment with propanolol (25 mg/kg, i.p.) increased PCP-elicited activity by 56% ($p<.05$). Pimozide (1 mg/kg, i.p.) decreased PCP-elicited activity by 57% ($p<.05$) while haloperidol (0.5 mg/kg) decreased PCP-elicitied activity by 90% ($p<.05$). Trihexyphenidyl (5 mg/kg, i.p.) and atropine (10 mg/kg, i.p.) significantly increased PCP-elicited activity by 93% and 43% respectively, while arecoline (10 mg/kg, i.p.) and physostigmine (0.4 mg/kg, i.p.) decreased PCP-elicited activity by 23% and 61% respectively. This data indicates that the stimulation of locomotor activity produced by PCP can be influenced by noradrenergic, dopaminergic, and cholinergic input. Supported in part by F.F.R.P. Grant #73-582 to M.K.; USPHS 16,127 and RCDA MH 47,808 to H.Y.M.

367 BEHAVIORAL CHANGES IN SELECTED MEMBERS OF A JUVENILE PRIMATE SOCIAL COLONY WITH CHRONIC IMIPRAMINE ADMINISTRATION. R.C. Casper*, R.F. Schlemmer, Jr., F.K. Siemsen*, D.L. Garver, & J.M. Davis (SPON: G.N. Pandey). Ill. State Psychiat. Inst. & U. of Ill., Chicago, Ill. 60612

Administration of imipramine (Imip) to young children has become increasingly common, yet effects of tricyclic drugs on the developing human brain have remained untested for obvious ethical reasons. In this study, the behavioral effects after daily Imip administration to three selected members (Ts) of a stable, juvenile primate social colony of six, peer-raised Stumptail macaques were examined. Following a three week baseline period, where Ts received n.g. sham-treatment with water, imipramine HCl, 1.67 mg. salt/Kg., was administered n.g. to Ts daily at 6 PM for four weeks. Behavioral observation by an experienced, "blind" observer occured the following mornings during baseline and Imip treatment periods. All three animals treated with Imip engaged in more social and solitary play, more social grooming, and more social activity than during their respective baseline periods. Likewise, huddling and resting periods decreased in these animals during Imip treatment. Increased vigilance was also noted in all Ts. Since it has been shown previously in adults of this species that increased vigilance is modulated by norepinephrine (NE) (Garver et al, Am. J. Psychiatry 132:33, 1975) and since the NE hypothesis also underlies the antidepressant effects of Imip the same neurotransmitter is likely to be involved in mediating the observed stimulating effects of Imip in these monkeys.

368 EPISODIC HYPERKINESIA IN MONKEYS FOLLOWING CHRONIC HALOPERIDOL. Bernard Weiss. Dept. Rad. Biol. Biophysics, U. Rochester Sch. Med. Dent., Rochester, N.Y. 14642.

Drugs employed as chemotherapeutic agents in psychiatry often evoke disorders of movement. Early in treatment, these disorders resemble Parkinsonism. Later, they fade, to be replaced by what have been called tardive dyskinesias. These tend to persist, sometimes not even becoming apparent until the drug is withdrawn. The present study represents another attempt to determine the degree to which parallel phenomena are observed in laboratory primates. Specimens of Cebus apella and Saimiri sciurea were maintained on various doses of haloperidol. After several months of administration, both groups developed movement disorders. In the cebus monkeys, the pattern was dominated by violent uncontrolled movements that flung the animals about their cage. These episodes of hyperkinesis tended to last only a few minutes and to occur 2-6 hours after daily oral administration of 0.5 mg/kg or more. Several episodes might recur during a single day. The squirrel monkeys tended to develop bizarre postures, athetosis, and transient dystonias. The implications of these results are examined from two vantage points: drug-induced movement disorders in humans, and the possible neurochemical substrates, which probably involve dopamine.

Examples will be shown in an accompanying motion picture film.

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369 A COMPARISON OF THE EFFECTS OF CLOZAPINE, CHLORPROMAZINE, AND COMPAZINE ON CATALEPSY, ACTIVITY, AND SEVERAL OPERANT TASKS. J.L. Howard, K.W. Rohrbach*, G.T. Pollard*, and F.E. Soroko*. Department of Pharmacology, Wellcome Research Labs, Research Triangle Park, North Carolina 27709.

Clozapine has been shown to be an efficacious neuroleptic with a different pharmacological profile than existing neuroleptics (e.g. Simpson, Cur. Ther. Res. 1974, 16, 679). Because the behavioral effects of Clozapine have not been widely explored, we compared Clozapine to chlorpromazine (CPZ) and Compazine in producing catalepsy, in affecting spontaneous and d-amphetamine stimulated activity, in acquisition and performance of a two-way active avoidance response and in bar pressing for food reward on a multiple fixed interval/fixed ratio schedule using male Long-Evans rats. All three compoundsproduced dose-related decreases in spontaneous activity; however, whereas CPZ and Compazine antagonized d-amphetamine-stimulated locomotor increases, Clozapine potentiated amphetamine's action. Clozapine also did not produce catalepsy as did the other two compounds. All three compounds slowed acquisition and reduced performance of the avoidance task in a dose-dependent manner. Clozapine, however, did not produce an increase in omits at doses decreasing avoids as did the other two neuroleptics. No qualitative differences were observed among the three neuroleptics in their effects on the FI/FR schedule.

It was of interest to determine whether the behavioral differences between Clozapine and other neuroleptics were due to its stronger anticholinergic action. This was tested by combining an anticholergic, scopolamine, with CPZ in those tests in which a difference between CPZ and Clozapine had been observed. The addition of 0.5 mg/kg of scopolamine to CPZ reversed the catalepsy caused by CPZ alone and changed the suppression of d-amphetamine's effect on locomotor activity to a potentiation. In behaviors in which CPZ and Clozapine had qualitatively similar effects, the addition of scopolamine to CPZ produced the same action as CPZ alone.

370 CLOZAPINE INCREASES RAT SERUM PROLACTIN LEVELS. H. Y. Meltzer, S. Daniels* and V. S. Fang*. Depts. of Psych. and Med., Univ. of Chgo. Pritzker Schl. of Med., Chicago, Ill. 60637

Clozapine, an effective anti-psychotic drug, has a different pattern of effects on rat brain amine metabolism than classical neuroleptic drugs whose most important biochemical action is believed to be dopamine receptor blockade. Clozapine does not produce extrapyramidal side effects as do the neuroleptic drugs. This effect of the neuroleptics and their capacity to increase serum prolactin has been attributed to their dopamine receptor blocking properties. We therefore decided to determine if clozapine increased rat serum prolactin levels. Serum prolactin was determined by a double antibody radioimmunoassay. Rats were killed by decapitation. Clozapine, 5 mg/kg i.p. increased serum prolactin in male Sprague-Dawley rats from 7.1±S.D. 4.6 ng/ml to 57.6±S.D. 20.3 ng/ml, 30 min. after injection. Clozapine 10, 50 and 100 mg/kg i.p. produced mean serum prolactin levels that were in the range of 143-164 ng/ml and which were not significantly different from each other. Serum prolactin after 10 mg/kg clozapine was significantly greater than after chlorpromazine, 5 mg/kg or haloperidol 0.5 mg/kg. The effect of drugs which affect dopamine and serotonin metabolism on the serum prolactin increase following clozapine will be discussed. The increase in serum prolactin may be due to clozapine's ability to produce dopamine blockade or to inhibit nerve impulse-dopamine release, or both. The capacity of clozapine to affect brain serotonin and norepinephrine metabolism and its strong anti-cholinergic properties are probably not involved in its ability to increase serum prolactin.

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371 DIFFERENTIAL EFFECTS OF CHLORDIAZEPOXIDE (CDP) ON HYPER-REACTIVITY AND VI RESPONSE RATES IN SEPTAL MICE. L.J. Standish*and R.S. Feldman, Psychology Dept., U. Mass., Amherst, Mass. 01002

Mice with septal lesions were tested for 8 days for hyper-irritability (septal rage) before and after i.p. doses of CDP (20, 40 and 60 mg/kg). They were also tested on a VI 40 sec. schedule for food reinforcement after daily drug treatment. The results showed that septal rage was suppressed proportional to dose for all drug tests. VI rates were also suppressed proportional to dose but after 2-3 days response rates returned to baseline then proceeded to overshoot baseline levels. CDP has a transient sedative effect that is correlated with a transient reduction of brainstem NE turnover. This accounts for the transient drop in VI response rates. CDP also has a persistent anxiolytic or disinhibitory effect correlated with persistent reduction of brainstem 5-HT turnover. This could account for the persistent suppression of septal rage. This would also account for the overshoot of VI responding once tolerance to the sedative effect unmasked the anxiolytic or disinhibitory effect. However, Lilly-110140, which specifically blocks 5-HT re-uptake and potentiates 5-HT effects, failed to reverse CDP-induced attenuation of septal rage, while it does reverse CDP-induced disinhibition of punished responding. CDP is also reported to stimulate glycine receptors which may account for CDP's muscle relaxant, anti-convulsive and anxiolytic effects. It is suggested that septal rage may be due to a deficit involving glycine activated neural systems.

372 AGE-SPECIFIC RESPONSES TO A SINGLE INJECTION OF RESERPINE TO RATS. Jean DiRaddo, Dept. of Psychology, Univ. of Rochester, Rochester, N.Y. 14627.

As an initial step in an investigation of some characteristics of the functional pools of dopamine and noradrenaline across ages, a developmental analysis of the recovery of the locomotor response after reserpine has been performed. Long-Evans hooded rats (aged 7,14,17,21,28 days, and 10 wks) were injected with reserpine (2.5 mg/kg or 5 mg/kg,I.P.) or the drug vehicle (glacial acetic acid and dextrose, I.P.) at the same time of day in all studies. At 2,5,12, and 24 hrs after the injections, each animal was placed in an activity meter for a 20 min period. The pattern of locomotor response was dose- and age-specific. Animals were considered inactive, active, or hyperactive in relation to their littermate controls tested in the same session. At 14 days of age, animals receiving the higher reserpine dose showed a slight hyperactivity 12 hrs after the injection, with activity levels below controls at other times. Those with the lower dose at this age were inactive at all times tested. At 15-17 days of age, those with the higher dose were active at 2 hrs and hyperactive from 5-24 hrs. Ss with 2.5 mg/kg reserpine, however, were inactive previous to 24 hrs, at which time they showed a marked hyperactivity. The most striking hyperactivity was seen in 21 day old Ss 5 hrs after the lower dose, at other times they were relatively inactive. Some of those receiving 5 mg/kg reserpine showed a much less marked hyperactivity at 2 or 5 hrs. At 28 days of age, reserpinized rats were generally inactive, except for some slight hyperactivity at 5 hrs in those with the higher dose. The age-specific nature of these responses may be related to developmental changes in the relationship between different transmitter pools or to changes in the rate of axoplasmic flow. Neurochemical studies utilizing radioactive precursors and amines are now in progress to examine possible age-specific correlates of these responses in the cortex and the striatum.

373 IN VITRO INDUCTION OF 3H-RESERPINE BINDING TO SUBCELLULAR COMPONENTS OF THE RAT FOREBRAIN BY Δ9-TETRAHYDROCANNABINOL. K.M. Johnson*, B.T. Ho, W.L. Dewey and L.S. Harris. Texas Res. Inst. Mental Sci., Houston, TX 77025, and Dept. of Pharmacol., Med. Col. of Va., Richmond, VA 23298.

Δ^9-Tetrahydrocannabinol (Δ^9-THC) has been reported to attenuate both reserpine-induced serotonin depletion and reserpine-induced hypothermia. In an effort to determine the nature of this interaction, we have examined the binding of reserpine to a crude synaptosomal fraction of rat forebrain after preincubating with Δ^9-THC or its vehicle. Δ^9-THC preincubation led to a dose-responsive increase in the amount of ^{3}H-reserpine (^{3}H-R) bound to this fraction, with the maximum increase (93%) being observed at 100 uM Δ^9-THC. In subsequent experiments, the crude synaptosomal preparation was further fractionated in order to determine the subcellular localization of the bound ^{3}H-R. Preincubation with Δ^9-THC [100 uM] produced a shift in the localization of ^{3}H-R from the incubation medium and the microsomal supernatant (decrease of 66%) to the crude mitochondrial (CM) pellet (increase of 154%). The CM pellet was subfractionated both by differential centrifugation after osmotic shock and by layering on a five-step discontinuous sucrose gradient and centrifuging at 80,000 x g. Osmotic shock with 0.032 M sucrose and centrifugation revealed that the Δ^9-THC-induced increase in ^{3}H-R was contained in both the synaptic vesicle fraction (247%) and the fraction containing myelin, ruptured synaptosomes and mitochondria (324%). Separating the CM fraction into five component parts showed that Δ^9-THC increased the ^{3}H-R bound by about 275% in the three fractions containing myelin, membrane fragments or mitochondria. Even more dramatic increases ($>$ 10 fold) were observed in the two fractions containing cholinergic and non-cholinergic nerve endings. Although other possibilities exist, these data suggest that Δ^9-THC retards the action of reserpine by an induction of silent reserpine receptor sites. (Supported by USPHS Grants Nos. DA-00326 and DA-00490).

374 SIGNIFICANT INCREASES IN DOPAMINE AND NOREPINEPHRINE LEVELS AFTER PROLYL-LEUCYL-GLYCINAMIDE IN RATS SUBJECTED TO THE BEHAVIORAL DOPA POTENTIATION TEST. M.A. Spirtes, Richard M. Kostrzewa, Nicholas P. Plotnikoff* and Abba J. Kastin. Veterans Administration Hosp., and Tulane Univ. School of Med., New Orleans, and Abbott Labs., Chicago.

After the experiments of Plotnikoff et al. (Neuroendocrinology 14: 271, 1974) indicated that prolyl-leucyl-glycinamide (melanocyte stimulating hormone release inhibiting factor, MIF-I) failed to influence dopamine levels in the caudate nucleus of rat brain, it was decided to investigate the effect of MIF-I on dopamine (DA) and norepinephrine (NE) levels in the striatum of rats studied in the dihydroxyphenylalanine (DOPA)-behavioral potentiation test. Groups of six Charles River male albino rats, 150 g each, were pretreated with oral pargyline and then injected i.p. with saline, MIF-I, or thyrotropin releasing hormone (TRH) 90 min before sacrifice followed by DOPA 1 hr later. The animals were then sacrificed by decapitation 30 min after the DOPA. Within 10 min, the striatum and other brain areas were removed, frozen on dry ice, and weighed. NE and DA were determined according to the method of Hogans (Biochemistry of Catecholamines, University Park Press, Baltimore, 1973, p. 230-232). Three doses of MIF-I were used, 0.1, 0.3 or 1.0 mg/kg or two doses of TRH, 0.5 and 2.0 mg/kg i.p. Pargyline, when used, was given at a dose of 45 mg/kg p.o. DOPA, when used, was injected i.p. at a dose of 100 mg/kg. Controls of saline alone, pargyline + saline, saline + DOPA, and pargyline + saline + DOPA were also prepared. Results indicated that TRH did not significantly influence the DA or NE levels during the DOPA behavioral potentiation test whereas MIF-I increased ($p < 0.05$) DA levels to the same degree (ca 100%) for all three doses. NE levels increased by about 70% at both the low and high dose of MIF-I. It is not clear whether the increased DA levels found in the striatum after administration of MIF-I are related to the clinical effects of MIF-I in Parkinson's disease.

375 BRAIN CATECHOLAMINES AND THE ANTINOCICEPTIVE ACTION OF (±)9-NOR-9β-OH-HEXAHYDROCANNABINOL. Alan S. Bloom*, W. L. Dewey, L. S. Harris, and K. Brosius*. (SPON: G. H. DeVries). Dept. of Pharmacol., Med. Col. of Va., Richmond, Virginia 23298.

We have previously reported (Fed. Proc. 34, 787, 1975) that compounds such as morphine and other narcotic analgesics that are active in the mouse tail flick test increase catecholamine synthesis as measured by the conversion of ^{3}H-tyrosine to ^{3}H-norepinephrine (NE) and ^{3}H-dopamine (DA). (±)9-nor-9β-OH-hexahydrocannabinol (HHC) has a potency (ED50=7 mg/kg) in the mouse tail flick test that is about equal to that of morphine (ED50= 5.8 mg/kg). Studies with the (-) isomer suggest that is the active form of the compound. Naloxone partially (32%) antagonized this activity. 9-nor-9α-OH-HHC was inactive in the tail flick test as was 15 mg/kg of Δ^{9}-tetrahydrocannabinol (THC). (±)9-nor-9β-OH-HHC significantly increases accumulation of newly synthesized catecholamines in a dose dependent manner. At 30 minutes after its administration, a 20 mg/kg dose increased the accumulation of newly synthesized NE and DA to 170% and 218% of vehicle control, respectively. This effect also was antagonized by naloxone. These increases were not accompanied by changes in the endogenous levels of NE and DA. The same dose of Δ^{9}-THC or the α-isomer did not significantly increase catecholamine synthesis. The drug treatments also did not alter endogenous tyrosine concentration or the specific activity of ^{3}H-tyrosine. These studies suggest that (±)9-nor-9β-OH-HHC is a compound that possesses some of the properties of the narcotic analgesics including antinociception and the activation of catecholamine containing neurons and that these effects are antagonized by naloxone. These data also suggest our earlier hypothesis that agents which are active in the mouse tail flick test increase the synthesis of brain catecholamines. (Supported by USPHS grants no. DA00490 and T22DA00128).

376 EVIDENCE FOR A RESERPINE-LIKE ACTION OF Δ^{9}-TETRAHYDRO-CANNABINOL (THC). Stuart M. Deikel and Brooks Carder*. Dept. Psych., UCLA, Los Angeles, 90024.

A model of THC action, involving a reserpine-like release and ultimate depletion of catecholamines, is proposed. This interpretation is supported by several experimental results. Pretreatment with a monoamine oxidase inhibitor, followed by THC, produced straub tail and excitatory effects on intracranial self-stimulation behavior. Further, rats made tolerant to THC exhibited cross-tolerance to the hypothermic effects of the reserpine-congener tetrabenazine (TBZ) Finally, behavioral cross-tolerance between THC and TBZ, in an unlearned swimming escape task, was demonstrated. The relevance of these data to a reserpine-like model of cannabis action is discussed. Possible discrepancies between the effects of reserpine and THC are also noted.

377 THE EFFECTS OF Δ9-THC ON PRENATAL TOXICITY AND MATERNAL BEHAVIOR IN CBA MICE. Daniel L. Ely and Flora Watson. Dept. Biology, Univ. of Calif., Riverside and Dept. Physiology, Univ. of So. Calif., Los Angeles.

A dose response relationship was found between Δ9-THC (20-200 mg/kg I.V. and I.P.) and fetal reabsorption in 121 female CBA mice. The percentage of normal litters decreased from 93% to 31% as doses increased from 20 mg/kg to 80 mg/kg (day 7, 8 or 9 of gestation). The decline in the percentage of normal litters was generally due to fetal reabsorption but a small percentage of litters showed retarded development, subcutaneous blood clots, hydroencephaly and decreased birth weight, although litter size was normal. A dose response relationship was observed between Δ9-THC (single injection 1 day postpartum) and the amount of time a mother spent with her young. There was a decrease in the amount of time she spent with her young from 80% to 40%, and at all doses the mothers failed to maintain a clean nesting area. A dose response relationship was also observed between Δ9-THC and pup retrieval and grooming behavior. At the higher doses (60-80 mg/kg) pup retrieval was eliminated when measured 24 hours post injection and pup grooming was significantly decreased. These behaviors returned to baseline values after 7 days with the exception of the animals at the high doses (80 mg/kg). Analysis of 6-hour segments of spontaneous motor activity rhythms showed a suppression of activity for 12 hours post injection and a significant increase in activity in the following 12 hours (20 mg/kg, I.V.).

378 AMPHETAMINE-INDUCED EXCITATION: DIRECT OR INDIRECT ACTION. K. Blum, J.E. Wallace*, J.D. Eubanks*, H.A. Schwertner* and W.W. Morgan*. Depts. Pharmacol. Pathol. and Anat., H. Sci. Ctr. Univ. Texas, San Antonio, Texas 78284.

The biogenic amines dopamine (DA), norepinephrine (NE) and the catecholamine releasers, d-amphetamine (AM) and tyramine (Ty) were investigated for their ability to produce convulsions elicited by handling in mice following intracerebral injection according to the method of Goldstein (1974). At the dose range of 5 to 100 μg NE is greater than DA in its ability to induce convulsions in mice. The slopes of the first derivative of the dose response for NE is similar to Ty suggesting similarity in site of action. The slopes of the first derivatives for dose-response of DA is not similar to NE. Evidence supporting the indirect NE-mediated theory of central AM action is suggested by our finding that the tyrosine hydroxylase inhibitor, α-methyl-para-tyrosine (MPT) at a dose of 80 mg/kg injected daily for 4 days reduced NE levels by 64% and significantly reduced the convulsion response of AM by 51%. However, preliminary analysis of AM-induced convulsions indicates dissimilarity of the slopes of the dose-response data as compared to NE. In other experiments, 6-hydroxydopamine (6-HD) was intracerebrally (i.c.) injected into mice at an initial dose of 200 μg and then 48 hours later. This compound caused an acute degeneration of adrenergic nerve terminals on the brain as evidenced by the absence of brain catecholamines. In contrast to our other findings with MPT, 6-HD treatment did not alter the AM-induced convulsion response. Thus, the release of catecholamines after AM and CNS excitation may be due to a completely independent mechanism. Our work further confirms the work of Havlicek et al., (1974) who recently showed that although MPT reduced AM-induced hypermotility in rats, 6-HD failed to produce any changes in the AM response (Research supported by Air Force Grant #AFOSR-71-2074 and NIDA Grant #1-T01-DA00290-01.

379 AMPHETAMINE EFFECTS ON MOTOR BEHAVIORS OF CHRONIC DECEREBRATED RATS. Luis A. Baez and Robert Petroff*. Dept. Psych., Southern Illinois Univ., Carbondale, Ill. 62901

The ability of amphetamine to enhance locomotor activity and to elicit stereotypic behaviors in animals has been linked to actions of this drug on central monoaminergic neurons, primarily the ascending noradrenergic and dopaminergic pathways. In view of recent reports that amphetamine stimulates locomotion in thalamic rats (Huston and Borbely, Physiol. Behav., 1974, 12, 433-448), and may elicit stereotypic behaviors in mesencephalic cats (Marcus and Villablanca, Proc. West. Pharmac. Soc., 1974, 17, 219-222), it appeared of interest to investigate the actions of this agent in decerebrated rats, a preparation which has been reported to exhibit a rather broad range of behaviors (Woods, J. Neurophysiol., 1964, 27, 635-644). Male, adult, Long-Evans rats were anesthetized with sodium pentothal and the brainstem was transected bilaterally, at the level of the superior colliculus, with a hand-guided spatula. Mortality was high, but those animals which survived to the chronic stage were eventually able to walk and engage in other complex behaviors. When animals were walking and appeared to be in good condition, they were injected with saline or various doses of d-amphetamine. Behavior was observed directly or evaluated using a time-lapse video tape recorder. Chronic decerebrated rats were behaviorally responsive to amphetamine administration, although the pattern of responding differed from that of sham-lesioned animals. Our findings indicate that some of the components of amphetamine-induced "behavioral arousal" can be integrated at relatively low levels of the central nervous system, suggesting the invovement of monoaminergic projections to brainstem structures and/or to the spinal cord.

380 PHARMACOLOGICAL AND BEHAVIORAL TOLERANCE TO METHYLPHENIDATE-INDUCED ACTIVITY IN RATS. W. Gibson Wood, Henry Schreiber*, Ramiro Villescas*, and Richard Carlson*. Dept. of Psychology, Texas Tech University, Lubbock, Texas 79409.

Kalant, LeBlanc, and Gibbins (1971) have proposed that the stimulus for the rate of tolerance development is not the concentration of the drug but the amount of functional impairment it produces. Functional impairment has been defined as the amount of disturbance related to the functional demand made on the organism. For example, an animal that is placed in a novel environment or forced to perform a task following the administration of a sympathomimetic or sedative-hypnotic drug would show more impairment as a result of the drug-induced stimulation or depression than an animal that is simply injected with either drug and left in its home cage. It would also be expected that the animal receiving the drug and the exposure to the novel environment would develop tolerance at a faster rate than the animal injected with the drug and returned to its home cage. In a test of this hypothesis by the present authors, it was found that animals receiving chronic injections of methylphenidate (MI) or saline (SI) and returned to their home cages, when both groups were injected with methylphenidate and tested in a Y-maze for behavioral activity, responded at an equivalent rate. In contrast, animals chronically injected with methylphenidate (MT) or saline (ST) and tested, when both groups were injected with methylphenidate and tested did not show a difference in responding. That the rate of the development of tolerance can be affected by more then simply exposure to the drug was supported by the significantly higher level of activity of the MI group compared to the ST group. It was also shown that habituation or adaptation to the test environment altered an animal's initial response to the drug. The ST group when injected with methylphenidate and tested, responded significantly lower than the saline injection group, which also received the drug for the first time. The results support the hypothesis that the rate of the development of tolerance is dependent on the adaptation to the functional impairment produced by the drug and not simply an adjustment or alternation in an animal's metabolism to the pharmacological action of the drug.

381 PARADOXICAL EFFECTS OF AMPHETAMINE ON PRE- AND POST-WEANLING RATS: TRANSITION FROM CONSPECIFIC-DIRECTED AROUSAL TO NON-DIRECTED AROUSAL. Patrick K. Randall* and Byron A. Campbell, Dept. of Psychology, Princeton University, Princeton, N.J. 08540

Time-lapse video tape photography was employed to investigate the effect of d-amphetamine sulphate (0,0.25,0.5,1.0, and 2 mg/kg) on locomotor activity in 15 and 30 day-old albino rats, tested either alone or in the presence of an anesthetized adult rat. Fifteen day-old rats tested alone showed hypersensitivity to amphetamine with a greater magnitude of response, prolonged time course, and dose-response curve to the left of 30 day-old animals similarly housed. Those 15 day-old subjects tested in the presence of an anesthetized adult, however, showed no increase in activity at any dose employed. Instead, the drug appeared to enhance the tendency for the pup to approach and stay in contact with the anesthetized adult. In contrast amphetamine induced locomotor was not inhibited by the anesthetized adult in 30 day-old rats. They also showed a dose dependent decrease in time spent in contact with the anesthetized adult. A second experiment tested more directly the ability of amphetamine to enhance behaviors directed toward the anesthetized adult in the fifteen day-old rats. Fifteen and 30 day-old rats were observed in the presence of a periodically moving stimulus animal. Fifteen day-old, but not 30 day-old subjects showed a dose related increase in the tendency to follow the anesthetized adult. These results suggest that in 15 day-old rats increases in arousal may be "canalized" into behaviors directed at conspecifics, and that the disappearance of the canalization reflects the changing neuronal and behavioral organization of the animal during development.

382 STIMULUS PROPERTIES OF d-AMPHETAMINE IN THE RAT: INTERACTION WITH THYROTROPIN-RELEASING HORMONE (TRH). Catherine N. Jones, Lester D. Grant, Arthur J. Prange, Jr. and George R. Breese. Neurobiology Program and Dept. Psychiatry, Univ. North Carolina, Chapel Hill, North Carolina 27514

Recent research has demonstrated that d-amphetamine and TRH have several pharmacological effects in common and that TRH potentiates some of the effects of d-amphetamine in the rat. The present study examined the possibilities (1) that TRH might have stimulus properties similar to other psychoactive drugs (i.e., that the hormone can act as a discriminative stimulus), (2) that the stimulus properties of TRH might be similar to those of d-amphetamine, (3) that TRH might potentiate the stimulus properties of d-amphetamine. Five groups of rats were trained on a two-lever operant discrimination using either d-amphetamine (0.8 or 1.6 mg/kg) or TRH (1, 5 or 10 mg/kg) vs. saline as cues. Subjects readily acquired the discrimination based on d-amphetamine vs. saline, but failed to acquire the TRH vs. saline discrimination. Following acquisition of the d-amphetamine discrimination, subjects were tested for generalization following administration of TRH alone or TRH + d-amphetamine. TRH alone (1, 5 or 10 mg/kg) produced responding predominantely on the lever previously reinforced under saline. TRH administered one hour prior to d-amphetamine (0.8 mg/kg) significantly increased the proportion of responses on the lever previously reinforced under d-amphetamine. These results indicate that TRH does not have stimulus properties at the doses tested, but that TRH can potentiate the stimulus properties of d-amphetamine. (This research was supported by NIMH Grant MH-11107, Alfred P. Sloan Foundation, Career Development Award MH-00013, NIH Grant HD-03110 and NIH Grant NS-09844.)

383 COMPARATIVE EFFECTS OF CHRONIC d AND l-AMPHETAMINE ON SELECTED MEMBERS OF A PRIMATE SOCIAL COLONY. R.F. Schlemmer, Jr., D.L. Garver, K.L. Preston*, and J.M. Davis. Ill. State Psychiat. Inst. & U. of Ill., Chicago 60612.

To study further the behavioral effects of d-amphetamine (d-A) and l-amphetamine (l-A), chronic equimolar doses of d-A and l-A were given to two selected members (α & β-females) of a stable social colony of five, feral, adult Stumptail macaques. In the first experiment, l-A, 1.5 mg base/Kg. in time-release form, was administered n.g. every 12 hours for 11 consecutive days. Following a 4 week wash-out period, d-A, 1.5 mg base/Kg in time-release form, was administered as in the first experiment. Behavioral observation occurred daily in the AM for 1 week prior to and throughout the 11 day treatment period in both experiments by the same "blind" observer. Both isomers produced stereotyped behavior as a major effect, inducing equal amounts of stereotyped behavior from day 5 of treatment until the end of each experiment. Both d-A & l-A produced similar effects in the distancing scores of the individual treated animals, the β-female (β) becoming isolated and the α-female (α) "shadowing" untreated animals. d-A & l-A both induced maximal activity scores in α & β. d-A induced hypervigilance in both α & β whereas l-A failed to alter vigilance scores in α & only induced marginal hypervigilance in β. Results of this study demonstrate that d-A & l-A are approximately equipotent in inducing abnormal, psychotic-like behavior in monkeys exemplified by equal induction of inappropriate and stereotyped behaviors and equivalent effects on distancing scores. However, d-A was more potent than l-A in inducing hypervigilance. It has previously been shown in this species that d-A induced hypervigilance is modulated by norepinephrine (NE) but stereotyped behavior is modulated by dopamine (DA). Results of this study appear to demonstrate that d-A & l-A are approximately equipotent on DA systems, but that d-A may be more potent than l-A on NE systems.

384 INTERACTIONS OF SEROTONIN AND CATECHOIAMINE ANTAGONISTS IN THE CONTROL OF ACTIVITY. Robert E. Davis, and Ernest W. Kent, Dept. of Psychol., Univ. of Illinois at Chicago Circle, Chicago, Illinois 60680.

Microinjections of 6-hydroxydopamine (6OH-Da), 8μg bilaterally, were made into areas A9 and A10 of the anterior mesencephalon following systemic administration (PCA) 1,2,5 mg/kg; parachlorophenylalanine (PCPA) 400 mg/kg; lysergic acid diethylamide (LSD) 50, 100, 150 mg/kg; cinnanserin (CIN) 15, 30, 60 mg/kg). Measurements were made of norepinephrine, dopamine (DA) and 5-hydroxytryptamine (5HT) levels in the striatum and basal forebrain as well as multiple aspects of activity. Administration of PCPA, LSD, and CIN were found to elevate emotionality and to decrease initiation and absolute level of general activity. PCA was found to elevate emotionality and decrease general activity at lower doseages (1, 2 mg/kg) while the highest dose (5 mg/kg) depressed activity initially (days 1-4) but enhanced activity over days 7-40. This biphasic response is interpreted in relation to possible permanent neurotoxic action of high doseages of PCA (Harvey, Science, 1974). Multivariate analysis (step down F) was used to study the interaction of DA and 5HT antagonists. In agreement with enhanced amphetamine produced activity following indoleamine antagonism (Green and Harvey, J. Pharm. Exp. Ther., 1974), DA and 5HT antagonists exert synergistic actions on locomotor activity.

385 SNIFFING BEHAVIOR ELICITED BY CHOLINERGIC (MUSCARINIC) RECEPTOR ACTIVITY IN THE RAT OLFACTORY TUBERCLE. Sarah F. Leibowitz and Jeffrey M. Falk*. The Rockefeller University, New York, NY 10021.

Using the technique of administering drugs directly into the brain, we have found that sniffing behavior in the rat can be dramatically altered by cholinergic (muscarinic) receptor activity in the olfactory tubercle. When administered into this region, the cholinergic stimulant carbachol has been found to elicit a vigorous sniffing response within the first minute after injection. This response, which may be directed at the cage or simply into the air, continues over a period of 15 to 30 minutes. Its magnitude and duration are found to be dose-dependent, with the lowest effective dose being approximately 10 ng. A strong sniffing response can also be observed with the anticholinesterase agent, physostigmine (0.6 and 3.0 μg free base) and with the putative cholinergic neurotransmitter, acetylcholine (5 μg acetylcholine chloride), in combination with physostigmine (0.2 μg free base). Little or no response has been observed with the compound nicotine, nor with the other putative neurotransmitters, dopamine, norepinephrine, epinephrine, serotonin, and histamine. The sniffing induced by carbachol has been found to be antagonized by local administration of the muscarinic receptor blockers, atropine and scopolamine, but unaffected (or even potentiated) by blockers of nicotine (mecamylamine), dopamine (haloperidol), alpha-adrenergic (phentolamine), beta-adrenergic (propranolol), serotonin (cinanserin), and histamine (brompheniramine) receptors. These data provide preliminary evidence for a cholinergic (muscarinic) link, in the olfactory tubercle, which participates in the mediation of sniffing behavior in the rat. (This research was supported by USPHS grant MH 13189 and by funds from the Grant Foundation.)

386 INTRAVENTRICULAR NOREPINEPHRINE AND DOPAMINE: DIFFERENTIAL EFFECTS ON SLEEP IN THE RAT. Ernest Hartmann and George Zwilling*. Boston State Hospital, Boston, 02124.

In a series of studies on a total of 19 male albino Norwegian rats we investigated the effects of small amounts (0.01 to 300 micrograms per animal) of norepinephrine (NE) and dopamine (DA) administered intraventricularly in the rat. In some studies phenoxybenzamine (PB) was administered orally, two hours before the IVT infusions. In one study normetanephrine effects were investigated.

A 26-gauge indwelling cannula was placed in a lateral ventricle at the same time that electrodes were implanted for sleep recordings. After recovery, animals were adapted to the recording situation and then were recorded continuously for up to 504 hours. On every recording day, the rats received an infusion of 30 ul CSF given over a ten-minute period. The solution contained NE, DA or simply artificial CSF (placebo).

Normetanephrine, 0.3 and 30.0 ug, had no effect. NE and DA had significant effects, almost entirely limited to the first two hours after administration. NE produced a dose related increase in waking (W), reaching 190% of control at 300 ug and a decrease in desynchronized sleep (D) to 40% of control at 300 ug. These effects were not blocked by PB 40 mg/kg orally. DA produced no significant effect on W but a significant increase in D-time, up to 170% of control. S-sleep was not altered by any of the amines administered.

387 BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF SELECTIVE BRAINSTEM LESIONS: Stanley Lorens, Christer Köhler[x], Bolek Srebro[x], and Hans Guldberg[x]. Dept. of Pharmacol., Psychol., and Physiol., Univ. of Bergen, MFH-Bygget, 5000 Bergen, NORWAY.

Electrolytic lesions were produced in either the dorsal raphe nucleus (DR), median raphe nucleus (MR), dorsal tegmental nucleus (DTG), ventral tegmental nucleus (VTG), or the pontine raphe area (B5,6). Open field activity, two-way (shuttle) conditioned avoidance (CAR) acquisition, and regional forebrain 5-hydroxytryptamine (5-HT) concentrations were examined 24-32 days postoperatively. Enhanced locomotor activity was observed only after the MR and VTG lesions. CAR acquisition was facilitated after the VTG, DTG, and B5,6 lesions. DR lesions did not affect either behavioral measure. The DTG lesions did not affect forebrain 5-HT, while B5,6 lesions lowered only thalamic 5-HT. The MR and VTG lesions reduced hypothalamic, thalamic, hippocampal, and neocortical 5-HT, suggesting that ascending 5-HT fibers from the MR penetrate the VTG as the latter nucleus does not contain 5-HT perikarya. Although the DR lesions did not affect the behavioral parameters studied, they did result in significant reductions in the 5-HT content of all forebrain regions assayed (including the amygdala-pyriform area and striatum), except for the hippocampus. Facilitated CAR acquisition was not related to regional 5-HT changes. Increased open field activity, however, may be associated with reductions in hippocampal 5-HT, although recent studies employing para-chlorophenylalanine and 5,7-dihydroxytryptamine do not readily support this conclusion.

388 ANTAGONISTIC ACTION OF TETRAHYDROAMINOACRIDINE(THA) AND OTHER CHOLINERGIC AGENTS TO N-METHYLPIPERIDYL PHENYLISOPENTYNYL GLYCOLATE(PB), A PSYCHOTOMIMETIC DRUG. K. Lowy, L.G. Abood, and Holly Raines*. Center for Brain Research, Univ. of Rochester Med. Center, Rochester, N.Y. 14642.

With the use of PB, a psychotomimetic agent possessing anticholinergic properties, a study was undertaken to determine the ability of various cholinergic agents to antagonize the central effects of the glycolate. Behavior was assessed in cats, using a computer-controlled program which measured various quantifiable parameters, such as the number of responses to an auditory stimulus, errors in sound localization, auditory threshold, and lateral preference for a right and left lever (Lowy et al, Neuropharmacol. 13, 707, 1974). PB, at doses as low as 5 µg/kg, markedly decreased the number of responses and significantly influenced lateral preference. THA, an anticholinergic-analeptic drug, counteracted the effect of PB on the number of responses but not on lateral preference. Nicotinic drugs, such as nicotine and piperidine, did not antagonize the actions of PB; furthermore, when the cats were pretreated with 2 mg/kg atropine, an antimuscarinic drug, there was no protection against PB. The antagonistic action of THA was evidently restricted to only a part of the brain functions affected by PB. The central effects of PB would not appear to involve only cholinergic mechanisms. (Supported by MH 20142 and Council for Tobacco Research)

389 THE EFFECT OF CHRONIC LEAD TREATMENT ON THE SPONTANEOUS AND EVOKED RELEASE OF ACH FROM BRAIN TISSUE. P.T. Carroll*, E.K. Silbergeld and A.M. Goldberg. Johns Hopkins University, School of Hygiene, Baltimore, Md. 21205

Mice chronically exposed to lead during initial periods of development demonstrate increased levels of spontaneous motor activity. The pharmacological responses to a number of drugs indicate a decrease in central cholinergic activity and an increase in monoaminergic activity. Studies utilizing peripheral nervous tissue have shown a decreased evoked and an elevated spontaneous release of ACh by lead. The possibility was therefore examined that both evoked and spontaneous ACh release in brain might be similarly altered. The results indicate that lead administration inhibits the K^+ depolarized release of both choline and ACh. The inhibition of Ch release is twice that of ACh. Administration of methylphenidate (40 mg/kg), previously reported to decrease the lead-induced hyperactivity reverses the inhibition of both K^+ induced choline and ACh release caused by lead. Omission of Ca^{++} significantly inhibits the K^+ depolarized release of ACh without significantly altering choline release. The spontaneous release of ACh in lead-treated animals was significantly increased from 67 to 97 nMoles/g/hr. Inhibition of K^+ induced release of ACh by lead may occur by two different mechanisms: 1) lead may reduce the availability of choline for ACh synthesis; 2) lead may antagonize the role of calcium in ACh release. Supported by NIEHS grants 00034 and 00454 PTC is recipient of a Smith, Kline and French Fellowship. EKS is a Joseph P. Kennedy Fellow in Neurosciences.

390 MECHANISMS BY WHICH QUIPAZINE ALTERS BRAIN 5-HYDROXYINDOLE METABOLISM. Jacob H. Jacoby*, Robert A. Howd*, Marc S. Levin* and Richard J. Wurtman (SPON: J.D. Fernstrom). MIT, Cambridge Mass. 02139.

Quipazine (2-[1-piperazinyl] quinoline) is a new potential antidepressant agent, which appears to act both peripherally and centrally as a serotonin (5-HT) agonist. In this study, quipazine (10 mg/kg i.p.) was shown to increase 5-HT and decrease 5-hydroxyindoleacetic acid (5-HIAA) levels in the whole brain, several brain regions, and the spinal cord 1 hr after its administration to rats. In animals with transected spinal cords, quipazine induced extensor reflex activation, similar to that observed following 5-hydroxytryptophan (5-HTP) administration; neither chlorimipramine nor Lilly 110140 (which block 5-HT reuptake) produced this effect. These effects of quipazine and 5-HTP could be blocked by methiothepin. In slices of rat cerebral cortex, quipazine inhibited uptake of H^3-5-HT (EC_{50} 10^{-6}M) and H^3-norepinephrine (EC_{50} 2 x 10^{-6}M). Quipazine had no *in vivo* monoamine oxidase inhibitory activity. It was equipotent with Lilly 110140 in inhibition of 5-HT uptake, and less potent than chlorimipramine (EC_{50} 10^{-7}M). These observations suggest that quipazine directly activates serotonin receptors in the central nervous system; however, additional mechanisms, including an inhibition of amine uptake, may contribute to its *in vivo* activity.

(This study was supported by USPHS grant #AM 14228 and NIMH Fellowship #NS54909.)

391 EQUAL CONCENTRATIONS OF 3H-5-HYDROXYTRYPTAMINE AND 3H-TRYPTAMINE AFTER INJECTION OF 3H-L-TRYPTOPHAN INTO HIPPOCAMPUS. H. Jackman and M. Radulovački. Dept. Pharmacol., Univ. Ill. Med. Ctr., Chicago, Ill. 60612.

Administration of L-tryptophan (TRYP) to laboratory animals increases brain 5-hydroxytryptamine (5HT) and, to a lesser extent, tryptamine (TRYPT) (Axelrod and Saavedra, 1974). The findings in these reports deal mainly with whole brain analysis without describing the proportion of newly synthesized 5HT to TRYPT in specific brain structures. In this study we attempt to find the proportion of newly synthesized 5HT to TRYPT in the hippocampus (HIPP) after local administration of TRYP.

3H-L-TRYP (330 ng in 5 µl) was injected into the ventral HIPP of cats via an implanted cannula guide. HIPP extracellular fluid was sampled using a push-pull technique modified from Myers (1972). Our modification allowed discrete 50-µl samples of extracellular fluid to be removed at distinct points in time. The HIPP fluid was assayed for 3H-labeled 5HT, 5-hydroxyindole-3-acetic acid (5HIAA), TRYPT and indole acetic acid (IAA). Thin-layer chromatography was used for separation and liquid scintillation counting for measuring metabolite concentrations. Samples were removed every 30 min for 3 hr starting 15 min after 3H-L-tryptophan injection. The results show labeled 5HT and TRYPT appear in approximately equal concentrations at each test point with decreasing concentrations through the 3-hr test period. Labeled 5HIAA and IAA were also found in equal concentrations at each test point, decreasing over the total test period. However, 3H-5HIAA and 3H-IAA concentrations were three times the concentrations of 3H-5HT and 3H-TRYPT.

These data raise the question as to whether administration of TRYP produces effects predominantly through increased 5HT metabolism, or also through increases in tryptamine metabolism. (Supported by PHS Grant NS 10921)

392 THE EFFECT OF BENZODIAZEPINES ON BOTH ACTIVATED AND ACTIVATOR DEFICIENT PHOSPHODIESTERASE. Richard Lehne, Erminio Costa and Petko Uzunov. (SPON: N. H. Neff). Laboratory of Preclinical Pharmacol., NIMH, Saint Elizabeths Hospital, Washington, D.C.

Diazepam (1×10^{-5}M) augments the increase in cyclic AMP content elicited in bullfrog sympathetic ganglion by preganglionic stimulation (Suria et al., 1974). We decided to measure the inhibition of a purified high Km phosphodiesterase (PDE) by diazepam and related benzodiazepines (BD) in the presence and absence of PDE activator (PDEA). PDEA lowers the Km of PDE using cyclic AMP as a substrate. Ki values were determined for Diazepam and 10 related analogues. These values were compared with those determined for hexobarbital, phenetharbital, diphenylhydantoin and aminophylline.

The Ki values of diazepam for both cyclic AMP and cyclic GMP PDE were 70 µM in the absence and 40 µM in the presence of PDEA. These values were about 5 fold below the Km of the PDE for cyclic AMP and were about 7 fold above the Km of PDE for cGMP. Diazepam is a competitive inhibitor of PDE using either substrate, both in the absence and presence of PDEA. The Ki values for activator deficient PDE using cyclic AMP as a substrate were 12,000 µM for hexobarbital and phenetharbital, 944 µM for diphenylhydantoin and 233 µM for aminophyllin. The addition of PDEA fails to lower the Ki values of the barbiturates and raises the Ki of aminophylline to 544 µM.

We conclude that diazepam unlike aminophylline is a more potent inhibitor of an activator-saturated PDE hydrolysing cyclic AMP.

The Ki values and the character of the inhibition of 10 other related BD were studied under the same conditions.

393 CHOLINERGIC MECHANISMS AND SHORT-TERM MEMORY IN THE MONKEY. Raymond T. Bartus and H. J. Johnson*. Psychopharmacology Section, Parke-Davis Res. Labs., Ann Arbor, Mi. 48106

Recent evidence suggests that cholinergic mechanisms are directly involved in the expression of rodent short-term memory (STM). Yet in work with non-human primates several authors failed to confirm this involvement. Using the delayed-matching-to-sample (DMS) procedure to measure STM, the anti-cholinergic agent scopolamine showed no specific effects on STM. That is, the detrimental effects of scopolamine were no greater at longer retention intervals (when STM mechanisms are presumably more important) than they were at the shorter intervals (when efficient STM is presumably not necessary). Thus, it was generally concluded in the primate studies that acetylcholine is not directly involved in STM, despite the contrary evidence reported for rodents.

However, potential methodological problems inherent in the procedures used with primates might have been responsible for the differences reported. The most serious is that in order to successfully perform the DMS task, not only is effective STM required, but also a great deal of sensory discrimination. It is conceivable that if sufficient impairment of discriminatory ability occurs with scopolamine, any effects on STM, might be sufficiently obscured so that they are not measured.

A preliminary study was therefore conducted in which 8 Rhesus monkeys were trained on visual discrimination problems and then tested under several doses of scopolamine, as well as saline, sodium pentobarbital, and chlorpromazine. It was found that scopolamine did indeed effectively disrupt performance of highly trained discrimination tasks at relatively mild doses (as low as 0.015 m/k). For this reason, investigations were conducted to re-evaluate the effects of scopolamine on STM.

An automated indirect delayed response (DR) procedure was used. This computed-controlled apparatus was designed to minimize some of those variables which potentially confound an accurate assessment of primate memory capabilities. Furthermore, the DR procedure has the additional advantage of not requiring nearly as much sensory discrimination as does the DMS, since the monkey need only perceive where the conditioned stimulus appears and remember this position during the retention interval. The DMS on the other hand, requires the monkey to encode which of two or more possible stimuli occurred before the delay and then discriminate among the alternatives when the retention interval terminates. Thus, it was hoped the DR test might provide a more accurate assessment of the effects of scopolamine on STM, unconfounded by its effects on discrimination.

Three naive Rhesus monkeys were trained to observe the conditioned stimulus (green light) which was presented on one of nine stimulus-response panels (arranged in a 3 X 3 matrix) and then respond to that panel sometime later, when the opportunity occurred. Retention intervals of 0, 2.5, 5, and 10 sec were generated in a quisi-random manner to control for possible interactions of drug metabolism, fatigue, etc., with delay. Two doses of scopolamine (from 0.01 to 0.05 m/k), and several control days were run.

It was found that, contrary to earlier reports using the DMS procedure, a clear interaction of drug and retention occurred in this situation. Under scopolamine, greatest impairments occurred on the longest delays, with little or no effect with zero second retention. Furthermore, the impairments observed on the longer delays were even greater with the highest dose of scopolamine. These data therefore lend strong support to the notion that cholinergic mechanisms play an important role in the expression of STM in primates.

394 CENTRAL ACTION OF SOMATOSTATIN: ROLE OF HIPPOCAMPUS. Milan Rezek* Viktor Havlicek, Kenneth R. Hughes*, and Henry Friesen*. Dept. of Physiology, University of Manitoba, 770 Bannatyne Ave., Winnipeg, Man., R3E 0W3.

We have previously reported that intraventricular (IV) infusion of somatostatin (SRIF) in normal and hypophysectomized rats considerably alters the sleep-waking pattern and markedly changes behavior and motor function. In an effort to identify the brain structure which might be mediating the effect of SRIF we have selected the hippocampal formation for the initial cerebral tissue infusions. Several doses of SRIF (0.01,0.1,1.0,5.0, and 10.0 μg/ml/min) were infused into the dorsal hippocampus and dental gyrus (DH: A-3.5,L-2.0,V-2.5; DG: A-2.0,L-4.5,V-4.5) of the unrestrained, freely moving rats implanted with chronic infusion cannulas whose tips were used as monopolar electrodes for recording local EEG changes at the site of infusion. The animals were also implanted with epidural cortical and subcutaneous muscle (m. masseter)electrodes and were monitored for behavioral, motor and EEG changes before and after the administration. Infusion of 0.01 μg produced a freezing response in most of the animals shortly before the end, and for a limited period after the infusion. The freezing response was often associated with the quiver of the lower jaw and the tremor of the m. masseter. With increasing dose (0.1 μg) the animals initially displayed a similar pattern of behavioral changes but freezing and subsequent sniffing and horizontal exploration were accompanied by stereotyped manifestations such as chewing and biting which recurred at various later periods. Also, a moderate reduction in REM sleep and the first signs of EEG dissociation from behavior (high voltage, hypersynchronized or flat almost isoelectric EEG pattern in waking animals) were observed, especially in DH animals. The increase in intensity and duration of freezing, tremor and oral stereotypy along with additional manifestations of stereotype behavior (scratching, stepping, tail arching) and first signs of coordination difficulties was produced by 1 μg of SRIF. Voracious and prolonged eating was observed in 66% of DH animals. This was the only infusion site and dose at which this response was most frequently observed. The duration of REM sleep was further reduced (1st hour - to 21.2 % of control value after DG and to 12.8% after DH infusion) while EEG dissociation lasted longer. A dose of 5 μg markedly aggravated coordination difficulties and often resulted in a loss of balance. The animals were only marginally asleep and seldom progressed into a REM sleep. The pattern of EEG dissociation was further prolonged and amounted to 20.3% and 40.3% of total waking time in DG and DH animals respectively and also occurred during the sleep period. All manifestations were more apparent following DH infusions. At a dose of 10 μg, the coordination difficulties developed into a contralateral paraplegia-in-extension (80% of DH animals) and in one case prolonged rolling seizures were observed. These severe motor pathologies were correlated with a flat, EEG pattern interrupted by episodes of high voltage, hypersynchronized and spike discharge EEG pattern. Paraplegia or seizures did not develop after DG infusions although all these animals experienced severe coordination difficulties. The total time of EEG dissociation represented more than 50% of the total waking time and more than 40% of sleeping time (DH infusions). The REM and slow-wave sleep were considerably reduced following DG infusions and often eliminated for the entire observation period after DH infusions. In contrast, the administration of four cyclic SRIF analogues in a dose (5 μg) at which SRIF produced major behavioral, motor and EEG changes had no observable effect on any of these variables. These results provide further evidence for the central non-endocrine action of hypothalamic peptides.

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395 REVERSAL OF THE EFFECTS OF ANTIANXIETY AGENTS USING BLOCKERS OF γ-AMINOBUTYRIC ACID (GABA) R.W. Piwonka, J.F. Healey*, and P.C. Canniff*
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A common link may exist between antianxiety agents and GABA: 1) the benzodiazepines and barbiturates enhance primary afferent depolarization; 2) GABA and diazepam effect similar changes on cerebellar cGMP levels (Costa et al., Fed. Proc. 34: 298, 1975). If antianxiety agents act through GABA-mediated systems, then GABA blockers should reverse the effects of these agents, whereas blockers of other central inhibitory systems should not reverse those effects. Several antianxiety agents potentiate with tetrabenazine to cause loss of righting reflex in mice placed on their backs. Diazepam, 1 mg/kg i.p., followed after 30 min by a standard 100 mg/kg i.p. tetrabenazine dose produced a loss of righting in 96% of mice tested. Subcutaneous administration of picrotoxin, 2.0 to 5.0 mg/kg, or bicuculline, 1.1 to 2.3 mg/kg as putative synaptic GABA blockers; or 3-mercaptopropionic acid, 21 to 43 mg/kg as an inhibitor of GABA synthesis restored righting in a dose-dependent fashion to these mice. Strychnine, 0.4 to 1.1 mg/kg failed to restore righting in any mice pretreated with diazepam and tetrabenazine (n=50). When the dose of GABA antagonists was fixed, and the diazepam dose ranged from 0.6 to 2.0 mg/kg, that reversal of the loss of righting by the GABA antagonists was then dose-dependent upon diazepam. Other antianxiety agents behaved in a similar fashion to diazepam. Chlormezanone, 90 mg/kg, with tetrabenazine produced loss of righting ability in 95% of the mice tested. The GABA antagonists, but not strychnine, restored righting ability over a similar dose range. Phenobarbital, 45 mg/kg, and tetrabenazine produced in 96% of the mice loss of righting; and restoration of righting was produced by the GABA antagonists, but not by strychnine. The data support the concept that antianxiety agents use a common mechanism which involves GABA.

396 TAILS OF EATING, DRINKING, SEX AND MATERNAL BEHAVIOR: A NIGROSTRIATAL DOPAMINE STORY. Seymour M. Antelman*, Henry Szechtman*, Neil Rowland* and Anthony R. Caggiula*. (SPON: E. B. Goldstein). Dept. Psychiatry, Western Psychiatric Inst. and Clinic, Univ. Pittsburgh, Sch. Med., and Psychobiology Program, Dept. Psych., Univ. Pittsburgh, Pittsburgh, Pa.15260

We have recently discovered that a mild, seemingly nonpainful pinch to the tail of a rat can induce biologically significant consummatory behaviors. The particular behavior which emerges as a function of the pinch depends on the available goal objects. For instance, food sated animals display eating in the presence of pellets & drinking in the presence of a tube containing milk, or another palatable (though not necessarily nutritive) solution. In the presence of rat pups, tail-pinch induces maternal behavior, whereas the presence of a receptive female induces copulation in naive male rats. In addition to the foregoing, we have also shown that tail-pinch can induce hyperphagia and obesity in normal rats & recovery from the aphagia which accompanies lateral hypothalamic lesions. Similarly, we have successfully reversed the copulatory deficits seen in male rats following the administration of 6-hydroxydopamine. Other behavioral experiments in our laboratory indicate remarkable parallels between tail-pinch-induced behaviors & similar behaviors seen following electrical brain stimulation.

Tail-pinch-induced behaviors occur with exceptional reliability, having now been demonstrated (typically within seconds) in virtually every one of approximately 1000 animals tested to date. A pharmacological analysis of the tail-pinch phenomenon suggests that it is critically dependent on the integrity of the nigrostriatal dopamine system.

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397 BEHAVIORAL CHANGES IN SELECTED MEMBERS OF A PRIMATE SOCIAL COLONY AFTER ACUTE & CHRONIC ADMINISTRATION OF PHENCYCLIDINE (PCP). J.P. Bederka, Jr.*, R.F. Schlemmer, Jr., J.A. Jackson*, K.L. Preston*, & J.M. Davis, U. of Ill., & Ill. State Psych. Inst., Chicago, Illinois 60612.

PCP is a psychotomimetic agent which has seen common street-drug usage, both alone & as an adulterant, & has been reported to cause schizophreniform psychoses in some users. Yet, the behavioral pharmacology of this agent has not been rigorously investigated. In this study, the behavioral effects of acute & chronic dosing with PCP in two selected members of a primate social colony of five, feral, adult Stumptailmacaques were examined. In the 1st experiment, acute, dose-dependent behavioral changes in doses of 25-1000 µg (base)/Kg were studied. In the subsequent experiment, the effects of chronic PCP administration, 50 µg/Kg daily for 2 weeks were studied. Behavioral observation by a "blind" observer occurred for one hour, beginning 15 minutes after i.m. administration of PCP. Baseline values (B) were determined prior to each experiment with normal saline being substituted for PCP. The 1st experiment revealed that PCP, in general, induced three dose-dependent states. At the lower dose, 25 µg/Kg, PCP induced an increase in self-grooming with a decrease in social activity when compared to B. Between 50 & 100 µg/Kg, PCP induced ataxia increased huddling & resting with eyes open, & decreased social activity. Animals treated with higher doses of PCP, 500-1000 µg/Kg, were immobilized by the drug, continuously stared into space, & failed to respond to any attempt at social interaction by control animals including repeated threats & attacks by controls. Preliminary results from the chronic administration of PCP indicate consistently decreased social activity with increased huddling & resting with eyes open compared to B. Furthermore, non social stereotyped behaviors developed in the chronically treated animals. None of the chronic PCP induced behaviors show evidence of tolerance. These results indicate that PCP induces abnormal behavioral changes in monkey social colonies which may be useful in studies on this and related psychotomimetic agents.

398 EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL ON CA-1 FIELD POTENTIALS FROM RAT HIPPOCAMPUS. Richard M. Vardaris, Dept. Psych., Kent State University, Kent, Ohio 44242, and Timothy J. Teyler, Dept. Psych. Soc. Rel., Harvard University, Cambridge, Mass. 02138.

It has been reported that delta-9-tetrahydrocannabinol (THC, the major active principle of marihuana) may enhance the amplitude of gross evoked responses at various loci in the brain. However, little is known concerning the parameters and underlying mechanisms of this enhancement.

Preliminary evidence has indicated that the drug can potentiate retrograde amnesia in rats by enhancing electrically-elicited hippocampal spike-and-wave activity. Since the functional organization of the hippocampus is reasonably well understood this structure provides a good model system for studying the effect of THC on the brain.

The intact hippocampal preparation utilized unanesthetized, paralyzed rats with suction aspiration of the tissue overlying the hippocampus. Extracellular population responses of the CA1 pyramids were elicited by antidromic (alveus) and orthodromic (Schaffer collaterals) stimulation within lamellae. Input-output functions were determined for each model of stimulation and, in most experiments, were repeated with the recording electrode advanced into the dendritic layer of CA1 to obtain a population EPSP.

After establishing these levels of responding, 2 mg/kg THC was injected intraperitoneally as an aqueous suspension in the presence of polyvinylpyrrolidone (PVP). After 30 min, post-drug input-output functions were obtained for the antidromic and orthodromic CA1 response. In parallel control experiments the same procedures were followed but only the PVP vehicle was administered.

It was found that THC affected both orthodromically and antidromically activated CA1 population potentials. The slope of the input-output functions for EPSP amplitude was increased such that, at asymptote, the post-drug response was more than a millivolt greater than the pre-drug level. There was no apparent effect on threshold. A similar pattern of change was obtained in the antidromic input-output function for amplitude. Latencies of both orthodromic and antidromic responses increased by several milliseconds. It was concluded that THC enhanced CA1 field potentials and that the effect was probably post-synaptic. The increased latency could be explained by the highly lipophilic drug having a direct effect on myelin. The vehicle alone (PVP) had no apparent effect on amplitude or latency, as the amplitudes simply declined slightly as a function of time and deteriorating physiological status.

The above phenomena are being studied in the hippocampal explant, a preparation well suited to the investigation of drug effects in neuronal systems as the interpretation of results is not complicated by the presence of pharmaceutical agents. The results of the explant experiments appear to confirm those obtained in the intact preparation.

Future experiments will investigate the drug's effects in other monosynaptic and polysynaptic circuits within the lamella. In addition the mechanisms of amplitude enhancement and latency increase will be sought.

399 SUBSTRATE PREFERRED INHIBITION OF BRAIN MONOAMINE OXIDASE BY SELECTED NEUROAMINE-DERIVED ALKALOIDS. L.R. Meyerson, K.D. McMurtrey* and V.E. Davis. Baylor Col. of Med. and VA Hosp., Houston, TX 77211.

Studies were conducted to examine the effects of tetrahydropapaveroline(THP), salsolinol(SAL) and various hydroxylated and methoxylated tetrahydroprotoberberine alkaloids on monoamine oxidase(MAO) forms A and B in rat brain homogenates. The substrates utilized were serotonin, a specific substrate for type A MAO; tyramine, a substrate for both type A and B MAO; and benzylamine, a preferred substrate for type B MAO. The concentrations of THP, SAL, 2,3,9,10-tetrahydroxyberbine and 2,3,10,11-tetrahydroxyberbine producing 50% inhibition (I50) of the oxidation of serotonin were 1.0mM, 0.25mM, 0.24mM and 0.04mM, respectively. However in marked contrast, the (I50) concentrations of these alkaloids with benzylamine as substrate were 4.4mM, 50mM, 5.6mM and 13mM, respectively. These findings indicated that SAL and the tetrahydroxyberbines were substrate preferred inhibitors of type A MAO whereas THP was a nonspecific inhibitor of rat brain MAO. Kinetic data revealed that THP, SAL and 2,3,10,11-tetrahydroxyberbine inhibited the oxidation of serotonin in a typical competitive manner with apparent K_i values of 0.82mM, 0.11mM and 0.05mM, respectively. When benzylamine was utilized as substrate, 2,3,10,11-tetrahydroxyberbine was a competitive inhibitor with an apparent K_i value of 3.8mM. THP and SAL noncompetitively inhibited benzylamine oxidation with apparent K_i values of 5.0mM and 52mM, respectively. When the hydroxyl groups at the 2,3,9,10, and 11 positions of the berbine ring system were sequentially replaced by methoxyl groups the potency and selectivity of their MAO inhibitory property declined markedly. Thus, the interaction of these alkaloids with the metabolic pathways of neurotransmitters suggests that these compounds may be of relevance in the modification of central synaptic function. (Supported by USPH Grant #AA-00226).

400 SOME NEUROBIOLOGICAL ASPECTS OF 6-METHOXY-1,2,3,4-TETRAHYDRO-β-CARBOLINE IN CF1 MICE. Neil S. Buckholtz, William O. Boggan, and Jerrold S. Meyer. Dept of Psychiatry and Behav. Sci. and Dept of Biochem., Medical University of South Carolina, Charleston, S.C. 29401

6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THBC) has been reported to elevate levels of brain serotonin (5-hydroxytryptamine, 5HT) approximately two-fold within two hours after injection without affecting levels of norepinephrine (McIsaac et al., J. Neurochem., 1972, 19, 1203-1206). We have replicated this finding in our laboratory, have attempted to determine the mechanism for the 5HT elevation, and have characterized some endocrinological and physiological effects of 6-MeO-THBC. Two possible mechanisms for the elevation of brain 5HT are inhibition of monoamine oxidase (MAO) and inhibition of reuptake of 5HT. We examined both of these in vitro. 6-MeO-THBC had about half the ability to inhibit MAO as pargyline at concentrations of 10^{-5}-10^{-6} M. At concentrations of 10^{-6}-10^{-7} M, 6-MeO-THBC inhibited uptake of radioactively labeled 5HT into a crude homogenate, but inhibition was much less than that of either chlorimipramine or Lilly 110140 which are more selective inhibitors of 5HT uptake. These results suggest that at least part of the elevation of 5HT seen after 6-MeO-THBC may be attributable to the MAO and reuptake inhibiting properties of 6-MeO-THBC.

6-MeO-THBC (100 mg/kg, i.p.) increased plasma corticosterone approximately three-fold over control levels at one hour after injection. 6-MeO-THBC (100 mg/kg) was also effective in decreasing rectal temperature, the maximum decrease occurring one hour after injection. These data show that 6-MeO-THBC has effects on several neurobiological systems and these effects may be mediated through 5HT.

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401 CYSTEINE AND DOPA AS INHIBITORS OF THE FORMATION OF TETRAHYDRO-β-CARBOLINES BY 5-METHYLTETRAHYDROFOLIC ACID-MEDIATED ENZYME. R.-L. Lin* and N. Narasimhachari. Galesburg State Research Hospital, Galesburg, Illinois 61401.

It was demonstrated in our earlier communication that the enzymatic reaction products of the indolethylamines obtained with 5-methyltetrahydrofolic acid (MTHF) differ from those obtained with S-adenosylmethionine (Lin and Narasimhachari, Res. Commun. Chem. Pathol. Pharmacol. 8: 535, 1974). The separation of these two enzymes has also been reported (Lin and Narasimhachari, Biochim. Biophys. Acta, 385: 268, 1975). Other investigators have now reported the formation of tetrahydro-β-carbolines, such as tryptoline (Barchas et al., Arch. Gen Psychiatry, 31: 862, 1974; Meller et al., Science 187: 171, 1975) or pyridoindoles (Mandel et al., Science 186: 741, 1974) as the major product of the MTHF-mediated enzymatic reaction with indolethylamines. It has been suggested that enzymatically formed formaldehyde (HCHO) condenses with indolethylamines nonenzymatically to produce tetrahydro-β-carboline. We report in this communication results of trapping of HCHO enzymatically formed from MTHF as well as condensation experiments with ^{14}C-HCHO and these studies confirmed the suggested mechanism. Cysteine reacts rapidly with HCHO to form thiazolidine-β-carboxylic acid (Ratner and Clarke, J. Am. Chem. Soc., 59: 200, 1937), and is a better trapping agent for HCHO than indolethylamines tested, but is also superior to dimethylcyclohexandione and semicarbazide, the reagents most commonly used. We found that because of this easy and rapid reaction, cysteine inhibited the formation of tetrahydro-β-carbolines from indolethylamines whether yielded enzymatically with MTHF or nonenzymatically with HCHO. A similar inhibition by compounds containing SH-group, such as homocysteine, and penicillamine as well as dopa was observed. Data on relative rates of inhibition by various compounds will be presented.

Thiazolidine-β-carboxylic acid methyl or ethyl ester was identified by GC-MS as the product when cysteine methyl or ethyl ester was incubated with MTHF in the presence of the enzyme. The results also showed that the inhibition of the formation of tetrahydro-β-carbolines by cysteine and dopa resulted from competition for HCHO formed enzymatically from MTHF. The substrate specificity reported by several investigators merely represents differences in the rates of condensation between HCHO and various amines. It is unlikely that the formation of tetrahydro-β-carbolines in vivo occurs in tissues where the relative concentration of cysteine is much higher than that of indolethylamines. This MTHF-mediated HCHO-forming enzyme might regulate the level of MTHF in tissues rather than to serve as the HCHO donor for the formation of tetrahydro-β-carbolines.

402 5-METHOXY-N,N:DIMETHYLTRYPTAMINE (5 MeO-DMT) SHARES INHIBITORY EFFECTS WITH MESCALINE BUT NOT EXCITATORY EFFECTS ON SHUTTLEBOX ESCAPE/AVOIDANCE IN RATS David M. Stoff, J. Christian Gillin and Richard J. Wyatt. Laboratory of Clinical Psychopharmacology, NIMH, IRP, St. Eliz. Hosp., Wash., D.C. 20032.

5 MeO-DMT, a rapidly acting psychedelic agent, has been implicated as a potential endogenous schizogen. It is reportedly found in urine of schizophrenics, correlated with clinical exacerbation of symptoms, and can be synthesized from 5-methoxytryptamine, a constituent of rat brain and human cerebrospinal fluid. We studied the behavioral effects of 5 MeO-DMT and mescaline on acquisition of shuttlebox escape/avoidance in four different strains of rats: (1) Long-Evans hooded; (2) Sprague-Dawley/Zivic-Miller (ZM); (3) Fischer 344/Mai (F344);and (4) Roman High Avoiders (RHA). One min after 5 MeO-DMT, 10 mins after mescaline or saline (ip) rats were given a 100 trial acquisition test in the shuttlebox. Each rat was retested for 100 trials under saline 24 hrs later. Results were analyzed in terms of percentage avoidance responses over the initial 100 trial test as well as the retest: (1) in hooded rats, 5 MeO-DMT (0.25-4.0 mg/kg) had no effect but mescaline (40.0 mg/kg) had an excitatory effect (i.e., increased % avoidance responses) on the initial acquisition test; 5 MeO-DMT (8.0-16.0 mg/kg) induced toxic effects (e.g., forelimb tremors, posterior muscle spasms, shivering, abnormal gait and posture, backward locomotion; (2) in ZM, 5 MeO-DMT (4.0 mg/kg) had an inhibitory effect (i.e., decreased % avoidances) and mescaline (40.0 mg/kg) had an excitatory effect; both effects were not present on retest; (3) in F344, 5 MeO-DMT (0.5-4.0 mg/kg) and mescaline (10.0-40.0 mg/kg) had inhibitory effects which disappeared for 5 MeO-DMT but remained for mescaline on retest; and (4) in RHA, neither 5 MeO-DMT (4.0 mg/kg) nor mescaline (40.0 mg/kg) influenced avoidance. For saline rats, there were marked differences in avoidance performance with RHA>F344>ZM>Long-Evans. Thus, 5 MeO-DMT was ineffective in rats who were poor avoidance performers (i.e., Long-Evans hooded, ZM) while mescaline was excitatory in these strains; both 5 MeO-DMT and mescaline shared dose-response inhibitory effects in rats who were good avoidance performers (i.e., F344). These results for 5 MeO-DMT are similar to previous data reported for N,N:Dimethyltryptamine (DMT): neither of the tryptamine derived agents possess excitatory effects on shuttlebox escape/avoidance, contrary to mescaline and LSD, while all these drugs share inhibitory effects (Stoff et al., Psychopharm. 1974, 36, 301; Soc. for Neurosci., 1974, 4th Ann. Mtg). If excitatory effects on shuttlebox escape/avoidance are mediated by catecholamines (CA) and inhibitory effects via serotonin, then 5 MeO-DMT and DMT's inability to produce excitation may be related to its lack of effect on CAs (Anden et al., J Pharm. Exp. Ther., 1971, 179, 236) whereas the ability to stimulate serotonergic receptors in shared by 5 MeO-DMT, DMT, LSD and mescaline (Aghajanian et al., J Pharm. Exp. Ther., 1970, 1971, 178; Fuxe et al., Eur. J. Pharm., 1972, 19, 25).

403 3-METHOXYTYRAMINE, MESCALINE, N',N'-DIMETHYLTRYPTAMINE: EFFECTS OF INJECTION INTO THE NUCLEUS ACCUMBENS AND NEOSTRIATUM OF RATS. Russell E. Dill, Roy L. Dorris* and Ingrid Thonnard-Phillips*. Depts. MicroAnat. & Pharmacol., Baylor Dent. Col., Dallas, 75226.

Male albino rats were bilaterally cannulated in the caudate/putamen and nucleus accumbens. Subsequent unilateral intrastriatal (i.s.) injection of two hallucinogenic drugs, mescaline and N',N'-dimethyltryptamine, and a metabolite of dopamine, 3-methoxytyramine (3-MT) produced contralateral choreiform movements. The bilateral injection of each of these compounds into the nucleus accumbens produced catalepsy. Pretreatment (12 hrs) with an MAO inhibitor, pheniprazine (5mg/kg i.p.) significantly ($P<0.01$) enhanced the motor effects of i.s. 3-MT. Pretreatment (2 hrs) with haloperidol (5mg/kg i.p.) blocked the effects of i.s. 3-MT and significantly ($P=0.02$) enhanced the motor effects of i.s. carbachol. These data support the hypothesis that a defect in brain MAO could lead to the shunting of dopamine metabolism to its O-methylated metabolite, 3-MT, with consequent alteration of brain function such that psychotic symptoms could appear.

404 CHARACTERIZATION OF THE UPTAKE OF 3-METHOXYTYRAMINE (3-MT) BY BRAIN TISSUE IN VITRO. J. H. Gordon and M. K. Shellenberger. Department of Pharmacology, and Kansas Center for Mental Retardation, Kansas University Medical Center, Kansas City, Kansas 66103.

Since 3-MT is known to be physiologically active, the accumulation of this compound in drug-induced or naturally occuring toxic states may be of importance both experimentally and clinically. To date the means by which the brain handles this dopamine (DA) metabolite has not been characterized. This study has examined one aspect by utilizing tritiated 3-MT in synaptosomes from rat striatum and slices of both striatum and cerebellum. No accumulation of 3-MT could be demonstrated in synaptosomes but DA (0.1 µM) uptake was inhibited by 3-MT ($IC_{50} = 1.3 \times 10^{-5}$M). Studies in brain tissue slices showed: 1) 3-MT accumulation was active at a high concentration (10 µM) of amine; 2) the accumulated amine was easily washed out of the tissue slice; 3) the uptake was saturable with high values for K_m and V_{max}; 4) agents, such as metanephrine, which inhibit peripheral extraneuronal amine uptake had similar effectiveness against 3-MT uptake. It was concluded that: 1) the mechanism for the accumulation of 3-MT is similar in structurally different areas of the brain; 2) the 3-MT accumulation in the brain is similar to the extraneuronal accumulation of amines in the periphery; 3) the 3-MT is accumulated by a mechanism which is not a part of the nerve terminal. (Supported by USPHS Grants MH 21405, GM 403 and K02-MH-70184).

405 ABSENCE OF TOLERANCE AND CROSS-TOLERANCE TO THE STEREOTYPE-INDUCING EFFECT OF AMPHETAMINE IN THE RAT. M. Marlyne Kilbey and Everett H. Ellinwood, Jr. Box 3838, Duke University Medical Center, Durham, N. C. 27710 .

Previous investigations of tolerance to amphetamine effects produced contradictory results (Hug, 1972: Kalant, et al., 1971). Recently Segal and Mandell (1974) reported that chronic administration of amphetamine produced increasingly augmented locomotion (0.05 mg/kg) and stereotyped behavior (2.5 mg/kg), i.e., reverse tolerance. In Study 1, to further investigate this phenomena ten female rats received daily i.p. injections of amphetamine, increasing systematically from 4.0 to 9.4 mg/kg over a 10-day period. Nine Ss received saline daily. On Day 11 Ss were tested with 4.0 mg/kg d-amphetamine. On Days 1 and 11, behavioral ratings were made using a rating scale previously described (Ellinwood and Balster, 1974). While amphetamine induced stereotypies ($p \leq 0.001$); these did not differ from Day 1 to Day 11 for the group administered amphetamine daily. Cross-tolerance was measured in a similar paradigm with daily doses of l-amphetamine ranging from 10.0 mg/kg (Day 1) to 23.6 mg/kg (Day 10) administered to 13 rats and saline to 12 rats. On Day 11, half of each group received 3 mg/kg d-amphetamine while the remaining Ss received 6 mg/kg d-amphetamine. Behavioral ratings were taken. The pretreatment factor was non-significant ($p \leq 0.07$), while the amphetamine dose levels differed significantly ($p \leq 0.001$). These data do not support a hypothesis of reverse tolerance to amphetamine-inducing stereotyped behavior effects.

406 THE EFFECTS OF ROUTE OF ADMINISTRATION AND NALOXONE PRETREATMENT ON THE FREQUENCY AND MODE OF DEATH OF MORPHINE IN THE RAT. N. R. Kramarcy and D. R. Meyer (SPON: R. P. Smith). Neuropsychopharmacology Program, University of Louisville, Louisville, Ky. 40208.

The administration of a single dose of morphine (MS) to drug naive organisms typically results in a profile of pharmacologic responses, which include both depressant and stimulant actions. Previous reports suggest that the typical action of MS in the rat is biphasic: initial depressant action is followed by stimulation. This phenomenon is dependent upon dose as well as route of administration. Generally at low doses, MS stimulant activity predominates, and at high doses MS depressant activity predominates. Route of administration is also critical. Intraperitoneal (ip) or intravenous (iv) administration promote MS depressant activity, whereas subcutaneous (sc) administration promotes MS stimulant activity. Pretreatment with many narcotic antagonists such as naloxone and naltrexone have all been reported to antagonize MS depressant but not MS stimulant activity. To further ascertain the influence of route of administration and narcotic antagonist pretreatment in MS pharmacologic activity, several experiments were conducted to examine the effects of these factors on responsiveness to lethal challenges of MS.

In the first experiment, two groups (grp) of male Sprague-Dawley rats, 60 days old, received a single injection of MS (either sc or ip). After MS administration, the animals were observed for 1, 2, 3 & 24 hr post-injection to determine lethality dose-response curves. When MS was administered sc the predominant result was behavioral stimulation with no loss of righting reflex. The animals were cataleptic, although respiratory depression and cyanosis were observed. Frequent myoclonic jerks were observed within 1 to 3 hr post-injection, and death by convulsion occurred between 3 & 24 hr post-injection. The LD_{50} dose was 775 mg/kg and death was entirely by convulsions. When MS was administered ip the predominant result was behavioral sedation (hypnotic) with loss of righting reflex. At lower doses catalepsy was predominant, but at higher doses respiratory depression was predominant. There were little or no myoclonic jerks observed, and death by respiratory depression occurred within 1 to 2 hrs post-injection. The LD_{50} dose was 475 mg/kg--and death was entirely by respiration depression.

The second experiment investigated the effects of naloxone pretreatment on MS (ip) lethality. Five grp of animals were pretreated with naloxone (ip) in one of the following doses: 1.0, 10.0, 100.0, 200.0, or 300.0 mg/kg 5 min. before a single MS (ip) challenge. After MS administration animals were observed for 1, 2, 3 & 24 hrs post-injection to determine lethality dose-response curves. Low doses of Naloxone (1.0, 10.0 & 100.0 mg/kg) had a protective effect. Latency of death was increased, the LD_{50} dose of MS was increased and the mode of death was altered. With Naloxone pretreatment the predominant result was behavioral stimulation and catalepsy. The number of animals exhibiting loss of righting reflex decreased, although respiratory depression and cyanosis were still observed. Frequent myoclonic jerks were observed as well. At low doses of MS death was due to convulsions and at high doses deaths were due to respiratory depression. Deaths occurred between 3 & 24 hrs post-injection. The LD_{50} dose of MS was raised to 500, 545, & 670 mg/kg by the various naloxone doses respectively, and the mode of death (60 to 80%) was convulsions. Higher doses of Naloxone (200.0 & 300.0 mg/kg) exhibited synergistic activity with the MS challenge. Latency of death decreased, the LD_{50} dose of MS decreased and the mode of death was almost entirely due to convulsions. All animals were cataleptic and few lost righting reflex. Frequent myoclonic jerks were observed. Deaths occurred within 1 hr post-injection. The LD_{50} dose of MS was decreased to 570 & 275 mg/kg respectively, and the mode of death (80 to 100%) was convulsions.

407 A TIME SERIES ANALYSIS OF ORAL SELF-ADMINISTRATION OF D-AMPHETAMINE IN THE RAT. D. R. Meyer and R. P. Smith. Neuropsychopharmacology Program, University of Louisville, Louisville, Ky. 40208.

A new methodological procedure will be described that has been developed for use in long-term longitudinal studies designed to assess the effects of both acute and chronic drug self-administration upon normal circadian regulatory systems. The primary emphasis will be concerned with the investigation of subsequent behavioral manifestations, such as sleep-wakefulness cycles, feeding and drinking patterns, and somatic and psychologic toxicities. The use of appropriate data analysis procedures such as Fourier analysis, autocorrelation techniques, regression analysis, and ANOVA techniques will be discussed.

Data from two preliminary studies of d-amphetamine self-administration will be presented. In experiment 1, two groups of animals were exposed to either water or d-amphetamine (A) solutions for eleven weeks. During this time, the A group was exposed to the following doses (.05, .10, .20, and .40 mg/cc). In experiment 2, four groups of animals were exposed to either water or A solutions for two weeks. During this time, the three A groups were exposed to one of the following doses (.10, .20, or .40 mg/cc). During both experiments fluid intake and locomotion were monitored every two hrs. Food intake and urine output were monitored daily, and body weight was monitored weekly.

The results of both experiments indicated that there was a dose-related disruption of normal circadian fluid consumption and locomotor patterns; a dose-related disruption of normal urine output and water metabolism; a dose-related anorexic effect and body weight loss; and a dose-related increase in the frequency of both somatic and psychologic toxicities.

In general, the magnitude of the A effects were greater during the chronic exposure than during the acute exposure. These results indicated that during chronic A exposure the somatic and psychologic toxicities of the drug were potentiated. The relationship between these drug-induced toxicities and the A-induced toxic psychosis in humans will be discussed.

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408 EFFECTS OF PRENATAL METHADONE ADMINISTRATION ON MATERNAL AND PERINATAL MORTALITY AND GROWTH. Donald E. Hutchings*, Howard F. Hunt*, James P. Towey*, Tove S. Rosen*, and Howard S. Gorinson* (SPON: Dennis D. Kelly) Psychiatric Institute, 722 West 168th Street, New York, N.Y. 10032

The effects of oral methadone administration during pregnancy in the laboratory rat were investigated at four dose levels. 5, 10, 15 or 20 mg/kg was administered via gastric intubation beginning on Day 8 of pregnancy to avoid possible interference with implantation. Each drug group began at 5 mg/kg; one group was maintained at 5 mg throughout pregnancy, while the other drug groups received 5 mg increments at 4-day intervals until their terminal dose levels were attained. All groups received the final dose on Day 22 of pregnancy. An intubation control group received sterile water alone on the same gestation days and a non-treated control group was left undisturbed during pregnancy. All experimental and control litters were fostered at birth to untreated mothers. Higher dose levels of methadone reduced maternal weight gain during pregnancy and increased maternal mortality and the incidence of stillbirths and resorptions. Birth weight was a function of dose level and litter size; birth weights were significantly reduced for 10 and 15 mg pups with the 15 mg group also showing reduced litter size. The 20 mg groups had the smallest litter sizes but these pups were heavier than those in any other experimental or control group. The small pups in the 10 and 15 mg groups made up their weight deficits by weaning at 28 days, but the 20 mg pups remained the heaviest of all at weaning. The experimental and control groups showed no differences in post-natal mortality. A second experiment determined the blood levels of methadone in treated mothers and their litters. Naive rats received 5, 10, and 15 mg/kg dose regimens as in the first experiment. Mothers and litters were sacrificed 24 hours prior to predicted time of parturition and maternal and litter blood specimens analyzed separately by gas liquid chromatography. Maternal and litter blood levels for the 5, 10 and 15 mg/kg groups approximated blood levels in humans receiving daily methadone maintenance doses of 20-40, 40-80, and 80-120 mg, respectively.

409 THE BEHAVIORAL EFFECTS OF FETAL AND NEONATAL EXPOSURE TO METHADONE IN THE RAT. M.K. Etkin*, L.V. Grove*, J. Harry*, J.H. Johnson and J.A. Rosecrans
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Eleven pregnant rats were removed from breeding cages and exposed to methadone via drinking water (0.125 mg/ml). An equal number of non-drugged female pregnant rats served as yoked controls. Rats were maintained on methadone throughout the prenatal period. Within 24 hours of birth, all litters were randomly culled and cross fostered among control and methadone treated mothers such that each mother had eight pups--four males and four females. Three of the mothers receiving methadone were taken off the drug, while four of the remaining mothers were maintained on the drug during the postnatal period. Five of the yoked control mothers were placed on the methadone drinking regimen, while the remaining animals were maintained as controls during this period. Thus, all possible pre- and postnatal interactions were accounted for with the result of 16 experimental groups, 6-14 pups each. Because of the high mortality rate among pups and mothers, two groups were not formed. These were pups and mothers receiving methadone prenatally and withdrawn from the drug postnatally. Methadone drinking mothers received an average of 12.6 mg/kg methadone during pre- and postnatal periods. All pups were weaned at 23 days of age and emotionality and spontaneous activity tested at days 28 and 65-71 after birth. These experiments did not yield many significant results, except in animals exposed to methadone pre- and postnatally via mothers receiving methadone throughout this period. Rearing in both sexes was reduced by 20-30% at all times tested, suggesting a decrease in arousal levels. At 105-120 days of age, rats were exposed to a choice situation in which animals had access to both a solution of methadone (0.125 mg/ml) or quinine sulfate (0.04 mg/ml) as their source of fluid intake for 10 days. Male rats initially chose methadone more often than female rats, but intake declined rapidly such that control rats chose quinine sulfate more often by day 10. The early exposure of rats to methadone generally reduced differences between sexes in terms of drinking choice, and in one group, significantly reversed the preference according to sex. This effect occurred in rats (methadone or control prenatally CMC, MNC) cross fostered with controls who were exposed to methadone for the first time. These mothers were unable to care for their young; and, as a result, weaning weights of these pups were 30% lower than all other groups. Female rats in this group consistently drank more methadone (70-80%) than quinine sulfate throughout the study. In male rats, on the other hand, preference for methadone was less than that of control rats. This study essentially showed that prenatal exposure to a drug such as methadone can have some effects on later behavior, but that postnatal effects of drugs should also be considered. In this study, it may not have been the drug itself which produced later behavioral effects, but could be the result of stress induced by mothers on pups because of their first experience with a drug such as methadone. (Supported by NIH Grant No. R01-DA-00296-02).

410 ORAL DYSKINESIAS: ENHANCED SENSITIVITY TO METHAMPHETAMINE IN RHESUS MONKEYS WHO HAD PREVIOUSLY CONSUMED METHADONE. Robert D. Eibergen* and Kristin R. Carlson. Dept. Pharm., Sch. Med., Univ. Pittsburgh, 15261.

Chronic methamphetamine (MA) intoxication produces a developing series of stereotyped behaviors in the rhesus monkey. Initially, 2-10 mg/kg elicits biting and chewing, grooming and picking of the skin, and searching movements. After several months of intoxication, 10-20 mg/kg elicits oral dyskinesias, e.g. repetitive wide mouth opening, lateral jaw displacement, and tongue rolling and protrusion, behaviors which are very similar to the symptoms of tardive dyskinesia in humans. In the present experiments, we examined the types of stereotyped behaviors elicited by MA, as a function of prior methadone consumption, in adult male rhesus monkeys. Seven monkeys had orally consumed 1-3 mg/kg methadone HCl (MD) once daily for 10-22 months and subsequently had been drug-free for 2-17 months. Eight control monkeys had not received MD. Intramuscular injections of MA were administered to all monkeys on a schedule of MA on 4 consecutive days followed by no drug on the next three days. Starting at 1 or 2 mg/kg, MA doses were increased with each weekly cycle. In 6 of the 7 former MD monkeys, 2 mg/kg produced intense oral dyskinesias; 4 monkeys exhibited dyskinesias on the first injection day at that dose and the other 2 required only 4 injections. The same oral dyskinesias were elicited by MA on over 40 subsequent occasions. The dyskinesias persisted for up to 36 hrs. after 2 mg/kg MA, and up to 72 hrs. after 4 mg/kg. A sudden loud noise, an apparently stressful stimulus, exacerbated the dyskinesias; this stress response is a common finding in cases of human tardive dyskinesia. Conversely, 2 mg/kg MA did not elicit oral dyskinesias in any control monkey. Moreover, injections of up to 5 mg/kg on over 40 subsequent occasions failed to produce dyskinesias, and instead continued to elicit jerky body movements and mild chewing motions.

Two of the above control monkeys were then injected with MD twice daily for 45 days; the dosage was gradually incremented from 0.5 to 5.0 mg/kg. An additional control monkey received saline (SAL) during this time. On the 11th day following termination of MD or SAL, the monkeys were again injected with 2 mg/kg MA. At this time, MA elicited oral dyskinesias in the 2 monkeys who had received MD, but not in the SAL monkey.

It is clear from these experiments that chronic treatment with MD potentiates the monkey's subsequent response to MA. The enhanced sensitivity to MA in monkeys withdrawn from MD for 2-17 months has clinical implications for methadone-maintenance patients, since it indicates that chronic MD consumption can effect very long-lasting changes in brain function. A possible mechanism for the development of enhanced sensitivity to MA in our experiments is suggested by the resemblence of the oral dyskinesias elicited in former MD monkeys to symptoms of tardive dyskinesia in humans. Tardive dyskinesia is believed to result from chronic blockade of striatal dopamine receptors by the lengthy use of neuroleptic drugs. The receptors are thought to become supersensitive, such that dopamine produces an exaggerated response. MD also is known to block striatal dopamine receptors. It is possible that chronic administration of MD renders the striatal dopamine receptors supersensitive to the dopamine released by MA. (Supported in part by PHS grant MH20121)

411 DEVELOPMENTAL CHANGES IN THE LOCOMOTOR STIMULANT EFFECTS OF dl-AMPHETAMINE Linda Patia Lanier and Robert L. Isaacson. Dept. of Psychology, Univ. Florida, Gainesville, Fl. 32611

The effects of dl-amphetamine on locomotor behavior was examined in Long Evans hooded rats of different ages. Separate groups of rats were tested for five consecutive days beginning on postnatal Days 18, 34, or 45 in the open field. On the first and last day of testing no injections were given. On the second through fourth test days different groups of rats were tested 15 min. after systemic administration of saline, 2, 5, or 10 mg/kg dl-amphetamine. All doses of amphetamine significantly increased the open field activity of animals tested beginning on postnatal Day 18. However, a surprising nonresponsiveness to amphetamine at any dose level was observed in animals whose testing was initiated on postnatal Day 34. Activity was significantly increased after 2 and 5 mg/kg but not 10 mg/kg dl-amphetamine when rats were tested beginning on postnatal Day 45. These results are interpreted as reflecting a curvilinear sensitivity to dl-amphetamine in the developing rat as measured by locomotor activity. In a subsequent study the relative responsiveness of the animals to the two isomers of amphetamine were tested over 6 dose levels (range: 0.25-8.0 mg/kg) of each drug in animals tested at a responsive age (postnatal Days 18 & 19). The d-isomer enhanced locomotor activity at all but the 0.25 and 1.0 mg/kg dose levels while only the 4.0 mg/kg dose level of the l-isomer produced an enhancement.

412 ACUTE EFFECTS OF MORPHINE AND CHLORPROMAZINE ON ACQUISITION OF SHUTTLE BOX CONDITIONED AVOIDANCE RESPONSE. Abdulrahman M. Ageel,* Lincoln Chin, Clinton L. Trafton, Byron C. Jones* and Albert L. Picchioni.* The University of Arizona College of Pharmacy and Department of Psychology, Tucson, Arizona 85721.

Morphine sulfate or chlorpromazine hydrochloride in s.c. doses of 0.25 to 24.0 mg/kg and 0.0625 to 4.0 mg/kg, respectively, were administered to naive rats 30 minutes prior to initiation of acquisition of conditioned avoidance response (CAR) under massed trials. Three CAR task difficulty levels, created by manipulation of the duration of conditioned and unconditioned stimuli, intertrial interval and shock intensity, were used in conjunction with graded doses of morphine and chlorpromazine. Chlorpromazine, in a dose related manner, caused decrement in CAR acquisition in all tasks. Morphine, in comparison, produced a biphasic dose response. For a given task difficulty, low doses of morphine enhanced acquisition, whereas higher doses inhibited acquisition. The dosage range of morphine required to produce the biphasic dose response on acquisition of CAR increased with increasing task difficulty. It is noteworthy that doses of morphine which inhibited acquisition in one task effectively enhanced acquisition in a more difficult task. These results emphasize the need to consider potential interactions between dose levels and task difficulty in the application of drugs in learning paradigms.

413 DIFFERENTIAL ANTAGONISM BY LITHIUM OF MORPHINE AND D-AMPHETAMINE EFFECTS ON SUBSTANTIA NIGRA SELF-STIMULATION. J.M. Liebman and D.S. Segal. Psychiatry Dept., Sch. Med., UCSD, La Jolla, 92037.

The effects of chronically administered lithium (Li) on electrical self-stimulation (SS) in substantia nigra were investigated. After stable baseline rates of responding for SS were established, lithium chloride was administered daily to rats for 10 days at doses of 1.5, 2.0 and 2.5 meq/kg, s.c. Self-stimulation tests took place 2 hr after Li injection on the 1st, 3rd, 5th, 7th and 10th days of treatment, and lasted 30 min. No effect was observed at the lowest dose of Li. At 2.0 meq/kg, Li significantly reduced SS only on the 3rd and 5th days of treatment. The progressive reduction in SS observed at 2.5 meq/kg Li appeared to be confounded with the appearance of Li-induced toxicity and weight loss produced by this dose. Another group of rats was pretreated with morphine sulfate (15 mg/kg) daily for 10 days and tested on alternate days 3 hr after injection. The pronounced morphine-induced facilitation of SS was then challenged by 11 days of concurrent daily Li (1.5 or 2.0 meq/kg) and morphine treatment. Testing was continued on alternate days as before. Lithium (2.0 meq/kg) significantly reduced morphine-facilitated SS during the entire treatment and this reduction was more marked than that observed with Li treatment alone. In contrast, the large increase of SS induced by d-amphetamine (0.5 mg/kg) was not significantly antagonized by 10 days of chronic Li (2.0 meq/kg) treatment. Thus, Li may antagonize morphine-induced reinforcement processes more strongly than d-amphetamine-induced reinforcement. These results are in notable contrast to the reported enhancement of morphine analgesia by Li (Jensen, 1974). Further, these findings suggest that different mechanisms may underlie morphine- and d-amphetamine-induced facilitation of SS.

414 COMPARISON BETWEEN METHADONE AND MORPHINE SELF-ADMINISTRATION BEHAVIOR. Toreen E. Werner, Stanley G. Smith and W. Marvin Davis. Department of Pharmacology, School of Pharmacy, University of Mississippi, University, MS 38677.

Adult male Sprague-Dawley derived rats were implanted with chronic indwelling jugular cannulas. Five groups of subjects (N=5 each) were then allowed access on CRF schedule to saline, or to 0.01, 0.03, 0.1, or 0.3 mg/kg/infusion of methadone hydrochloride. Following six days of access to methadone, those groups were switched to equal doses of morphine sulfate. This allowed a within-group analysis of methadone-to-morphine potency differences. In a second set of subjects, three groups were allowed access to 0.03, 0.1, or 0.3 mg/kg/infusion of morphine for 6 days. This allowed a between-groups analysis of potency differences. The results for both within-groups and between-groups comparisons show that the number of infusions taken was an inverse function of the unit dose; i.e., the higher the dose the lower the number self-infused. Data for the amount of drug self-administered (mg/kg/session) showed a direct relationship to unit dose; i.e., as the dose was increased so did the amount self-administered. Also, for both within-groups and between-groups comparisons, more responses were emitted for morphine than for methadone; i.e., both number of infusions of morphine and amount self-administered were significantly greater than for methadone.

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415 SEROTONERGIC INVOLVEMENT IN THE TEMPERATURE LOWERING EFFECTS OF METHAQUALONE. William O. Boggan. Dept. Psychiatry. Medical University of South Carolina, Charleston, 29401.

Methaqualone (2-methyl-3-o-tolyl-4(3H)-quinazolinone) produces a profound, dose dependent and time dependent fall in the rectal temperature of mice tested at 22°C. At a dose of 75 mg/kg methaqualone reduces rectal temperature of C57BL/6J mice by approximately 7°C at the peak time of action, one hour. This effect is manifest within fifteen minutes and lasts for approximately 8 hours. The effect is not blocked by SKF525A, a drug known to block the liver microsomal enzymes responsible for the major degradation of methaqualone, thus suggesting that methaqualone rather than a metabolite is responsible for the temperature effect. The temperature lowering effect by methaqualone can be blocked if the animals are kept at 38°C, suggesting that the drug blocks the ability of the mice to thermoregulate. Pretreatment of the animals with the tryptophan hydroxylase inhibitor, parachlorophenylalanine, significantly attenuates the methaqualone effect while pretreatment of the mice with the serotonin uptake inhibitor, Lilly 110140, significantly potentiates the temperature lowering effect of methaqualone. Neither pretreatment produced any effects when given alone to control animals. These latter data imply serotonergic involvement in methaqualone's action on body temperature.

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416 MORPHINE AND PGE_2: EFFECT ON IN VIVO cAMP LEVELS IN BRAIN. Philip F. VonVoigtlander and Elizabeth G. Losey* Research Laboratories, The Upjohn Co., Kalamazoo, Mich. 49001.

Subsequent to intravenous PGE_2 (1 mg/kg), male mice were sacrificed by microwave irradiation. Regional brain dissection and assay for cAMP revealed significant elevations of cAMP in the striatum and mid-hind brain but not in the cerebral cortex or thalamus. Dose-response and time course studies indicated that 1 mg/kg PGE_2 caused an optimal elevation in striatal cAMP one minute after administration. Subcutaneous morphine sulfate at high doses (30 and 10 mg/kg) also resulted in elevated striatal cAMP levels; however, low doses (3 and 1 mg/kg) did not. Pretreatment with morphine (10, 3, 1 or 0.3 mg/kg) did not alter the cAMP response to PGE_2. Thus the theory that morphine analgesia results from an antagonism of PGE_2-induced cAMP elevation in brain (Collier and Roy, Nature 248:24, 1974) is not supported by these data. In addition, morphine (30 and 10 mg/kg)-induced elevations in cAMP were not blocked by the narcotic antagonist, naloxone (10 mg/kg). Thus this morphine effect is probably not mediated by a specific morphine-receptor mechanism. Experiments examining cAMP responses to PGE_2 in morphine-tolerant mice will also be discussed.

417 UPTAKE OF ^{14}C-MORPHINE BY NEURONAL AND GLIAL CELLS ISOLATED FROM RAT BRAIN. Lawrence E. DeBault and Rafiq Waziri. Dept. of Psych., College of Med., Un. of Iowa, Iowa City, Iowa, 52242.

In a previous report (DeBault and Waziri; Transactions Am. Soc. Neurochemistry, p. 181, 1974) the ^{14}C-morphine uptake by mouse neuroblastoma (NEB), rat glioma (C_6) and mouse fibroblast (L929) was shown to be concentration and cell line dependent. In these and subsequent experiments it was also shown that C_6 glial cells could take up more ^{14}C-morphine than NEB neuronal cells under all three experimental conditions; naive, morphine tolerant, and morphine withdrawn states. The relative ^{14}C-morphine uptake in all three cell lines was greatest for tolerant > withdrawn ≥ naive. In an effort to relate these findings to a whole animal model, the techniques of Sellinger et al. (Nature New Biol. 230:253, 1971) were utilized to isolate neuronal and glial cells from the brains of naive, tolerant and withdrawn rats, and the cells' in vitro ^{14}C-morphine uptake measured. On a per cell basis, isolated glial cells from naive, tolerant and withdrawn rats took up significantly more ^{14}C-morphine than did neuronal cells isolated from the same animals. Though there was no statistically significant difference in the ^{14}C-morphine uptake by glial cells between naive, tolerant and withdrawn rats, there was a trend for glial cells from tolerant and withdrawn rats to take up more labeled morphine than similar cells from naive rats. There was also a trend toward higher glial to neuronal uptake ratios in tolerant rats as compared to naive ones. These preliminary data indicate that there is a similarity in the morphine uptake characteristics between cell lines of neuronal and glial origin and isolated neuronal and glial cells from rat brain. These findings may also indicate that glial cells play an important role in the neural mechanisms of tolerance to opiates.
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418 IN VITRO EFFECTS OF NARCOTICS ON SYNAPTIC TRANSMISSION IN RAT SUPERIOR CERVICAL GANGLIA. James E. Forbes*, William L. Dewey and Louis S. Harris. Dept. of Pharmacol., Med. Col. Va., Richmond, Va. 23298.

The effect of narcotics, narcotic-antagonist analgesics and pure narcotic antagonists on synaptic transmission in the isolated rat superior cervical ganglia was studied in vitro. The superior cervical ganglia, sympathetic trunk and postganglionic trunks were dissected from adult male albino rats. The blood supply to the ganglia was left intact until the ganglia was actually removed. After being removed, the ganglia was bathed in Eccles-Krebs solution, pH 7.2, oxygenated with 95% O_2 - 5% CO_2 at a temperature of 38°C. Evoked postganglionic potentials were elicited by stimulating the sympathetic trunk with biphasic supramaximal stimuli of 0.5 msec duration at a frequency of 1/sec. The evoked postganglionic potentials were recorded from the external or internal carotid artery nerves with a suction electrode. Morphine inhibited the postganglionic potentials by 60% at a concentration of 6.6×10^{-4} M and 30% at a concentration of 3.3×10^{-4} M. The morphine inhibition was not antagonized by naloxone at concentrations of 0.3×10^{-4} M to 1.5×10^{-3} M. Other narcotic analgesics including meperidine (1.8×10^{-3} M), levorphanol (6.5×10^{-4} M) and methadone (7.2×10^{-4} M) also blocked the evoked postganglionic potentials. The narcotic-antagonist analgesics, pentazocine (1.2×10^{-2} M), cyclazocine (1.8×10^{-5} M) and nalorphine (1.6×10^{-3} M) also inhibited the evoked postganglionic potentials. The pure antagonist nalocone did not produce inhibition of the evoked postganglionic potentials in concentrations up to 1.5×10^{-3} M, however naltrexone (7.9×10^{-4} M) did block the evoked postganglionic potentials. It was concluded that the in vivo actions of the narcotic analgesics were non-specific. (Supported by Grant No. DA00326-1 and Training Grant No. T22 DE 00116-01 and Grant No. DA00490).

419 CAFFEINE ACCUMULATION IN BRAIN AREAS. Patricia J. Bernthal* and H. Dix Christensen* (Spon: J. I. Moore). Depts. Pharm., and Psychiat. and Behav. Sci., Univ. of Okla. Health Sciences Center, Oklahoma City, Okla. 73190.

Caffeine concentrations in different brain structures were determined at the time of peak plasma level in male rats. Maximal concentrations of caffeine in plasma occur one hour after gastric intubation. The peak concentration after 1.5 mg/kg of caffeine is 3.15 ± 0.82 μg/ml; the half-life is 3.7 hours. Caffeine levels were measured by a recently developed radioimmunoassay. The assay has a linear range from 0.1-30 ng. All known caffeine metabolites cross react with the antiserum less than 1% with the exception of theophylline (8%). The procedure consists of homogenizing the brain tissue in saline, adjusting the pH to 11.5 with 2.5 N sodium hydroxide, and extracting the caffeine with chloroform, which is then evaporated. The caffeine residue is then diluted with a phosphate buffer. Antiserum (0.1 ml of a 1:70,000 final dilution) and 1,3-dimethyl-7-propyl-xanthine-^{3}H (60 pg, 62.6 Ci/mMol) are incubated with the samples for two hours at 4°C. The free caffeine is then separated from the bound caffeine by the addition of dextran-coated charcoal. The labelled bound caffeine derivative is then counted in a liquid scintillation counter, and the amount of caffeine in the sample is determined.

Mean and standard error for the brain caffeine levels were as follows: medulla, 0.63 ± 0.13 μg/g; pons, 0.46 ± 0.05; cerebellum, 0.49 ± 0.04; midbrain, 0.56 ± 0.08; hypothalamus, 0.61 ± 0.13; hippocampus, 0.56 ± 0.08; striatum, 1.10 ± 0.20; cortex, 0.56 ± 0.05. Only the striatum had a significant accumulation of caffeine compared to other brain levels ($P < .05$).

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420 STATE DEPENDENT EFFECTS OF LYSERGIC ACID DIETHYLAMIDE AND CYCLAZOCINE ON THE CONDITIONED EMOTIONAL RESPONSE. William T. Chance* and John A. Rosecrans. Dept. Pharmacol., Med. Coll. Va., Richmond, 23219.

Wray (Psychopharmacologia 26: 29, 1972) has stressed the importance of the use of animal models in the assessment of psychotomimetic properties of potential therapeutic agents. On the basis of similar disruption of rats' performance in continuous discriminative avoidance by lysergic acid diethylamide (LSD) and cyclazocine (CYCL), he postulated a similar mechanism of action for the two drugs. This disruption was furthermore interpreted to be a demonstration of psychotomimetic effects of CYCL in animals.

Since a decrement in the performance of a fear-motivated task may result from a variety of reasons e.g., analgesia, fear reduction, locomotor deficiency, or drug dissociated effects, LSD and CYCL were investigated using a conditioned emotional response (CER) paradigm to assess state dependent effects. Nine groups of 10 rats each were individually placed in a Lafayette chamber and presented with a four-sec duration CS (white noise) overlapping and terminating with a two-sec US (0.6 ma shock). Six CS presentations were administered to each rat with an intertrial interval (ITI) of 11 sec. Three groups of rats were trained 30 min after the injection of 0.3 cc normal saline, three after 1.0 mg/kg LSD, and three after 2.0 mg/kg CYCL. Three additional groups of 10 rats each were injected with saline, placed in the chamber, and presented with the CS alone. Three days after training, all rats were individually placed in a Woodward activity cage and presented with the CS (12 presentations, 11 sec ITI) 30 min after the injection of the appropriate drug. Activity was measured for the duration of the CS presentations (3 min). Each of the 3 groups trained after the injection of a particular drug was tested under either that same drug or was switched to one of the other drug states during testing. Thus the 3 groups that were trained under CYCL were tested after the injection of either saline, CYCL, or LSD.

The CER procedure was found to reduce activity to 37.7% of control values. In the no-shock condition LSD significantly decreased activity, while CYCL was without effect. LSD also significantly decreased both performance and acquisition, since activity was reduced during testing as compared to training under the drug. Although CYCL had no significant effect on performance in the control group, the drug completely obviated acquisition and recall, possibly through motivational changes. Thus there was no difference between activity of the CER groups and the no-shock controls in any of the CYCL conditions (training, testing, or training and testing). There was evidence for a weak state dependent effect of LSD, but no indication of drug dissociation was found under the CYCL state. No generalization of the CER was evident when the rats were trained under CYCL and tested under LSD. A moderate degree of generalization was found, however, when the rats were trained under LSD and tested after the injection of CYCL.

These results suggest that CYCL produces disruption of fear-motivated tasks by affecting motivating systems, possibly through fear reduction or analgesia. Thus even after the rats have learned the task their performance under the CYCL state could be disrupted by lack of motivation. Disruption under the LSD state seems to occur primarily because of its effects on performance. LSD significantly decreased the rats' activity even in the no-shock condition, and this decrease probably resulted because of the psychotomimetic effects on locomotor activity. Thus it seems that the drugs may have been causing similar effects in Wray's study, with avoidance behavior being disrupted by CYCL not because of psychotomimetic effects, but by changes in motivation.

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421 METHAQUALONE STIMULATES PITUITARY-ADRENOCORTICAL ACTIVITY IN MICE. Jerrold S. Meyer and William O. Boggan. Dept. of Psychiatry, Medical University of South Carolina, Charleston, S.C. 29401.

The effect of methaqualone administration on plasma corticosterone concentration was studied in adult male C57BL/6J mice. Methaqualone was intubated orally as a suspension in 1% gum acacia. Vehicle alone was given to control mice. Plasma corticosterone content was determined by a competitive protein-binding radioassay. Methaqualone (25, 50, 75, or 100 mg/kg) produced a marked dose-dependent rise in plasma corticosterone. After 75 mg/kg methaqualone, plasma corticosterone peaked at approximately 1 hour post-intubation and returned to control values by 4 hours following drug administration. This effect was completely blocked by dexamethasone pretreatment, indicating that ACTH release was required and that the primary action of methaqualone was not on the adrenal cortex. Methaqualone is primarily metabolized by the liver microsomal enzyme system. An inhibitor of this system (SKF 545A) failed to block the adrenocortical response to methaqualone, suggesting that this response is stimulated by methaqualone itself rather than by some major metabolite of the drug. Because methaqualone produces severe hypothermia in mice, some subjects were tested for adrenocortical response to the drug while being maintained at normal body temperature (38°C) in a thermostatically controlled oven. These animals showed highly significant corticosterone rises compared to oven maintained controls, thus demonstrating a dissociation between the hypothermic and pituitary-adrenocortical effects of methaqualone. The exact site(s) of action of this drug, as well as the potential mediation of these effects by central neurotransmitter systems, remain to be elucidated. (Supported by NIDA Grant DA-01035 to W.O.B. and by General Research Support Grant RR05420 from NIH to the Medical University of So. Carolina).

422 POSSIBLE INVOLVEMENT OF ADRENOCORTICOTROPHIC HORMONE IN THE MAINTENANCE OF ORAL MORPHINE INTAKE IN RATS. Zalman Amit, David Ziskind* and Deborah Levitan*. Center for Research on Drug Dependence, Concordia University, Montreal, Quebec, Canada.

Several investigators (e.g. Kerr & Pozuelo, J. Mayo Clin. Proc., 47: 621-628, 1972) have implicated the hypothalamus in the control of drug intake in rats. Given the intimate connections between the hypothalamus and the pituitary gland, a study was designed to examine the putative role of the pituitary in drug intake. Male rats were hypophysectomized by the transauricular method. Following recovery, these animals were presented with a choice between 0.02% morphine dissolved in a 5% sucrose solution and equiaversive 0.0125% quinine dissolved in sucrose of the same concentration (5%). Two additional groups of hypophysectomized rats were given a series of injections of either adrenocorticotrophic hormone (ACTH) or growth hormone. Hypophysectomized animals did not drink the morphine/sucrose solution, but did not avoid the quinine/sucrose solution. The ACTH-treated rats did not differ from intact controls in their consumption of morphine. Growth hormone did not seem to have an effect on morphine consumption in hypophysectomized animals. Since one of the main sites of action of ACTH is the adrenal glands, we further examined the role of ACTH in morphine intake by adrenalectomizing intact rats. We found that adrenalectomy did not block morphine intake in these rats. Furthermore, pilot data revealed that hypophysectomy did not reduce intake of sodium pentobarbitol, and had only a minor effect on ethanol intake. These results argue against post-surgical general debilitation as a cause of blockade of morphine drinking, and support the notion of specific involvement of ACTH in the regulation of morphine intake in rats.

423 CATECHOLAMINES IN THE CINGULATE CORTEX AND HYPOTHALAMUS AND EFFECT OF OPIATE ADMINISTRATION AND ADDICTION IN SQUIRREL MONKEYS. E.T. Angelakos*, J.D. Irvin and S. Jacobs (SPON: J.L. Osterholm). Hahnemann Medical College, Phila., Pa., and Univ. Calif., Santa Barbara, Calif.

Norepinephrine (NE) and Epinephrine (E) were determined in various regions of the brain with the fluorometric technique. In addition tissue sections were examined with the fluorescence histochemical technique (formaldehyde reaction). In untreated animals the concentration of total catecholamines (CA) (NE + E) was 2 to 3 times higher in the cingulate cortex than in other parts of the cortex (0.05 to 0.08 vs. 0.15 to 0.22 μg/gm). This was confirmed histochemically where CA varicosities were found along the ventral margin of the cingulate cortex. In opiate addicted animals (5 to 7 days) regional brain CA levels were dependent on the time of sacrifice after the last dose. Up to two hours after the last dose CA levels in the medulla, pons and especially hypothalamus were depressed to about half normal. While three to four hours after the last dose there was a marked elevation (2x to 3x) in CA levels in the hypothalamus and cingulate cortex but not in other regions. These returned to normal by 6 to 7 hrs. Histochemical observations paralleled the chemical findings. In the cingulate cortex increase in CAs was associated with CA varicosities (and occasionally CA cells) throughout the ventromedial region. Addiction was tested with Naloxone which produced characteristic withdrawal symptoms but had no significant effect on regional brain CA levels. Similarly administration of a single dose of opiate had no significant effect on brain CAs. It is concluded that the changes in brain CA which are found mainly in the hypothalamus and cingulate cortex are related to addiction rather than to an acute opiate effect. (Supported by ONR Contract #305-965).

424 REPEATED ADDICT RELAPSE AND CLASSICAL CONDITIONING: AN EXPERIMENTAL APPROACH. Elliot A. Stein. Dept. of Physiology, Univ. of Maryland, SM, Baltimore, Maryland 21201.

Pavlov observed that after several injections of morphine in dogs, the sight of the experimenter or the syringe itself was sufficient to elicit many of the physiological responses to morphine. It has been postulated that addict relapse may, in part, be due to similar conditioning of morphine elicited reactions. A chronic rat model has been developed to test the hypothesis that morphine, acting as an unconditional stimulus, can be classically conditioned within the nervous system. An awake, freely moving rat, surgically prepared with an indwelling,intravenous catheter, EKG electrodes, and cortical and subcortical EEG electrodes, was subjected to a Pavlovian paradigm of a 1/sec click (CS), and a small (500 ug/kg) IV infusion of morphine. The CS was sounded for two minutes into an earphone mounted on the rats head. One minute after the onset of the click train, 0.38 mls of morphine was infused over a 30 second period. Before, during and after each two minute trial(2 trials/day), the heart rate, EEG, Auditory Evoked Potential (AEP) and general behavior were recorded. Unconditional (physiological) responses to morphine included:EKG-profound bradycardia, atrioventricular block, and atrial fibrillation;EEG-appearance of high voltage, slow wave spindle activity; Behavior-a stereotypic, transient catatonia;AEP- an increase in amplitude of both early & late components from all CNS loci examined. Conditional responses included behavioral signs of orienting and fixation, EKG tachycardia, & an increase of the late (30-50 msec) components of the AEP to saline similar to that seen to morphine. Results indicate that the direct effects of morphine can be classically conditioned within the nervous system, and may thus play an important role in repeated addict relapse.(Supported in part by USPHS Training Grant #GM-01075-12 & DOD Contract #DADA-17-73-C3030)

425 PROFILES OF OPIATE ABSTINENCE: RODENT STRAIN DIFFERENCES. Larry P. Gonzalez and Harold L. Altshuler. Dept. Neuropsychopharmacology, Texas Research Institute of Mental Sciences, Texas Medical Center, Houston, Texas 77025.

Five strains of rats, two outbred (Sprague-Dawley and Holtzman) and three inbred strains (Buffalo, Lewis, and Fisher) received subcutaneous implants of either morphine (75 mg) or placebo pellets, prepared according to the procedure of Gibson and Tingstad (J. Pharm. Sci., 59:426, 1970). The naloxone precipitated opiate abstinence syndrome (.4 mg/kg naloxone hydrochloride, subcutaneous) was observed after one, two, or three days of exposure to the implanted pellet. Fourteen abstinence signs were measured including activity rate, weight change, escape attempts, wet dog shakes, teeth chattering, writhing, yawning, penile erections, vocalization or hostility on handling, ptosis, eye twitching, lacrimation, rhinorrhea, and diarrhea. Inter-strain differences were present in both the pre- and post-naloxone periods. These differences were most evident in the frequency of occurrence of wet dog shakes, teeth chattering episodes, escape attempts, and activity counts. The abstinence profile following injection of naloxone was found to change with different lengths of exposure to the implanted morphine pellet. This change was statistically significant in four of the five strains observed, as determined by a multivariate analysis of variance. No significant effect of length of exposure was found in the response profile of Holtzman rats. For those strains showing a significant length-of-exposure effect, a discriminant function based on the abstinence syndrome profile was obtained. This function describes differences along the length-of-exposure dimension and may prove useful as a quantitative assessment of the abstinence syndrome.

426 SELF-ADMINISTRATION OF NORADRENERGIC AND DOPAMINERGIC AGONISTS. W. Marvin Davis and Stanley G. Smith. Department of Pharmacology, School of Pharmacy, University of Mississippi, University, MS 38677.

Reinforcing qualities were demonstrated for i.v. doses of 2 dopaminergic agonists, apomorphine HCl (APO; 60 µg/kg/infusion) and piribedil (500 µg/kg/infusion), and one noradrenergic agonist, clonidine HCl (CLO; 15 µg/kg/infusion). Ss were ad. male Sprague-Dawley derived rats. Tests for reinforcing effects employed both the self-administration (SA) method and a combined pavlovian and free operant paradigm for the establishment of conditioned reinforcement (CR) by methods as in Davis & Smith, Life Sci. 12: 185, 1973. The latter method permits a behavioral measure of reinforcing properties in the absence of drugs, so as to minimize the influence of other properties. Studies with APO also employed pretreatment with haloperidol (HAL; 5 mg/kg), a blocking agent toward central dopaminergic activity, and U-14,624 (600 mg/kg), an inhibitor of central noradrenergic activity via depletion of NE by inhibition of dopamine-β-hydroxylase. As only HAL prevented SA and CR, the dopaminergic basis for the reinforcing action of APO was supported. Pretreatment with HAL before piribedil also inhibited development of SA and CR. Groups pretreated with saline before APO and piribedil both acquired SA behavior and displayed CR. In experiments on CLO, pretreatment with an α-adrenergic blocking agent, phenoxybenzamine (15 mg/kg), inhibited development of both SA and CR, in contrast to pretreatment with saline. These studies suggest that pharmacologic activation of either central noradrenergic or dopaminergic systems can result in reinforcing effects.

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427 EFFECTS OF DOSE, DEPRIVATION AND SATIATION ON INTRAGASTRIC MORPHINE SELF-ADMINISTRATION. Stanley G. Smith, Toreen E. Werner and W. Marvin Davis. Department of Pharmacology, School of Pharmacy, University of Mississippi, University, MS 38677.

Adult male Sprague-Dawley derived rats were implanted with intragastric cannulas (Smith, Werner and Davis, Physiol. Psychol. 3: 220, 1975). Separate groups (N=5) were allowed to self-administer six different doses of morphine sulfate. The resulting data, unlike those from previous intravenous self-administration research, indicated that increases in dosage produced corresponding increases in the number of infusions taken. A second experiment examined the effect of intervals of deprivation up to 32 hours. Contrary to published research with oral or intravenous self-administration, shorter intervals of deprivation produced higher numbers of infusions. A third experiment examined the effects of satiation on self-administration of morphine in groups of rats. Noncontingent intragastric infusions were given prior to and midway in a 10-hr test session. Doses were 0, 50, 150 and 300 mg/kg of morphine for each infusion. The results showed enhancement of drug-taking behavior, not satiation effects. The disparities in the present results between intragastric findings and those obtained from intravenous or oral routes are discussed.

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428 KETAMINE SELF-ADMINISTRATION IN THE RHESUS MONKEY. J. Edward Moreton, Dept. Pharmacol. & Toxicol., Sch. Pharm., Univ. of Maryland, Baltimore, Md. 21201, and Richard A. Meisch,* Linda Stark,* and Travis Thompson,* Psychiat. Res. Unit, Dept. Psychiat., Sch. Med., Univ. of Minnesota, Minneapolis, Minn. 55455.

Intravenous self-administration of ketamine hydrochloride, a derivative of phencyclidine, was studied as a function of dose and response requirement on a fixed ratio (FR) schedule of reinforcement. Three male rhesus monkeys were prepared with a chronic indwelling jugular cannula and a protective leather harness (Pickens et al., J. Exp. Anal. Behav. 9:701, 1966) which permitted relatively unrestrained movement. Each dose level and FR parameter was in effect during daily two-hour sessions until the number of infusions/session remained stable for five consecutive days. The monkeys initiated self-administration of ketamine (0.2 mg ketamine base/kg/infusion) on a fixed ratio one (FR1) schedule of reinforcement. Following stabilization of drug intake the dose was held constant at 0.2 mg/kg while the FR was increased geometrically by two-fold until self-administration ceased. Self-administration was then reestablished at FR1. As the FR was increased from 1 to 256 or 512, drug intake decreased linearly. Response patterns were characteristic of FR responding for other reinforcers, particularly at higher FRs which resulted in lower drug intake and consequently less drug-induced disruption of operant behavior.

The ketamine dose was then varied geometrically between 1.6 and 8×10^{-4} mg/kg at FR1, FR8, and FR64. Total drug intake decreased linearly while response rate and number of infusions per session were inverted U-shaped functions of ketamine dose. At FR1 response rate increased until an optimal dose of 0.0125 mg/kg was reached which produced approximately 300 self-infusions per session for a total intake of 4 mg/kg. Further dose reductions produced decreases in response rate and number of reinforcements. Higher FR values required higher ketamine doses to maintain responding. That is, dose-response curves for FR8 and FR64 were shifted downward and to the left of the curve generated at FR1. Doses which maintained maximum responding at FR8 and FR64 were 0.025 and 0.1 mg/kg, respectively.

Gross behavioral effects produced by ketamine included heavy sedation to light anesthesia between self-injections at doses and FR values which resulted in drug intake from 10-20 mg/kg/session. Behavioral effects at lower drug intake ranged from light sedation to no apparent effects.

The present study demonstrated that intravenously administered ketamine will serve as a reinforcer in primates and maintain operant behavior in a manner similar to conventional and other drug reinforcers. The monkeys initiated self-administration at low doses of ketamine and maintained stable patterns of self-administration even at relatively low doses and high FR values.

In contrast with other drug reinforcers with central nervous system depressant effects, ketamine is not known to produce significant tolerance nor physical dependence, and may therefore represent a new class of reinforcers. (Supported by NIMH Grant MH 08565 to the University of Minnesota.)

429 EVALUATION OF METHADONE'S ABUSE POTENTIAL*. Khalil A. Khavari. Dept. Psychol. and Midwest Institute on Drug Use, The University of Wisconsin-Milwaukee, Wis. 53201.

In a series of studies, using rats, we investigated the abuse potential of dl-4, 4-diphenyl-6-dimethyl amino-3-heptanone hydrochloride by means of self-administration procedures. We observed the followings: rats ingest large quantities of methadone when the drug is mixed with their food (methadone concentration in food as high as 4 mg/g); solutions laced with methadone are generally refused (as little as .5 mg/ml of 10% sucrose); rats self-inject methadone via the intragastric, intraperitoneal, intramuscular, intravenous, and subcutaneous routes; rats with no previous history of drug treatment self-inject approximately the same amount of morphine and methadone; preliminary data indicate that methadone appears to be as desirable as morphine. We conclude that the abuse potential of methadone appears to be as great as that of morphine when self-injection is taken as an index of preference.

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*Supported in part by the National Institute on Drug Abuse (NIDA) Grant 1P01DA01080 and NSF Grant P2B0349 to K. A. Khavari.

430 INCREASED CNS SENSITIVITY TO FLUROTHYL AS A MEASURE OF PHYSICAL DEPENDENCE IN MICE FOLLOWING MORPHINE, PHENOBARBITAL, METHAQUALONE, AND ETHANOL TREATMENT. Alpern, H. P. and Greer, C. A.* Dept. Psych., Univ. Colorado, Boulder, Co. 80302.

Male C57BL/6 mice were administered either morphine, phenobarbital or ethanol in their drinking water or methaqualone in their food in order to make them dependent. During withdrawal onset of myoclonus, clonus and tonus was evaluated with flurothyl, a convulsant inhalant. Morphine, phenobarbital, methaqualone and ethanol treated subjects displayed significantly lower latencies for myoclonic and clonic convulsive behavior when compared with their respective controls. The increased CNS excitability observed is characteristic of physical dependence and it appears that the flurothyl technique employed has broad applicability to a number of agents characterized by their dependence producing properties.

431 EFFECTS OF OPIATES ON AVERSIVE ELECTRICAL STIMULATION OF THE MESENCEPHALON IN THE RAT. Agu Pert. Adult Psychiatry Branch, NIMH, Bethesda, 20014.

Sixty-five rats were implanted with bipolar platinum electrodes aimed for the mesencephalic periaqueductal gray matter and surrounding tegmentum at the level of the dorsal raphe nucleus. Following recovery, the animals were trained to escape from continuous electrical stimulation (0.2 msec rectangular pulses at 30 cps) of these areas in a standard toggle floor shuttle-box. The stimulation current was adjusted to produce mean escape latencies of 4-6 seconds. When performance had stabilized, the rats were divided into groups and injected with either 15 mg/kg morphine, 5 mg/kg levorphanol or 5 mg/kg dextrorphan 30 minutes prior to testing. A fourth group received 15 mg/kg of morphine followed by 2 mg/kg of naloxone 15 minutes prior to testing. This group was also tested with 2,20, and 40 mg/kg of naloxone alone 15 minutes prior to testing at one week intervals. Morphine and levorphanol were found to increase escape latencies while dextrorphan (inactive enantiomer of levorphanol) had no effect. Naloxone reversed the actions of morphine but had no effect on escape latencies by itself, even at the highest dose. Tolerance developed rapidly to the effects of morphine on escape latencies during five daily administrations. The time course of tolerance to this effect corresponded with the time course of tolerance to the analgesic effect of the same dose of morphine in the flinch-jump test. The findings are interpreted to imply that opiates may produce part of their analgesic actions by suppressing various mesencephalic regions which receive rich input from the somatosensory pathways (spinoreticular) that have been postulated to carry information concerning the protopathic aspects of pain.

432 INDEPENDENCE OF THE LACK OF EFFECT OF MORPHINE ON CENTRAL EVOKED PAIN FROM PROPERTIES OF BRAIN STIMULATION. J.P. Rosenfeld* and J.L. Vickery*, Northwestern University, Evanston, Ill., (SPON: A.P. Rudell)

Previous results showed that morphine given in doses producing clear analgesia for peripheral pain, fails to change nociceptive reaction threshold for pain produced by brain stimulation. Here, we approached the question of whether or not the lack of analgesia for central stimulation was related to unphysiological synchronizing aspects of brain stimulation. To this end, wave shape and frequency of brain stimulation were varied. The rats received 500 msec bursts of sinusoidal stimulation at 10 Hz and 500 msec bursts of (.5 msec biphasic) pulse stimulation at 300 pulses per second in reticular sites. Morphine failed to affect pain threshold for both kinds of stimulation.

433 CHANGES IN NOCICEPTIVE EVOKED ACTIVITY OF THE CAUDATE NUCLEI FOLLOWING LOCAL APPLICATION OF MORPHINE. C. Thomas Bennett, Thomas Bevan* and Kenneth Gall.* Biomedical Laboratory, Edgewood Arsenal, APG, MD 21010.

It is well established that there is a high concentration of opiate receptors in the caudate nucleus, dorsal medial thalamic area, and the periaqueductal region of the mid-brain. Almost nothing is known about the direct action of opiates on the electrical activity of these areas. Activity in these regions was recorded by means of a bipolar electrode-cannulae array, which permitted injection of various drugs directly at the recording site. The nociceptive stimulus was a 1.0 mA shock delivered to the tail of the rat.

It was found that nociceptive evoked activity was attenuated in the periaqueductal region and dorsal medial thalamic areas following local application of as little as 2.5 nanograms of morphine delivered in 0.25 µl of physiological saline. There was a decrease of at least 20% in the major positive and negative components of the evoked wave. Naloxone (2 mg/kg, I.P.) antagonized the effects of opiates on the nociceptive evoked activity. As much as 1 µl of physiological saline had no effect on the activity in these areas.

Morphine (2.5 nanograms in 0.25 µl saline) produced a more complex effect on activity recorded in the head of the caudate. The initial negative component was potentiated by at least 20% while the second positive component was attenuated. These effects were antagonized by naloxone. A muscarinic antagonist, atropine (2.0 nanograms) also produced facilitation of the initial negative components of the evoked activity in the caudate. This potentiation could be antagonized by arecoline. These data constitute the lowest reported effective use of morphine in an awake animal by a factor of 1000. In addition, these data support the contention that morphine exerts an influence on the extra pyrimidal motor systems.

434 MORPHINE-INDUCED "SPINNING" BEHAVIOR FOLLOWING MICROINJECTION IN MIDBRAIN RETICULAR FORMATION. Yasuko F. Jacquet, Abel Lajtha, and Ian S. Russell*. NY State Research Institute for Neurochemistry and Drug Addiction, Ward's Island, N.Y. 10035, and Medical Research Council, Unit on Neural Mechanism of Behavior, University College, London, England *.

Morphine injected via fine-gauge cannula in the midbrain reticular formation induced marked rotation behavior in rats. Sudden auditory and/or visual stimuli set off a train of rapid spins consisting of 10-12 pivots/5 sec on the ipsilateral hind leg. Morphine microinjection in sites 1 mm medial to this site resulted in no such effect, or resulted in an attenuated form of this behavior (e.g., slow circling around the perimeter of the bin). This "spinning" behavior appears to have 2 components: 1) a heightened arousal, with the rat being hyper-reactive and making vigorous "runs" to escape from sudden auditory and/or visual stimuli, but with 2) an impaired ability to move the ipsilateral hind limb, so that the net effect is a "spin" or "rotation." (A film will be shown of this effect.)

We previously reported (Science, 185, 1055 (1974)) that morphine microinjections in the periaqueductal gray resulted in a striking paradox: profound analgesia accompanied by an explosive hyperreactivity to sudden auditory and visual stimuli. These rats gave the appearance of being in a state of extreme fear, uttering shrill distress cries and making frantic, rapid leaps (of more than 60 cm vertically) although apparently unable to feel pain.

These behavioral effects have never been observed previously. Thus, morphine when microinjected directly into discrete CNS sites, gives rise to effects differing from those observed following systemic injections. This suggests that morphine has differential access to the various regions of the CNS when administered systemically.

435 EXCITATION BY MORPHINE IN THE CEREBRAL CORTEX. E. Puil*, B. Bioulac* and J.P. Lund. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Québec, Canada.

In decerebrated cats, the microiontophoretic application of morphine (50-100 nA for 0.5 to 3 min.) evoked a weak excitation of cerebral cortical neurones which were also sensitive to acetylcholine. This effect was more easily observed upon repeated applications of the narcotic and did not appear to be antagonized by naloxone (administered iontophoretically or intravenously). Iontophoresis of methylatropine (25-50 nA for 0.5 to 4 min.) prevented the activation of units by acetylcholine or morphine. Activation caused by glutamate was relatively unaltered by this antimuscarinic agent. Applications of morphine to neurones which were not spontaneously firing, or excited by test applications of acetylcholine (100 nA for 30 s), did not produce excitation but caused a depression of glutamate-evoked firing. These results suggest that the muscarinic effects of morphine in the cerebral cortex may not be related to the analgesia which occurs upon systemic administration of the compound.

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436 SYSTEMATIC MAPPING OF THE CENTRAL GRAY MEDIAL-THALAMIC AXIS OF THE RAT: EVIDENCE FOR A SOMATOTOPIC DISTRIBUTION OF MORPHINE SENSITIVE SITES WITHIN THE PERIAQUEDUCTAL GRAY. Tony L. Yaksh Thomas A. Rudy and Joseph C. Yeung*. Sch. Pharm., Univ. Wisc. Madison, WI, 53706.

Numerous investigations have been carried out to locate the site of action in brain of morphine by the employment of the microinjection technique. As a result, it is highly probable that the periaqueductal gray, particularly along its ventrolateral borders represents one important locus mediating the direct action of morphine in the production of the antinociceptive response. Little attention has been paid thus far, however, to the characteristics of the interaction of the drug with the hodolgy of the site at which it acts. Substantial information exists to suggest that the central gray is not a homogeneous structure with regard to its afferent or efferent connections. Such differences might serve to explain the localized changes in nociceptive responsiveness reported by others following electrical stimulation of the central gray.

To carry out these studies, 42 albino rats were implanted with arrays of 3 stainless steel 23 ga T.W. microinjection guides. Sites of implantation ranged from the level of the medial thalamus to the mouth of the aqueduct (AP +3.5 to AP -2.5 at distances ranging from 0.25 to 3 mm off midline). Following surgery, microinjections of morphine (1-20 ug/0.5 ul) were made at progressively deeper depths on different days in increments of 1 mm until either the deepest site or a response to the microinjection occurred. Changes in the animal's response on the hot plate, tail flick and its response to forcep pinch in all 4 body quadrants were observed at intervals of 5, 10, 40 and 60 min after the microinjection.

The following statements summarize the results of over 550 microinjection experiments carried out in 350 discrete brain sites. 1) Within the distribution of anatomical regions investigated (mesencephalic central gray through the levels of the medial thalamus) only those regions located within 1 mm of the periaqueductal gray were associated with a rapid (1-5 min latency) and long-lasting (1-3 hrs) elevation in the nociceptive threshold which was reversible by the injection of naloxone, either systemically (1mg/kg) or at the microinjection site (0.5 ug/0.5 ul). Sites lying within the medial thalamus or more lateral to the central gray produced no change in threshold with latencies less than that might be accounted for if the factor of diffusion were considered. 2) Within the central gray, we observed a crude rostocaudal, somatotopic distribution of sites producing an increase in the nociceptive threshold localized to the respective rostral of caudal regions of the body. While the predominant action of such injections was to effect a bilateral elevation in threshold, we have observed ipsilateral elevations in single body quadrants. This effect, when observed, has usually been limited to the facial region. 3) Microinjections of dibucaine (10 ug/0.5 ul) into morphine sensitive sites failed to exert any antinociceptive actions. 4) Electrical stimulation (4-40 Hz; 0.1-1.0 msec; 0.1-1.5 mA) was observed to elevate threshold in only a few of the morphine sensitive sites. Other sites, not sensitive to morphine did produce such elevations. These data suggest that the central gray is organized in a rostral caudal somatotopic pattern and that morphine may exert its antinociceptive action not by blocking the transmission of information through the central gray but by a modulatory action on other non-morphine sensitive regions.

437 THE EFFECTS OF CHRONIC COCAINE HYDROCHLORIDE ADMINISTRATION ON THE PRIMATE ELECTROENCEPHALOGRAM. P. E. Phillips*, H. L. Altshuler, D. W. Sanders*, and N. R. Burch*. Texas Research Institute of Mental Sciences and Baylor College of Medicine, Texas Medical Center, Houston, Texas 77025.

Period analysis of the primate electroencephalogram (EEG) was used to assess the effects of chronic cocaine HCl administration on the monkey brain. Cocaine, 3.0 mg/kg, was administered subcutaneously to 4 - 6 kg rhesus monkeys for 6 months, 3 times daily. Animals received intravenous challenge doses of cocaine, 3.0 mg/kg, once or twice weekly and their EEG responses measured by period analysis. Spontaneous major period, intermediate and minor period changes were analyzed with particular attention directed towards the differences in pre- and post-cocaine-dose EEG and changes in such differences over the course of the experiments. A uniform increase in pre-dose period counts was observed by the sixth week of the study, and the EEG response to cocaine was attenuated. Analysis of the frequency distribution of period counts during pre- and post-dose epochs also demonstrated an attenuated response to cocaine during the later weeks of the experiment. The ability of cocaine to alter responses to photic activation of the EEG also appeared to diminish after several weeks of chronic drug administration. The results obtained with these and other measures suggest that the chronic administration of cocaine to subhuman primates results in a diminished EEG response to the drug and, perhaps, the development of tolerance to some of the CNS effects of cocaine. (Supported in part by NIDA Grant No. 1RO1DA00799-01).

438 DURATION OF ACTION OF NALOXONE SUBCUTANEOUS PELLETS IN BLOCKING THE EEG AND BEHAVIORAL EFFECTS OF MORPHINE IN THE RAT. Gerald A. Young, J. Edward Moreton, David G. Hattan,* and Naim Khazan. Dept. of Pharmacol. and Toxicol., Sch. Pharm., Univ. of Maryland, Baltimore, Md. 21201.

It has been shown that naloxone pellets block relapse to morphine self-administration in post-addict rats (Moreton et al, Res. Commun. Chem. Pathol. Pharmacol., pub. June 1975). In the present study the duration of action of these naloxone pellets in blocking morphine effects was evaluated using electroencephalographic (EEG) and operant behavioral techniques.

Female rats were prepared with chronic cortical and temporalis muscle electrodes, implanted subcutaneously with 2 x 100 mg pellets of naloxone in the dorsal region between the shoulders and maintained in chambers equipped with swivel cable connectors for EEG and EMG recordings. Morphine sulphate, 10 mg/kg, was administered i.p. at noon every day. While such morphine injections induce cortical EEG slow waves (EEG slow bursts) and block the occurrence of REM sleep for about three hours in naive rats, in these experimental rats the naloxone pellets were effective in antagonizing morphine effects for at least ten days following pellet implantations.

In a second set of experiments, rats were operantly trained to lever press for food pellets on a variable interval one-minute schedule and implanted similarly with naloxone or placebo pellets. Saline, or morphine injections 2.5 to 10 mg/kg, were administered i.p. and the degree of disruption of the operant behavior was studied during daily 45-minute sessions. While morphine injections suppressed operant behavior in rats with placebo pellets, naloxone pellets were effective in antagonizing such morphine effects for an interval of up to two weeks. (Supported by National Institute on Drug Abuse Grant No. DA 01050.)

439 **HALOPERIDOL AND MORPHINE EFFECTS ON EVOKED RESPONSES IN CAUDATE NUCLEUS AND PINEAL BODY. H. Wachtendorf, T.F. Burks and N. Dafny. Neurobiology and Pharmacology, The Univ. of Texas Med. School at Houston, Houston, Texas 77025**

The effects of morphine on evoked responses were evaluated in caudate nucleus and pineal body, which differ markedly in neurotransmitter content. The caudate nucleus contains large amounts of dopamine and acetylcholine (ACh); the pineal body contains norepinephrine with only minute amounts of ACh. Also, the caudate is inside the blood-brain barrier while the pineal is outside the blood-brain barrier. Acoustic stimulation was used as a physiological tool to initiate synchronized polysynaptic activity in these structures. The averaged acoustic evoked responses following 32 repetitive click stimuli consisted of 5 components in both structures. The last 3 components (P_2, N_2 and P_3) were consistent and were evaluated as means of amplitudes (measured peak to peak in μV) before and after drug administration. Morphine (30 mg/kg, i.p.) significantly modified evoked responses in both structures. The three components (P_2, N_2 and P_3) of caudate nucleus responses were affected to about the same extent by morphine (73, 63 and 70% respectively), while in pineal body there was less effect on P_2 (43, 79 and 66% respectively for P_2, N_2 and P_3). In the pineal, the major effect of morphine was to increase response amplitudes in all three components, whereas the direction of change in caudate nucleus differed among the components. Because L-DOPA increases response amplitudes in caudate nucleus, the effects of haloperidol (1mg/kg, i.p.) were determined. Haloperidol decreased total responsiveness in caudate nucleus and also decreased response amplitudes. In pineal body, haloperidol did not alter responsiveness but decreased response amplitudes. Prior administration of haloperidol did not alter effects of morphine in pineal body. After haloperidol, however, morphine consistently increased response amplitudes in caudate nucleus. Atropine (.5mg/kg, i.p.) altered evoked response components in caudate nucleus and in pineal body, but did not affect the pattern of changes induced by morphine. These experiments demonstrate differential effects of morphine on evoked responses in caudate nucleus and pineal body and also indicate selective effects of haloperidol and atropine on responses to morphine. (Supported by U.S.P.H.S. Grant # DA0083.)

440 **MORPHINE MODIFICATION OF EVOKED POTENTIALS IN EXTRAPYRAMIDAL SYSTEM, N. Dafny and T.F. Burks. Departments of Neurostructure and Function and Pharmacology, The University of Texas Medical School at Houston 77025**

The effects of three dose levels of morphine on electrical responses to repetitive clicks stimuli were evaluated in unanesthetized rats. Semimicroelectrodes constructed of 60μ stainless steel wire were implanted during pentobarbital anesthesia. After recovery from surgery, field potentials evoked by an auditory stimulus were recorded in the caudate nucleus and substantia nigra before and after morphine administration in the freely behaving rats. Morphine caused dose-related bimodal changes in the amplitudes of the components of the responses in caudate nucleus. All three doses of morphine produced mixed responses (both increased and decreased amplitudes) in substantia nigra. At 10 mg/kg i.p., morphine depressed response amplitudes in caudate nucleus. The 30 mg/kg dose of morphine decreased caudate response amplitudes in some animals and increased them in others. At 50 mg/kg, morphine generally produced increased response amplitudes in caudate nucleus. At each dose level of morphine, the effects on response amplitudes began within 10 minutes of injection and persisted with only minor fluctuations during the subsequent 3 hr. Administration of naloxone (1 mg/kg i.p.) 1 hr. after morphine in other animals reversed the effects of the narcotic both at the 10 and the 50 mg/kg dose levels. Morphine thus causes different effects in separate structures within the extrapyramidal system. The responses to morphine in the caudate nucleus are bimodal, dose-related and are reversed by a narcotic antagonist. (Supported by U.S.P.H.S. Grant #DA 00803).

441 METHADONE: ITS INFLUENCE ON SUBCORTICAL STRUCTURES IN RAT BRAIN
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Effects of intravenous injection of methadone on gross electrical activity & on unit discharge were studied in cortical(somatosensory) & subcortical areas(amygdala, hippocampus, septum, thalamus, hypothalamus & midbrain reticular formation) in naive rats under flaxedil. From gross electrical recording it appears that with a small dose of methadone(0.5mg-0.75mg/Kg), the amygdala and the hypothalamus are the most sensitive subcortical structures studied so far. With moderate (0.75mg-1.5mg/Kg) and large(1.5mg & higher) doses, all subcortical structures may become involved. Following a moderate or large dose, the thalamus may become the generator for spike activity which resemble very much the interictal spike on EEG tracings. Methadone also altered markedly the evoked responses in somatosensory cortex following stimulation of amygdala and n. ret. thalami. Unit discharge in amygdala(n. basolat.) was altered dramatically. A large number of units which previously had very little spontaneous activity showed paroxysmal discharge. On the other hand, a small group of units showed burst activity only in the presence of spikes which presumably had their origin in the thalamus. Units were found in the midbrain reticular formation which either increased or decreased their spontaneous firing rates. Units were also found in the same area which did not respond to methadone. The data indicate that subcortical structures, especially the amygdala, undergo profound changes during methadone treatment. Causes of these changes are being investigated. (Supported by USPHS Grant MH 20946)

442 THE EFFECTS OF ACUTE ETHANOL TREATMENT ON THE REGIONAL TURNOVER OF CATECHOLAMINES IN RAT BRAIN. N. G. Bacopoulos*, R. K. Bhatnagar and L. S. Van Orden III. Dept. Pharmacology, University of Iowa, Iowa City, Iowa 52242.

The effect of acute administration of ethanol (2 g/kg ip., 20% v:v) on the turnover of brain norepinephrine (NE) and dopamine (DA) was studied. In one series of experiments, rats were injected intraventricularly with 50 µCi of ^{3}H-tyrosine. Two hours after ethanol injection, the specific activities of NE and DA in the telediencephalon were reduced by 50%. The specific activity of NE in the brain stem was the same in control and ethanol treated rats. The specific activity of tyrosine and the endogenous levels of NE and DA, were not altered by ethanol, either in the telediencephalon or in the brain stem (pons and medulla).

The turnover of NE and DA in four brain regions was estimated by inhibiting the synthesis of catecholamines with α-methyl-p-tyrosine (250 mg/kg and 125 mg/kg ip., 2 hrs later). Ethanol or saline was injected 15 min. before αMPT. NE and DA levels in frontal cortex, hypothalamus, striatum and brain stem were determined by an enzymatic-isotopic method at various times after αMPT. During the first two hours of ethanol intoxication the turnover of hypothalamic NE and striatal DA was reduced significantly. A slight but nonsignificant decrease of NE turnover was observed in the frontal cortex. NE turnover in the brain stem and DA turnover in the hypothalamus were not altered by ethanol treatment. Steady state levels of NE or DA were not altered by ethanol in any of the parts examined, with the exception of NE in the frontal cortex, which declined by 15% at two hours. The results of this study suggest that a depression of some central catecholaminergic systems may be associated with the effects of low doses of ethanol. (Supported by NIH grant GM12675 and GM00141.)

443 DIFFERENTIAL EFFECTS OF ETHANOL ON MULTIPLE-UNIT ACTIVITY OF SPECIFIC BRAIN AREAS. W. R. Klemm, Department of Biology, Texas A&M University, College Station, TX 77843.

Preliminary studies in this laboratory (Brain Res. 70: 361, 1974) suggested that recording of multiple-unit activity (MUA) was a sensitive method for testing the hypothesis that ETOH differentially affects neurons in specific brain regions. This present research developed a testing and analysis program for identifying possible primary target sites (i.e., brain areas with drug-induced MUA changes at the lower doses and at the shorter post-injection latencies).

Electrodes for concurrent EEG and MUA recording were chronically implanted in 13 brain areas of 14 rabbits. Each rabbit randomly received, with at least 5-day intervals, an intraperitoneal injection of 300, 600, 900, and 1200 mg/kg of 20% ETOH and a saline control volume equal to the 600 mg/kg dose. Recordings were made continuously for a 2-min pre-injection control period and a 15-min post-injection period.

Use of a Box and Jenkins time series analysis computer simulation led to the development of a sensitive manual quantification method for the continuous analog integrator display of MUA. Tentative results supported the hypothesis of differential responsiveness to ETOH, both in terms of brain areas affected and response latency. Some brain areas were relatively insensitive to ETOH, even in larger doses; other areas showed conspicuous short-latency (within about 1-2 min post-injection) MUA responses to even the lowest dose. These regional and temporal differences were not evident in the corresponding EEG, as analyzed visually. (Supported by NIAAA grant AA00373).

444 **ALCOHOL MODIFIED FIELD POTENTIALS IN BASAL GANGLIA CORRELATE WITH PLASMA ALCOHOL LEVELS.** **T. Rujirekagulwat, H.R. Matthews and N. Dafny. Neurobiology, The University of Texas Medical School at Houston 77025**

Electrophysiological experiments were performed in conscious rats implanted one week earlier with permanent electrodes in the head of the caudate nucleus and within the substantia nigra. The averaged acoustic evoked responses from 32 repetitive click stimuli were recorded simultaneously in both structures every ten minutes for a period of one hour prior to alcohol administration. Following these control recordings, each rat was given ethanol (1.67gm/kg, i.p. in 0.9NaCl). Blood alcohol levels over the subsequent 5 hour period had been previously determined in a group of weight matched control rats under identical conditions. Following injection, the blood alcohol level rose within 15 minutes to a mean of 1.7 mg/ml, and remained between 1.6 and 1.8 mg/ml for a period of 2 hours. Thereafter, the blood alcohol level declined linearly at 0.4 mg/ml/hr. The averaged auditory evoked responses were again obtained on each animal at three time periods following alcohol injection: 1) at 15-60 min., at 2-3 hr., and at 4-5 hr., when the blood alcohol level had declined to one-half of its maximum level. Results were averaged on-line with a Nikolet 1070 computer. Alcohol in general caused a significant reduction in all components of the averaged acoustic evoked response in both structures. The intensity of this reduction was directly correlated to the blood alcohol level and decreased over time. Moreover, about 80% of the recordings from the caudate nucleus (P_2 and N_2) demonstrated significant decreases in the amplitudes of the evoked responses, while less than 40% of the recordings obtained from the substantia nigra exhibited decreases. In conclusion, alcohol attenuated the averaged acoustic evoked responses with direct correlation to the alcohol levels in the blood. Different responsiveness was observed in the caudate nucleus as compared to the substantia nigra.

445 UNEXPECTED INTERACTIONS BETWEEN ALCOHOL AND D-AMPHETAMINE ON BEHAVIOR OF RATS. Richard H. Rech and Mary K. Vomachka* Dept. of Pharmacology, Mich. State Univ., East Lansing, Mich. 48824.

Amphetamines have generally been classed as analeptics that would counteract effects of CNS depressants such as barbiturates or alcohol. The present study, however, indicates that d-amphetamine (dA) combined with alcohol (Alc) or other CNS depressants may intensify and prolong impaired behavior in rats over that observed with the CNS depressant alone. In comparing the interactions of dA and Alc on rotarod performance (RR), 2 mg/kg dA + 1.5 G/kg Alc prolonged the impairment of RR beyond that noted with Alc alone. Doses of 4 or 6 mg/kg dA + 1.5 G/kg Alc were less disrupting than the 2 mg/kg dA + Alc. The combination of 8 mg/kg dA + 1.5 G/kg Alc greatly prolonged RR impairment (greater than 8 hrs), as well as inducing coma and lethality (within 24 hrs) in 2/3 of subjects. Rats treated with the same drug pair but not tested on RR showed lethality in only 1/6 of subjects. Other dose combinations of dA + Alc indicated a two-fold interaction: the low dose of dA appears to potentiate directly the disrupting effects of Alc on RR, while the high dose of dA induces a delayed impairment of RR (and lethality) when combined with Alc. Interactions of 2 mg/kg dA with pentobarbital or diazepam also showed enhanced disruption of RR over the effect of the depressant drug alone. However, 8 mg/kg dA + pentobarbital or diazepam did not result in the profound depression or lethality noted with the 8 mg/kg dA + Alc. Combinations of various doses of cocaine + Alc did not show the types of interactions seen with dA + Alc. Thus, the complete spectrum of interactions between dA and Alc is peculiar to this drug pair and is not duplicated with combinations involving related stimulants and depressants. (Supported by NIDA Contract ADM-45-74-146).

446 *In Vitro* Mechanisms of D-Amphetamine Sulfate Inhibition of Brain Protein Synthesis. M.A. Moskowitz, B.S. Baliga*, J. Zahringer*, H. Munro and R.J. Wurtman. Dept of Nutrition and Food Science, M.I.T., Cambridge, Mass. 02139.

We have previously shown that large doses of d-amphetamine sulfate disaggregate whole brain polyribosomes (an effect which can be blocked by pretreatment with drugs that block dopamine receptors) and inhibit *in vivo* brain protein synthesis in adolescent and adult rats. To determine the extent to which amphetamine inhibits *in vitro* protein synthesis, the rate of incorporation of isotopically labelled amino acids was measured in the presence of varying concentrations of amphetamine (0,1,5, and 10mM) and a cell-free system containing wheat germ ribosomal subunits utilizing m-RNA isolated from rat brain polysomes. At 5 and 10 mM concentrations, the rate of 14-C-leucine incorporation into protein was reduced by 30 and 50%, respectively. These observations suggest that high concentrations of amphetamine can inhibit protein synthesis *in vitro* by its direct action at the translational level of cellular protein synthesis.

447 NEONATAL MORPHINE TREATMENT: CONSEQUENCES IN ADULT FEMALE RATS. Theo Sonderegger and Emery Zimmermann. Dept. Anat., Sch. Med., UCLA, Los Angeles, 90024.

To study long-term effects of neonatal morphine (M) addiction female rats received twice daily s.c. injections of M on either Days 3-12 (to 8 mg/kg injection) (M_1) or Days 12-21 (to 16 mg/kg injection) (M_2). Controls received saline (S_1 or S_2) or no treatment (NT). All pups were handled and weighed daily; the overall mortality rate was 5%.

Body weights of M_1 and M_2 rats were temporarily suppressed ($p<0.02$) relative to those of their controls, but initially reduced M_1 and M_2 growth rates returned to those of the other groups during the treatment periods. On behavioral testing during Days 29-31 M and control groups did not differ on open-field measures but on Days 90-95 M_1 animals showed impaired learning of a conditioned emotional response compared to the other groups. On Day 40, but not on Day 145, M_1 animals showed increased ($p<0.05$) levels of plasma corticosterone 30 min following injections of naloxone (5 mg/kg). On Day 158 both M_1 and M_2 animals demonstrated diminished analgesic responses to M when tested with the hotplate technique. Compared to their controls on Day 170, M_1 and M_2 animals showed intact corticosterone responses to M (40 mg/kg) challenge.

These findings in part confirm earlier work and indicate that neonatal addiction to M produces temporary as well as certain long-lasting alterations of developmental, pituitary-adrenal, nociceptive, and behavioral responses in the female rat. (Supported in part by USPHS grant DA 826 & U. Neb. Research Council funds)

448 REGIONAL BRAIN LEVELS OF CYCLIC NUCLEOTIDES, GAMMA AMINOBUTYRIC ACID (GABA) AND GLUTAMATE DURING CHRONIC BARBITURATE INGESTION AND WITHDRAWAL. R.H. Lenox*, H.L. Wray*, G.J. Balcom*, T.D. Hawkins* and J.L. Meyerhoff, Div Neuropsychiatry, Dept Neuroendocrinology, Walter Reed Army Inst. of Res, Washington, D.C. 20012

The neurochemical basis of chronic barbiturate depression and subsequent abstinence convulsions has not been established. Attempts to relate changes in whole brain and cortical GABA concentration to barbiturate intoxication and withdrawal have been generally unsuccessful. Recent studies have shown cyclic nucleotides to be possible mediators of the action of several neurotransmitters in the central nervous system (CNS). We have studied the levels of two putative neurotransmitters, GABA and glutamate, as well as cyclic adenosine 3',5'-monophosphate (cAMP) and cyclic guanosine 3',5'-monophosphate (cGMP) in thirteen regions of the rat brain during both chronic barbital ingestion and withdrawal. Male albino rats maintained at 300 gm were given a sweetened (4% sugar) sodium barbital solution as sole source of liquid for three weeks, reaching maximum intake levels of 450 mg/kg/day. An isocaloric control group was run simultaneously. After determining appropriate microwave inactivation parameters, animals were sacrificed by high power microwave irradiation during chronic intoxication and at 36 hours after withdrawal of the drug. The latter time was found to correlate with the greatest sensitivity to audiogenic convulsions. Levels of GABA, glutamate and cAMP remained unchanged vs control values in all groups. cGMP, however, was decreased to levels as low as 25% of control in 9 out of 13 brain regions in animals on chronic barbital intake. During withdrawal, cGMP levels in all brain regions were equal to or higher than control values. These data suggest a possible relationship between the state of excitability of the CNS and cGMP levels in specific brain regions.

449 ACUTE AND CHRONIC MORPHINE ADMINISTRATION ON BRAIN γ-AMINOBUTYRIC ACID (GABA) LEVELS IN THE MOUSE. I.K. Ho and S.F. Tzeng* Dept. of Pharmacol. and Toxicol. Univ. of Miss. Med. Ctr., Jackson, Miss. 39216.

In male ICR mice receiving a s.c. administration of morphine sulfate, 15 mg/kg, brain levels of GABA were significantly higher than those of the control group, being 7 and 18% at 30 and 60 min. respectively. With the administration of morphine sulfate, 30 mg/kg, the brain levels of GABA were further increased to 11 and 20% at 30 and 60 min. after the administration. To monitor GABA levels during the development of tolerance to morphine animals were rendered tolerant-dependent by implanting a specially formulated 75 mg morphine pellet for the maximum of 72 hours. The control group received a placebo pellet for a same period of time. At 8 hr. after morphine pellet implantation, the GABA level was not significantly different from that of the control. However, in mice receiving a morphine pellet implantation for 24 or 72 hr, the brain levels of GABA were 10% lower than those of the control group. After abrupt withdrawal from morphine for 2 or 6 hr., the brain GABA level was further decreased to 80% of the placebo treated animals. At 24 hours after the removal of morphine pellet, the brain GABA level in morphine withdrawal group was still significantly lower than that of the control. The glutamic acid decarboxylase activity in morphine pellet implanted group was also inhibited more than 50%. The present studies further confirm our previous finding that the inhibitory neurotransmitter, GABA, is indeed involved in morphine analgesia and the development of tolerance to and physical dependence on morphine. (Supported by NIMH Grant DA-00563. I.K. Ho is a recipient of a Pharmaceutical Manufacturers Association Foundation Faculty Development Award in Basic Pharmacology).

450 FACILITATION OF MORPHINE DEPENDENCE BY BRAIN EXTRACT FROM DEPENDENT RATS. David H. Malin and Glen Radcliffe, Jr.* Univ. Houston at Clear Lake City and Baylor Coll. Med., Houston, TX 77025.

Brain extracts from morphine injected rats can reportedly confer a degree of tolerance on recipient animals (Int. J. Neuropharmacol. 5:183, 1966; Proc. Soc. Exp. Biol. Med. 130:287,1969). This study inquired whether extracts from morphine dependent rats would facilitate physical dependence formation in recipient rats. Donor rats were injected three times a day for 18 days with increasing doses of morphine sulphate. Aqueous extracts were prepared from their brains and those of control donors. Recipient rats, injected i.p. with these extracts, were then given several small priming doses of morphine (insufficient in themselves to induce marked dependence in normal rats). Dependence was determined by observing the severity of spontaneous withdrawal following the last injection, as measured by the number of wet dog shakes during a 15 minute "blind" test every day for 10 days. In each of six experiments with different doses of extract and different "priming" schedules, recipients of extract from morphine dependent donors made significantly more shakes than did the recipients of control donors. In some cases, the significant differences lasted over a week. In another experiment, naloxone-precipitated shaking was significantly elevated (three-fold) in experimental recipients. Effects of the extracts were dose dependent and reversible by morphine. Thus, morphine injection may induce synthesis of some brain substance that can cause or facilitate morphine dependence. Morphine itself is almost certainly not the active substance, since chemical assay of this type of extract has revealed the absence of any pharmacologically meaningful amount of morphine.

451 MORPHINE ANALGESIA: ATTENUATION AFTER 6-HYDROXYDOPAMINE LESIONS OF THE NIGRO-NEOSTRIATAL DOPAMINERGIC PROJECTION AND POTENTIATION AFTER LESIONS OF THE DORSAL NORADRENERGIC PROJECTION. Marion T. Price* and Hans C. Fibiger (SPON: D. P. Cain). Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, Canada.

The results of recent investigations have implicated both dopaminergic and noradrenergic neuronal pathways in the production of morphine analgesia. In the present study we examined the analgesic response to morphine in rats, using the tail flick test, after selective destruction of central catecholaminergic pathways with 6-hydroxydopamine (6-OHDA). Rats which were pretreated with desipramine hydrochloride (25 mg/kg) and then given bilateral 6-OHDA injections (8 µg/4 µl) into the zona compacta of the substantia nigra did not show an analgesic response to morphine (2 mg/kg) 10 days later. These lesions depleted striatal dopamine to 10 percent of control levels, but did not significantly affect brain noradrenaline (NA) levels. Lesions of the mesencephalic noradrenergic pathways (4 µg/2 µl) which reduced hypothalamic and cortical NA by more than 80 percent resulted in a potentiated and prolonged analgesia. In another experiment, the dorsal or the ventral noradrenergic pathways were selectively lesioned. Rats lesioned in the dorsal tegmental NA bundle demonstrated the exaggerated analgesic response to morphine, whereas tail flick latencies of those lesioned in the ventral NA bundle were not different from controls. These results (1) suggest that the integrity of the nigro-neostriatal pathway is an important neural substrate for morphine analgesia, and (2) implicate the dorsal noradrenergic pathway in previously reported demonstrations of potentiated morphine analgesia following pharmacological interference with central noradrenergic mechanisms.

(Supported by the Medical Research Council of Canada)

452 BRAIN REGION-SPECIFIC CYCLIC NUCLEOTIDE SYSTEMS IN NARCOTIC ANTAGONISM AND WITHDRAWAL. K. A. Bonnet and A. Sunshine*. Dept. Psychiatry., Sch. Med., NYU, New York, 10016

Regional changes in cyclic nucleotide levels have been monitored in acute and chronic morphine treatment, and during withdrawal precipitated by naloxone. Morphine produces decreases in cyclic AMP in hypothalamus, substantia nigra and periventricular grey which is accompanied by greater decreases in cyclic GMP. No such changes are seen on a challenge injection to tolerant animals, though steady state levels are altered. Naloxone produces elevations in caudate cyclic AMP and in pvg cyclic GMP in the drug naive animal. These effects are followed during precipitated abstinence in the chronic animal and relate to the neurochemistry and locus mediating abstinence.

453 THE EFFECTS OF NARCOTICS AND ADRENERGIC BLOCKING AGENTS ON THE ACCUMULATION OF ^{3}H-c-AMP IN RAT HYPOTHALAMIC SLICES. T.M. Badger* and T.J. Cicero* (SPON: E. Robins). Washington University School of Medicine, Department of Psychiatry, St. Louis, MO. 63110.

The effects of narcotics and several alpha and beta adrenergic blocking agents on the accumulation of ^{3}H-c-AMP in rat hypothalamic slices were evaluated. Alpha adrenergic blockers alone enhanced the accumulation of ^{3}H-c-AMP, whereas beta adrenergic blockers had no appreciable effect on its formation. Both alpha and beta adrenergic blockers did, however, block norepinephrine-induced stimulation of the adenyl cyclase system in hypothalamic slices. The narcotics, like alpha adrenergic blockers, also slightly enhanced the accumulation of ^{3}H-c-AMP in this system and antagonized the effects of norepinephrine on the accumulation of the ^{3}H-c-AMP. The reduced level of norepinephrine-induced stimulation of adenyl cyclase produced by the alpha blockers or narcotics was similar to the slightly enhanced accumulation of ^{3}H-c-AMP observed following incubation of hypothalamic slices in the presence of these agents alone. On the basis of these observations, it may be that alpha blockers and the narcotics block norepinephrine's effects on the adenyl cyclase system in rat hypothalamus by directly stimulating the accumulation of ^{3}H-c-AMP themselves. (Grants: DA-00109; DA-00259; and AA-70801)

454 EFFECT OF SINGLE AND MULTIPLE INJECTIONS OF METHADONE ON LOCOMOTOR ACTIVITY OF MICE. Lawrence D. Middaugh and Carroll A. Santos III*. Dept. of Biochemistry and of Psychiatry and Behavioral Science, Medical University of South Carolina, Charleston, 29401.

Methadone has been reported to both increase and decrease locomotor activity in mice and rats. Two obvious variables which could contribute to the contrary results are drug dose and species or strain of rodent used. It has previously been reported that morphine will produce increases in activity for some strains of mice but have no effect or produce decreases in activity for other strains. In the current study we examined the effects of methadone on locomotor activity in three strains of mice.

Locomotor activity of Swiss-Webster, C57BL/6J, or DBA/2J mice was assessed for four hours following subcutaneous injections of methadone (2.5mg or 15.0 mg/kg). Both doses of the drug increased the activity of the Swiss-Webster and C57 strains. The heightened activity following methadone injection was due primarily to less reduction in activity across time than was observed for saline control animals. The higher dose produced high activity levels for a longer time than the lower dose. On the contrary, the high drug dose produced a transient decrease in the locomotor activity of DBA/2J mice. The Swiss-Webster strain was used to assess the effects of multiple injections of the drug on activity. Animals receiving daily injections of methadone (15 mg/kg) for 14 days and then tested for four hours following injection of the drug on Day 15 had activity patterns similar to animals receiving an initial injection of 2.5 mg/kg. Hence, tolerance to the heightened activity produced by the drug is evident after 14 days of exposure.

455 EFFECT OF SEROTONERGIC AGONISTS AND ANTAGONISTS ON MOTOR ACTIVITY IN RATS. Donald M. Kuhn[X] and James B. Appel. Dept. Psych., Univ. of S.C., Columbia, S.C. 29208.

The motor activity of groups of rats was recorded for 3 hours after treatment with drugs which have effects on the serotonergic neuronal system. Lysergic acid diethylamide (LSD), which stimulates 5-HT receptors, produced a dose-related (80 - 520 μg/kg) increase in motor activity. After larger doses of LSD (520 μg/kg) rats displayed several symptoms also seen with l-tryptophan loading after MAO inhibition; these include head weaving, reciprocal forepaw treading, and hindlimb abduction. The LSD-induced increase in motor activity was blocked by methiothepin (0.5 - 3.0 mg/kg), methysergide (5.0 mg/kg), and cinanserin (15.0 mg/kg), which are putative 5-HT antagonists. Pretreatment with p-chlorophenylalanine (PCPA - 3 x 100 mg/kg), 12 days prior to LSD, potentiated the LSD-induced increase in motor activity. Quipazine (2.5 - 10 mg/kg), a drug with serotonergic actions, also increased motor activity and was blocked by cinanserin, methysergide, and cyproheptadine. Quipazine treated rats demonstrated reciprocal forepaw treading and head weaving but to a lesser degree than seen after l-tryptophan-pargyline treatment. Grahame-Smith (J. Neurochem. 18: 1053, 1971) hypothesizes that the hyperactivity syndrome produced by l-tryptophan-pargyline treatment in rats is due to 5-HT spilling over into functional activity. Our data are consistent with the hypothesis that the 5-HT system is involved in the hyperactivity syndrome and offer behavioral evidence that LSD and quipazine directly stimulate 5-HT receptors. Since neither LSD nor, apparently quipazine, are substrates for MAO, pargyline pretreatment is not necessary for these drugs to produce hyperactivity.

456 BEHAVIORAL EFFECTS OF L-5-HYDROXYTRYPTOPHAN (L-5-HTP) FOLLOWING LESIONS IN THE MEDIAL FOREBRAIN BUNDLE (MFB): EFFECT OF 6-HYDROXYDOPAMINE (6-HDA). L. M. Yunger and J. A. Harvey. Dept. Biol., Univ. of Pittsburgh, Pittsburgh, PA. 15260 and Dept. Psych., Univ. of Iowa, Iowa City, IA. 52242.

Lesions in the MFB, which transsect the ascending serotonergic pathway, produce a decreased jump threshold to footshock. Both the serotonin content of telencephalon and the jump threshold can be returned to normal levels by 37.5 mg/kg L-5-HTP. Inhibition of the peripheral decarboxylation of L-5-HTP by RO4-4602 did not affect the ability of L-5-HTP to reverse the behavioral effects of MFB lesions. In contrast, the jump threshold of lesioned rats receiving 6-HDA, which produced an 80-95% depletion of catecholamines in telencephalon, was not significantly increased by L-5-HTP either alone or in combination with RO4-4602. None of these drug treatments had any effect on the jump threshold of controls. However, the accumulation of serotonin from L-5-HTP in the presence of RO4-4602 was significantly reduced (-38%) in control rats following 6-HDA pretreatment. These studies suggest that catecholamine-containing neurons may, in part, mediate 1) the formation of serotonin from L-5-HTP and 2) the behavioral effects of L-5-HTP following serotonin depletion. (Supported by USPHS grants MH-16841 and MH-10641).

457 SEROTONERGIC INFLUENCES ON ROTATIONAL BEHAVIOR IN RATS.
Lloyd N. Fleisher* and Stanley D. Glick, Dept. Pharmacol., Mt. Sinai Sch. Med., CUNY, N. Y., 10029

The effects of serotonergic (5-HT) drugs on circling (rotation) behavior in unlesioned rats were examined using an automated rotometer. D-LSD (0.062, 0.125, 0.25, 0.5, 1 and 2 mg/kg), 5-methoxy-N,N-dimethyltryptamine (MDMT; 1 and 2.5 mg/kg) and methysergide (10 mg/kg) elicited significant rotation which was consistent in direction and magnitude from week to week. L-LSD was approximately 8 times less potent than d-LSD in eliciting rotation. Cyproheptadine (5 and 10 mg/kg) failed to produce significant rotation. Rotation with 0.125 mg/kg d-LSD was significantly increased 3 and 10 days after p-chlorophenylalanine (300 mg/kg). L-tryptophan (200 and 400 mg/kg) had no effect on d-LSD (0.25 mg/kg)-induced rotation, but haloperidol (0.5 mg/kg) completely blocked it. D-LSD (0.25 and 0.5 mg/kg), MDMT (1 mg/kg) and methysergide (10 mg/kg) caused rotation in the same directions, but there was no consistent relationship between d-LSD and apomorphine (10 mg/kg)-induced rotation. These results implicate a modulatory influence for 5-HT on the dopaminergic activity of the neostriatum. D-LSD and MDMT do not appear to interact directly with neostriatal dopamine receptors, but may increase dopaminergic activity by decreasing 5-HT input.

458 INCREASED SEIZURE SUSCEPTIBILITY IN RATS AFTER SELECTIVE DESTRUCTION OF SEROTONERGIC NEURONS BY 5,7,DIHYDROXYTRYPTAMINE (5,7-DHT)
R.A. Browning, W.E. Hoffmann* & M.R. Christofersen*. Southern Illinois University School of Medicine, Carbondale, Ill. 62901

In view of the suggested role of serotonin (5-HT) in seizures, it was of interest to examine seizure susceptibility in rats with a chronic 5-HT deficit. An intraventricular injection of 200 µg 5,7-DHT was found to lower forebrain 5-HT and norepinephrine (NE) by 88 and 60% respectively, without influencing dopamine. Pretreatment with protriptyline HCl (PTL) prevented the effects of 5,7-DHT on forebrain NE without altering its effect on 5-HT. A 63% reduction in brainstem 5-HT was also observed after 5,7-DHT. Although brainstem 5-HT appeared to recover slightly after 45 days, no recovery was observed in forebrain 61 days after injection of 5,7-DHT. These data are consistent with the idea that 5,7-DHT produced a degeneration of 5-HT nerve terminals. Rats treated two hours earlier with PTL (15 or 20 mg/kg) were injected intraventricularly with 200 µg 5,7-DHT, and subjected to Metrazole or maximal electroshock seizures (MES) 35 to 45 days later. A slight (20%) reduction in NE was observed in animals receiving 15 mg/kg PTL, but the 20 mg/kg dose completely prevented this. In the Metrazole seizure test an increase in seizure susceptibility was evidenced by a significant prolongation of seizure duration ($P<.01$) and an increased incidence of death in experimental compared to control animals (30% vs 0%). In the MES test, 5,7-DHT-treated rats exhibited a shorter latency to tonic extension ($P<.001$) and a significant prolongation of tonic extension ($P<.01$). Unlike animals treated with 6-hydroxydopamine, these rats failed to exhibit a prolonged recovery phase. These results indicate an enhanced susceptibility to seizures in animals chronically and selectively deficient in brain 5-HT. (Supported in part by a grant from the Epilepsy Foundation of America).

459 A BEHAVIORAL SYNDROME FOR THE STUDY OF CNS SEROTONIN RECEPTOR ACTIVITY: MEDIATION BY LOWER BRAINSTEM; EVIDENCE FOR DENERVATION SUPERSENSITIVITY; AND MECHANISM OF ACTION OF P-CHLOROAMPHETAMINE. Barry L. Jacobs. Dept. Psychol., Princeton Univ., Princeton, N. J. 08540.

A behavioral model for studying the activity of postsynaptic serotonin receptors in the CNS is proposed. It consists of the response complex of tremor, rigidity, reciprocal forepaw treading, Straub tail, hindlimb abduction and lateral head weaving in the rat, and is produced by a variety of compounds that either increase the availability of synaptic serotonin or directly stimulate postsynaptic serotonin receptors. When this constellation of neurological signs is displayed simultaneously it is indicative of stimulation specific to serotonin receptors. The present investigations demonstrate that: 1) the entire syndrome is mediated by neuronal mechanisms present in the pons, medulla and spinal cord (complete transections were made at various levels of the neuraxis and animals were administered pargyline (50 mg/kg i.p.) plus L-tryptophan (150 mg/kg i.p.)); 2) the syndrome is useful for quantitatively demonstrating behavioral evidence for the development of denervation supersensitivity in the serotonin system (5-methoxy-N, N-dimethyltryptamine, in varying doses, was administered to control animals and 5, 6-dihydroxytryptamine pretreated animals); and 3) the syndrome is useful for studying the mechanism of action of "serotonergic" drugs (p-chloroamphetamine produces the syndrome after a short latency, and this effect is blocked by prior serotonin synthesis inhibition).

460 ANALGESIC EFFECT OF LILLY 110140, A SPECIFIC UPTAKE INHIBITOR FOR SEROTONINERGIC NEURONS. R. B. Messing, L. Phebus*, L. A. Fisher* and L. D. Lytle. MIT, Cambridge, Ma. 02139.

Rats were tested using a modified flinch-jump procedure to obtain response thresholds to painful electric shocks, after injection of Lilly 110140, a drug that blocks uptake of serotonin (5-HT) into brain neurons. Four groups of rats (N = 7) were given either the H_2O vehicle, or 5, 10 or 20 mg/kg of 110140, and tested 2, 24 and 48 hrs after the i.p. injection. The results (see table) show an analgesic effect at all doses tested for at least 48 hrs. Furthermore, the effect appears to be dose dependent, with the higher doses producing greater analgesia. These data support

Time (hrs)	Vehicle	5 mg/kg	10 mg/kg	20 mg/kg
2	.72±.04[a]	.98±.08*	1.08±.14*	1.02±.05***
24	.72±.07	1.00±.03*	1.15±.13*	1.09±.06**
48	.78±.07	1.03±.11	1.12±.07**	1.17±.04***

[a]All values are mean jump thresholds in mA ± S.E.M.
*$p<.05$, **$p<.01$, ***$p<.001$ compared to vehicle injected control group.

the hypothesis that brain 5-HT neurons may mediate the analgesic response to painful stimuli. (Supported in part by a postdoctoral fellowship from The Medical Foundation to R.B.M. and a grant from the W.T. Grant Foundation to L.D.L.)

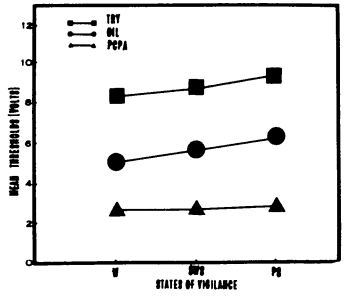
461 SENSITIVITY DURING STATES OF VIGILANCE IN RELATION WITH SEROTONIN LEVEL†

J.-P. Guillemot*, F. Leporé*, and M. Ptito, (SPON: J. Montplaisir), Dept. Psych., Univ. Montréal, and Univ. Québec à Trois-Rivières, Québec, Canada.

Clinical and experimental literature have indicated that increasing or decreasing the level of serotonin (5-HT) respectively decreased or increased somesthetic sensitivity. An alternate series of research by Jouvet, has shown this neurotransmitter to be implicated in the process of cortical synchronization characteristic of slow wave sleep (SWS). The aim of the present experiment was to determine whether these two phenomena, namely sensory modulation and cortical synchronization might depend upon a common mechanism. To test this hypothesis, we evaluated the threshold to a painful stimulus in sleeping (SWS and paradoxal sleep (PS)) and waking cats in relation to the inhibition and activation of the serotonergic system.

Method : The subjects were 6 adult naive cats. They were implanted with EEG, EOG and neck EMG recording electrodes in the classical manner. Stimulating electrodes were implanted s.c. on the back of the animals. 5-HT synthesis was respectively inhibited and activated by s.c. injections of 150 mg/kg/day of Parachlorophenylalanine (PCPA) and i.p. injection of 800 mg/kg/12 hours of DL-Tryptophan (TRY). The control group was injected the medium (oil) within which PCPA and TRY were suspended. The behavior of the animal was monitored with a closed circuit T.V. system, EEG, EOG and EMG electrical activity were amplified, displayed on a four channel oscilloscope and recorded on magnetic tapes. When necessary, they were also recorded on a polygraph. Pulsating DC electric shocks were given with a Grass stimulator. The thresholds were determined for each subject during waking, SWS and PS in the different drug states.

Results : Thresholds, expressed in volts, were analyzed using a factorial model of analysis of variance. Thresholds during the different levels of vigilance in relation to 5-HT level are shown in Fig. 1. The principal effects of drugs and states of vigilance are significant at the 0.001 level. The drug-state of vigilance interaction is significant. However a decomposition of this interaction effect shows that only the PCPA versus the other drug states when PS is compared to the two other states of vigilance is significant at the 0.01 level.



Discussion : These results indicate : 1) Inhibition or activating the serotonergic system with PCPA and TRY respectively decreases and increases the thresholds to painful electric stimuli. 2) Independently of drug states these thresholds are lowest during waking and highest during PS, with SWS holding an intermediate position. 3) The general lack of interaction between drugs and states of vigilance indicates that the serotonergic modulation of sensory input and of cortical synchronization during SWS probably do not depend upon a common mechanism. This of course is in agreement with electrophysiological studies of sleep which show that sleep is not a passive state during which the brain is less receptive to sensory stimulation but rather an active state whereby reticular structures actively synchronize brain activity. 4) The interaction at the level of PCPA vs the other drug states and PS vs the two other states of vigilance probably indicates that the 5-HT depletor effect of PCPA at this dosage level and for such a long duration decreases threshold to such a low level that even during PS the animal is excessively sensitive. The increase in threshold during PS which onewould need to insure the parallelism of the threshold curves does not thereby manifest itself.

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462 EVIDENCE FOR AN INHIBITORY FUNCTION FOR BRAIN SEROTONIN SYSTEMS IN THE LOCOMOTOR STIMULANT EFFECTS OF D-AMPHETAMINE. A.S. Hollister, G.R. Breese, C.M. Kuhn*, B.R. Cooper*, and S.M. Schanberg. University of North Carolina, Chapel Hill, N. C. and Duke University, Durham, N. C.

Recent research has demonstrated that inhibition of serotonin synthesis potentiates d-amphetamine stimulated motor activity, suggesting that serotonin systems in brain may perform an inhibitory function. This paper investigates further the effect of altering brain serotonergic activity on the locomotor stimulation produced by d-amphetamine. Pretreatment of rats with p-chlorophenylalanine was found to increase locomotor activity induced by d-amphetamine (3 mg/kg) nearly twofold. This increase was antagonized by 75 mg/kg 5-hydroxytryptophan (5HTP) administered one hour before d-amphetamine, but not by 100 mg/kg L-tryptophan. The serotonin receptor blocking agents, cyproheptadine and methysergide, also potentiated in a dose related fashion the locomotor response to 2 mg/kg d-amphetamine. In addition, a potentiation of d-amphetamine-induced locomotion was obtained after treatment with a tryptophan-free diet (1,2, 4,8 or 14 days) or after 48 hours of food deprivation. An involvement of serotonergic fibers in this response was inferred from the findings that two days of tryptophan-free diet or food deprivation greatly reduced brain serotonin turnover and that the potentiation was antagonized by increasing doses of L-tryptophan or 5HTP. Changes in the distribution of d-amphetamine and its metabolites were insufficient to account for the increased response to d-amphetamine after these brain serotonin manipulations. These results are consistent with the view that serotonergic systems may inhibit the locomotor effects of d-amphetamine in the rat. Such an inhibitory function for a brain monoamine system may explain the "paradoxical" calming effects of d-amphetamine in hyperkinetic children. (Supported by USPHS Grants MH-16522, HD-03110, MH-0013, MH-13688, and MH-11107, The Alfred P. Sloan Foundation and the PMA Foundation, Inc.)

463 BEHAVIORAL EFFECTS OF L-TRYPTOPHAN IN PIGEONS AND CONCURRENT NEUROCHEMICAL CHANGES IN NERVE ENDING FRACTIONS ISOLATED FROM THE TELENCEPHALON. J.E. Smith*, J.N. Hingtgen*, W.J. McBride and M.H. Aprison. The Institute of Psychiatric Research, Depts. of Biochemistry and Psychiatry, Indiana University Med. Ctr., Indianapolis, IN. 46202.

We have recently demonstrated that 300 mg/kg L-Tryptophan (Try) administered (i.m.) to pigeons working on a multiple fixed-ratio 50, fixed interval 10 min schedule for food reinforcement results in a period of behavioral depression that is temporally correlated with an increase in the levels of serotonin (5-HT) in the telencephalon (TEL). At various times after the administration of L-Try (0, 50, 90 and 150 min), pigeons working on the same schedule were sacrificed, their brains removed, dissected, and a crude synaptosomal fraction (P_2) prepared from the TEL. The contents of Try, 5-hydroxytryptophan (5-HTP), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), tyrosine (Tyr), dopamine (DA) and norepinephrine (NE) were then measured in this fraction. The levels of Try, 5-HTP, 5-HT, and 5-HIAA increased in the P_2 fraction after the injections of Try, but levels of Tyr, DA, and NE did not change. The course of the increase in the levels of 5-HT in P_2 from the TEL and its subsequent return to preinjection levels was temporally related to the onset of the decreased responding and gradual return to normal response rates. A small number of pigeons did not show any behavioral disruption after L-Try administration. When these birds were sacrificed at 90 min, increased levels of Try but not 5-HTP, 5-HT or 5-HIAA were found in the P_2 fractions from TEL. These data indicate that the behavioral disruption following L-Try administration is probably produced through serotonergic systems. (Supported in part by Research Grant MH-03225-16 and Postdoctoral Training Grant MH 10695 from NIMH).

464 L-TRYPTOPHAN (TRYP) NORMALIZES HYPERALGESIA BUT NOT FOREBRAIN SEROTONIN (5HT) LOSS INDUCED BY UNILATERAL LESIONS OF MEDIAL FOREBRAIN BUNDLE (MFB) IN RATS. Donald V. Coscina, Valerie Watt*, Damodar D. Godse* and Harvey C. Stancer*. Section of Neurochemistry, Clarke Institute of Psychiatry, University of Toronto, Toronto, Ontario, Canada M5T 1R8.

Reversal of hyperalgesia to footshock in rats with bilateral MFB damage correlates with forebrain 5HT repletion after systemic 5-hydroxytryptophan (5HTP) treatment (Harvey and Lints, J. comp. physiol. Psychol. 74: 28, 1971). However, such 5HT repletion reflects substantial non-neuronal as well as non-specific neuronal decarboxylation of 5HTP (Coscina, Warsh, Godse and Stancer, Res. Comm. Chem. Path. Pharmac. 7: 617, 1974). This questions the conclusion that reversal of MFB lesion-induced hyperalgesia is clearly due to brain 5HT repletion. To test this hypothesis further, we produced unilateral MFB lesions in 36 male albino rats (270-320 gm) by radio frequency heat production (56° C, 1 min). Three weeks later, 18 lesioned and 12 normal rats received 50 mg/kg TRYP in saline, i.p., 30 min before algesia testing on a hot plate (50° C, $\pm$ 1°). Another 18 lesioned and 12 normal rats received saline vehicle. Fifteen sec after recording paw-lick latencies (PLLs), rats were decapitated for fluorometric assays of hemispheric 5HT, norepinephrine (NE) and dopamine (DA) content. Saline-treated lesioned rats showed significant ($p < .002$) hyperalgesia compared to controls (M $\pm$ SEM PLLs = 5.5 $\pm$ 0.8 sec vs. 8.4 $\pm$ 1.1, respectively). TRYP reversed this sensitivity (8.5 $\pm$ 0.7 vs. 9.2 $\pm$ 1.2, respectively). MFB lesions reduced 5HT, NE and DA by 53, 31, and 80 % respective to controls (all ps $< .001$). TRYP produced 30% repletion of 5HT ($p < .001$) without affecting NE and DA; however, 5HT was still lower than normal ($p < .01$). These data support the suggestion that brain 5HT repletion can reverse MFB lesion-induced hyperalgesia but question a clear linear relationship between brain 5HT levels and responses to pain.

465 HYPERPHAGIA AND OBESITY IN RATS AFTER INTRAVENTRICULAR INJECTION OF 5,7-DIHYDROXYTRYPTAMINE. Charles F. Saller* and Edward M. Stricker. Dept. Biol., Univ. Pittsburgh, Pittsburgh, PA. 15260.

Adult male rats were given one intracerebroventricular injection of 200 µg 5,7-dihydroxytryptamine (5,7-DHT) after pretreatment with desmethylimipramine (DMI) (25 mg/kg, i.p.). Intakes of lab chow and water were decreased below control levels for 1-2 weeks, following which the lesioned rats began to consume 1.5-2 times their previous daily rations and their body weights increased by 8-12 gm/day. Rats were killed 140 days after injections, by which time they weighed 800-950 gm whereas controls weighed only 500-580 gm. Biochemical analyses revealed depletions of 70-85% serotonin (5-HT) in telencephalic tissue, whereas norepinephrine (NE) and dopamine levels were unchanged. A second group of rats, given a smaller dose of 5,7-DHT (100 µg, ivt.) after pretreatment with DMI, also became hyperphagic after a 1-2 week period of anorexia but gained weight at the rate of only 5-6 gm/day. They resumed normal intakes of chow and water after 2-3 months, at which time body weights had leveled off at 650-750 gm. Selective depletions of 50-60% telencephalic 5-HT were observed in these animals. A third group of rats, given 200 µg 5,7-DHT without DMI pretreatment, never became hyperphagic or obese. Their telencephalons showed depletions of 70-85% 5-HT but 40-50% NE losses as well. These results indicate that destruction of central serotonergic neurons can lead to a dramatic and prolonged increase in ad libitum food intake in rats. The 5-HT depletion required for the largest effects appears to be considerable. Furthermore, NE depletions, which by themselves do not disrupt food intake or alter body weight, can prevent the hyperphagia and resultant obesity, suggesting that some interaction between noradrenergic and serotonergic neurons is involved in the central control of food intake. (Supported by NIMH Grants MH-25140 and MH-20620.)

466 HYPERPHAGIA FOLLOWING NOREPINEPHRINE OR SEROTONIN DEPLETION IN RELATION TO HYPOTHALAMIC HYPERPHAGIA. Bartley G. Hoebel, J. Eric Ahlskog* and Frank P. Zemlan*. (SPON: Harry R. Kissileff). Dept. Psychology, Princeton University, Princeton, New Jersey, 08540.

A neurochemical dissection of the classical hypothalamic hyperphagia syndrome was undertaken. Female rats with electrolytic lesions of the ventromedial hypothalamus (VMH) that caused no norepinephrine (NE) depletion overate both in the daytime and at night and showed little or no diurnal feeding rythm. Rats with at least 90% depletion of NE resulting from 6-hydroxydopamine destruction of the ascending noradrenergic and perhaps also adrenergic bundles (NAB) were also hyperphagic but only at night. When animals were first given NAB lesions and then subsequently VMH lesions, the two effects were additive suggesting that the two syndromes have different neural substrates. A further separation of NAB and VMH hyperphagia occured when rats depleted of NE, not DA or serotonin (5HT), were tested with anorectic drugs. Following NAB lesions amphetamine was less potent and fenfluramine was moreso; this is opposite the known effects of VMH lesions. Female rats with 80% depletion of 5HT, but not NE or DA, following intraventricular paracholorophenylalanine were also hyperphagic, but they overate in the daytime. They were also hypersexual as measured by a significant increase in the frequency of lordosis when mounted. Thus these depletion effects may not be specific to feeding.

We conclude: 1) that the hyperphagia syndrome after NAB destruction is different from the classical VMH syndrome; 2) the circadian feeding pattern of serotonin depleted rats suggests a third type of hyperphagia distinct from the other two; and 3) VMH hyperphagia might thus be exacerbated if the lesion included these NE or 5HT systems.

467 A COMPARISON OF THE REGIONAL BRAIN DISTRIBUTION AND ESTIMATED WHOLE BRAIN TURNOVER RATE OF VANILLYLMANDELIC ACID (VMA) AND 3-METHOXY-4-HYDROXYPHENYLGLYCOL (MHPG). F. Karoum, N. H. Neff, and R. J. Wyatt, Labs. Clinical Psychopharmacology and Preclinical Pharmacology, NIMH, Saint Elizabeths Hosp., Washington. D. C. 20032

VMA and total MHPG were assayed in rat brain by mass fragmentography. Whole brain contained 13±2 and 599±20 pmol/g SEM (5) VMA and MHPG, respectively. Concentrations(pmol/g ±SEM (4)) in seven regions of the brain were:

REGION	VMA	MHPG	REGION	VMA	MHPG
Cerebellum	21±2	358±20	Midbrain	16±2	668±46
Medulla	13±1	722±47	Hippocampus	8±1	543±34
Hypothalamus	46±8	1173±108	Cortex	13±1	521±50
Striatum	86±3	445±8			

VMA represented about 1.5-5.5% of the deaminated metabolites in the brain regions except in the striatum where it represented about 16%. The rate of turnover of VMA and MHPG in whole brain were estimated from their decline after administering pargyline (75 mg/kg i.p.). VMA and MHPG were apparently formed at 33 and 230 pmol/g/hr, with turnover times of about 25 and 156 min, respectively. We conclude that VMA constitutes about 13% of the total deaminated amine metabolites formed in brain. Moreover, its short turnover time implies that it is probably eliminated from brain by transport.

468 EFFECTS OF PHENCYCLIDINE ON BIOGENIC AMINES IN RAT BRAIN. Robert C. Smith, Herbert Meltzer, Harry Dekirmenjian, John M. Davis. Illinois State Psychiatric Institute and Department of Psychiatry, University of Chicago 60637.

Since two of the important behavioral effects of phencyclidine (PCP)--inducing stereotyped behavior (SB) in rats and psychosis in man--are also produced by d-amphetamine (D-AMP) and methylphenidate (MP) it is relevant to investigate and compare the neurochemical effects of PCP. We investigated some effects of PCP on biogenic amines in rat brain. PCP significantly inhibited *in vitro* re-uptake of DA, NE and 5-HT in crude synaptosomal preparations of whole brain (NE, 5-HT) or caudate (DA). Approximate $ID_{50's}$: DA-5 x 10^{-7}M; NE-7 x 10^{-7}M; 5-HT-3 x 10^{-6}M. $ID_{50's}$ for D-AMP and MP (5-6 x 10^{-7}) were similar to PCP for DA re-uptake, and ID_{50} for MP (7 x 10^{-7}) was similar to PCP for NE re-uptake. There were no statistically significant effects of 10 to 20 mg/kg PCP on endogenous levels of whole brain NE, DA, or 5-HT 15 or 45 minutes after i.p. drug, although there was a trend for a small (10%) increase in NE and DA at 45 minutes. There was no significant increase in whole brain MHPG 15 minutes after 20 mg/kg PCP or probenecid + PCP. Studies of the effects of PCP on dopamine metabolism are being conducted. Our results suggest that re-uptake blockade may be one important effect of PCP on brain biogenic amines. Since several researchers have suggested that the effects of D-AMP and MP on inducing psychotic reactions in man and SB in rats may be mediated predominently by dopaminergic mechanisms, the similar potencies of PCP D-AMP, and MP on caudate DA re-uptake are consistent with the hypothesis that dopaminergic mechanisms may be important in these behavioral effects of PCP

469 EFFECT OF PROSTAGLANDIN, BACTERIAL PYROGEN AND NOREPINEPHRINE, INJECTED INTO THE HYPOTHALAMUS, ON THERMOREGULATION IN THE NEWBORN LAMB - Q.J. Pittman, W.L. Veale and K.E. Cooper, Division of Medical Physiology, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada.

Fever develops when prostaglandins of the E Series are injected into the cerebral ventricles or the anterior hypothalamic/preoptic area (AH/POA) of a number of species of animals. However, when prostaglandin E (PGE) was injected into the lateral ventricle of newborn lambs, fever often did not occur. To investigate this further, we have injected PGE, bacterial pyrogen and norepinephrine (NE) into the hypothalamus of newborn lambs at postnatal ages 50-70 hours. Shortly after birth, 12 lambs were implanted stereotaxically with an array of 4 guide tubes positioned bilaterally so that their tips lay above various hypothalamic loci. 1µl injections were made through a 27 gauge needle lowered to the appropriate depth. PGE_1 (0.2 µg) or PGE_2 (0.2 & 2.0 µg) was infused bilaterally into 23 hypothalamic sites and rectal temperature was measured for a minimum of 90 minutes thereafter. Though the injection sites (examined histologically) ranged throughout the medial hypothalamus from the preoptic area to the posterior nucleus, rectal temperatures varied less than $0.4^{\circ}C$ from the temperature prior to injection. Infusion of a bacterial pyrogen (S. abortus equi, 0.2 µg) into these same sites caused fevers to develop following 5 of the 23 injections. The sites from which febrile responses were obtained were in the AH/POA, but injections into this area in other lambs did not cause fever. Lambs that did not develop fever following central injection were often, if sensitized, able to develop fever after intravenous injection of 0.3 µg pyrogen.

Eight lambs were placed in a cold environment ($10^{\circ}C$), and NE was injected. Rectal temperature fell by $0.5\text{-}1.4^{\circ}C$ after 7 injections into the AH/POA, but 9 injections into other hypothalamic areas caused little temperature change. When 6 lambs were placed at a temperature of $30^{\circ}C$ and NE was injected, rectal temperatures ranged between +0.4 and $-0.45^{\circ}C$ of the temperature before the injection.

The results obtained from newborn lambs suggest that a.) fever may develop in the newborn lamb independently of the central involvement of prostaglandins, b.) the hypothalamus is relatively insensitive to the direct application of large amounts of bacterial pyrogen, and c.) NE, injected into the AH/POA, causes body temperature to fall when the lamb is thermoregulating in the cold.

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470 ALTERATION OF THE KINETIC CONSTANTS FOR NOREPINEPHRINE UPTAKE IN THE RAT CEREBRAL CORTEX BY ELECTROCONVULSIVE SHOCK OR BY ACUTE STRESS.

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Previous experiments in Swiss mice revealed that the apparent K_m for ^{3}H-dl-norepinephrine uptake in cerebral cortical synaptosomes was markedly increased by electroconvulsive shock (ECS) or by isolation-induced aggressive bouts between male mice (Science 180: 1050, 1973; Science 183: 220, 1973; Life Sci. 16: 45, 1975.) In male Sprague-Dawley rats, one single maximal ECS did not alter the apparent K_m or V_{max} for ^{3}H-l-norepinephrine uptake in the cerebral cortex within 5 min, as had been reported in the mouse. However, 24 hr after the last of a series of twice-daily ECS for 7 consecutive days (total of 14 maximal ECS), the apparent K_m had significantly increased by 36% and the V_{max} by 46%. This suggested that the affinity for synaptosomal reuptake of norepinephrine was reduced by ECS (elevated K_m) and that the total number of norepinephrine reuptake sites on the synaptosomal membranes had also increased (elevated V_{max}) as a result of chronic ECS pretreatment. When rats were tested 72 hr after the last of the chronic ECS treatments the kinetic constants had not returned to control (sham-shock) levels but actually showed a reversal of change in the kinetic constants. Thus the apparent K_m was significantly reduced by 22% and V_{max} by 23%, probably as a compensatory response of the normal rat brain. The decrease in affinity for norepinephrine reuptake 24 hr after chronic ECS in the rat, as in the mouse, may explain, at least in part, the antidepressant efficacy of ECS in human depressive illness.

A series of acute stresses in the rat were also tested for their effects on the kinetic constants for norepinephrine uptake immediately following the stress. Immobilization of rats for 1 hr failed to alter the kinetic constants for norepinephrine uptake in cerebral cortical synaptosomes. Similarly, cold stress (caging of the rat by itself for 30-45 min at 2-4°C in a refrigerator, after dousing the rat with a mild nonionic detergent solution to wet down its fur) failed to alter the apparent K_m or V_{max}. However, swim stress (10 min swim in 22°C water) increased the apparent K_m significantly by 44% and the V_{max} by 35%; body temp. had fallen from control levels of 38° to 30°C at the end of the swim period. Swim stress at other temperatures, as well as combined immobilization-and-cold stress are currently being tested.

Our data confirm that in the rat brain, as previously also seen in the mouse brain, the neuronal membrane norepinephrine reuptake mechanism, an active transport process, is in a dynamic state and can be altered by ECS or by certain stress states.

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471 THE EFFECT OF VISUAL STIMULATION ON THE RELEASE OF CATECHOLAMINES FROM THE VISUAL CORTEX IN THE CAT. Tomas A. Reader*, Jacques de Champlain, Lise Farley* and Herbert H. Jasper. Research Center in Neurological Sciences, Université de Montréal, Montréal, Canada.

The purpose of the present investigation was to determine whether endogenous norepinephrine (NE) and dopamine (DA) are released from the visual cortex in the cat. In addition, an attempt was made to study possible interactions between the cat's catecholaminergic and cholinergic systems.

Special nylon chambers covering a surface of about 1.1 cm^2 were placed on the cortical surface as previously described (G.C. Celesia and H.H. Jasper, J. Neurol., 16:1053, 1966; J.F. Mitchell, J. Physiol.(Lond.) 165:98, 1963). The chambers were perfused continuously with an artificial cerebrospinal fluid (K.A.C. Elliot and H.H. Jasper, J. Neurosurg., 6:140, 1949) by means of a Harvard pump at a rate of 6 ml/hr. Each sample was collected over a 30 min. period in tubes placed on crushed ice. In all the experiments,the EEG, the evoked potentials to electric and to photic stimuli, arterial blood pressure and end tidal CO_2% were determined in order to monitor the animals. The superfusates once collected were immediately acidified and maintained frozen until the assay was performed. The catecholamine contents of the superfusates was determined by a sensitive radiometric enzymatic assay based on the methylation of catecholamines by catechol-o-methyl transferase in the presence of (^{3}H-methyl)-S-adenosylmethionine and the (^{3}H)-derivatives are then isolated by organic extractions (J.T. Coyle and D. Henry, J. Neurochem., 21:61, 1973). The basal release of NE was of 22.49 ± 4.44 pg/min/cm^2 (N = 14) and that of DA was of 41.6 ± 9.18 pg/min/cm^2 (n = 14). Specific sensory stimulation of the visual system decreased the basal release of NE from 26.81 ± 5.63 pg/min/cm^2 (n = 10) to 12.63 ± 2.50 pg/min/cm^2 (53% reduction) and that of DA from 41.70 ± 12.42 pg/min/cm^2 (n = 10) to 15.76 ± 4.55 pg/min/cm^2 (62% reduction). This decrease in release could be blocked by nicotine and other cholinergic agents which in some cases inverted the sensory stimulation effect on the release of catecholamines. At the end of the experiments tissue samples were homogenized in 100 vol (w/v) of cold 0.1 N perchloric acid for the determination of endogenous amines. The endogenous contents of NE was of 213.57 ± 46.99 ng/gm (n = 7) and that of DA was of 248.71 ± 45.74 ng/gm (n = 7).

These results show: 1) the presence of noradrenergic and dopaminergic fibers in the visual cortex of the cat which liberate measurable amounts of NE and DA when the assays are performed with a sensitive and specific method; and 2) an interaction between catecholaminergic and cholinergic systems in which acetylcholine released by specific sensory mechanisms and/or general arousal could modulate catecholamine release.

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472 TRYPTOPHAN ENHANCEMENT OF TONIC IMMOBILITY: FURTHER EVIDENCE OF 5-HT INVOLVEMENT IN MOVEMENT SUPPRESSION. Jack D. Maser, Gordon G. Gallup, Jr.*, and Craig Harston*. Clinical Research Branch/NIMH, Rockville, Md. 20852 and Dept. of Psychology, Tulane University, New Orleans, La. 70118

The tonic immobility response (TI) is an innate, non-associative, defensive reaction involving suppression of movement. The response may last from seconds to several hours, depending upon the species and the independent variable. Drug data reported previously suggested that 5-hydroxytryptamine participated in this behavior. d-LSD, BOL-148, monoamine oxidase inhibitors, and p-chlorophenylalanine differentially modified TI duration. In the same order of magnitude, Aghajanian's laboratory reported suppressed raphe electrical activity following administration of these compounds. Other investigators have found no effect of norepinephrine or blockers of norepinephrine biosynthesis on TI. This report describes a dose-response function obtained for tryptophan injected intraperitoneally into chickens, one hour prior to behavioral testing. It was assumed (based on the work of Fernstrom & Wurtman, Science 1971: 173) that the tryptophan produced an increase in brain serotonin levels, thereby modulating the immobility reaction.

473 DO LESIONS IN CENTRAL CATECHOLAMINERGIC PATHWAYS CAUSE THE UNILATERAL LATERAL HYPOTHALAMIC SYNDROME? John C. Cheronis and Ruthmary K. Deuel. Dept. Peds., Univ. of Chicago, Chicago, 60637.

A syndrome consisting of contralateral lack of responsiveness to somatosensory stimuli, loss of visual placing of the contralateral forelimb and spontaneous ipsiversive rotation was found after unilateral lateral hypothalamic (ULH) lesions in rats. All components of the ULH syndrome appeared simultaneously as the animals recovered from anesthesia. This syndrome was originally reported by Marshall, et. al. (Sci. 174: 523, 1971) after ULH lesions and subsequently by Marshall, et. al. (JCPP 87: 808, 1974) after substantia nigra lesions. The latter were found to produce drops in telencephalic noradrenaline (NA) and dopamine (DA), presumably evidence of interruption of both major catecholaminergic (CA) systems.

To determine whether the ULH syndrome could relate to interruption of one or the other of these pathways by itself, unilateral radio frequency lesions were made in the locus coerulius (LC) or ventral tegmentum (VT). Immediately postoperatively rats with VT lesions did not demonstrate deficits in somatosensory responses or visual placing but did show marked ipsiversive rotation. Rats with LC lesions showed no elements of the ULH syndrome.

Since lesions of the VT (DA pathway) can lead to one component of the ULH syndrome without others, and since lesions of the LC (NA pathway) do not lead to any ULH syndrome components, the direct dependence of the full ULH syndrome on either CA pathway alone is unlikely.

474 HISTOLOGIC, ENZYMATIC, AND BEHAVIORAL EFFECTS OF SELECTIVE RAPHE LESIONS IN RATS. M. A. Geyer*, A. Puerto*, D. S. Segal, S. Knapp , and A. J. Mandell. Dept. Psychiatry, Sch. Med., UCSD, La Jolla, CA 92037

Selective lesions of the dorsal (B7), median (B8), or lateral (B9) raphe nuclei were made stereotaxically in 40 male rats four weeks before sacrifice. The extend of damage to each of the raphe nuclei was quantified histologically using a simplified formaldehyde histochemical method for the visualization of serotonin in cryostat sections. Detailed mapping of the distribution of the yellow-fluorescent raphe perikarya provided the basis for quantification. Tryptophan hydroxylase activity was measured in six forebrain regions from each animal, and the results were correlated with the percent damage to the raphe nuclei. Tyrosine hydroxylase activity was also assayed in five of the regions and was not significantly affected by any of the raphe lesions. Dorsal raphe lesions reduced tryptophan hydroxylase activity in the striatum, thalamus, cortex, and hypothalamus, but not in the septal nuclei or hippocampus. Damage to B8 resulted in decrements in this serotonergic enzyme activity in the septal nuclei, hippocampus, cortex, and hypothalamus, but not in the striatum or thalamus. Lesions of the scattered B9 cells had no significant effect on enzyme activity in any of these regions. The data suggest that two distinct, though perhaps overlapping, serotonergic systems innervate different parts of the forebrain: a mesostriatal pathway originating in B7 and a mesolimbic system derived from B8. The behavior of the lesioned rats was compared to that of sham-lesioned controls in a variety of experimental situations. Lesions of B7 or bilateral lesions of B9 had no significant effect on any of the behavioral measures. However, B8 lesions had marked behavioral effects similar to those previously found after combined raphe lesions or parachlorophenylalanine. Rats with median raphe lesions were hyperactive when placed in a novel environment and throughout the dark phase of the light/dark cycle. With respect to locomotor activity, the same rats were also hyper-responsive to amphetamine. Rats with B8 lesions also exhibited larger startle responses when placed in a stabilimeter and subjected to 30 air puff stimuli (30 sec ISI). The pattern of startle responding suggested that the presumably separable processes of sensitization and habituation were not differentially affected by the raphe lesions. Rather, in accord with the other behavioral indices, B8-lesioned rats showed an overall increase in responsivity, an effect opposite to that of intraventricularly infused serotonin (Geyer et al., Pharmacol. Biochem. Behav. 1975, in press). Furthermore, only B8-lesioned animals perseverated when given two non-reinforced trials in a Y-maze. These experiments suggest that the mesolimbic serotonergic pathway originating in B8 subserves some of the inhibition necessary to dampen behavioral responsivity.

475 REVERSAL OF SPONTANEOUS HYPERACTIVITY FOLLOWING EXPERIMENTAL CEREBRAL INFARCTION BY TREATMENT WITH DMI, d-AMPHETAMINE OR 6-HYDROXYDOPAMINE. R. G. Robinson and F. E. Bloom. Laboratory of Neuropharmacology, NIMH, Saint Elizabeths Hospital, Washington, D. C. 20032.

In order to study the behavioral and biochemical consequences of experimental cerebral infarction in the rat, the right middle cerebral artery was surgically ligated in a group of 50 rats. The resultant lesion (2x2x2 mm) involved only lateral parietal cortex but no subcortical tissue. With this experimental stroke, we reported (Nature. 255,332,1975) decreases in norepinephrine (NE) ipsilateral to the lesion in parietal and occipital cortex and in pons-mesencephalon both ipsilaterally and contralaterally. By 40 days after lesion, NE again approached control levels. Dopamine levels changed only in the ipsilateral brainstem, dropping continuously throughout the postoperative period. Histofluorescence studies indicate decreased intensity of locus coeruleus neurons and cortical axons ipsilateral to the lesion at 5 and 20 days but greater than normal cortical axon fluorescence by 40 days post-lesion. In addition, behavioral measures have revealed increased spontaneous horizontal activity per 24 hours from 2 to 20 days after vascular occlusion (Fig 1) without change in food or water intake. To pursue the possibility raised by these dynamic chemical and cytological changes that NE neurons may influence the expression of the post-stroke hyperactivity phenomenon, additional pharmacological experiments were done. Lesioned rats treated daily with desmethylimipramine (DMI), 10 mg/kg ip(Fig 1), showed no increase in spontaneous horizontal activity over sham operated rats treated

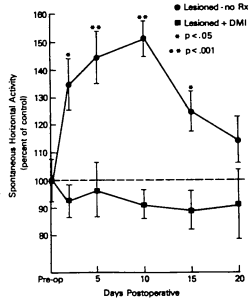

Fig. 1. The postoperative course of spontaneous horizontal activity following cerebral infarction. Untreated and DMI treated lesioned animals were compared with untreated sham-operated controls. N=8 for each point. p values represent t-test comparison with sham operated control.

with DMI or saline. Lesioned rats treated with d-amphetamine, 0.5 mg/kg ip twice daily, showed reduced spontaneous activity during the 3 hour post-injection period compared to similarly treated control animals. In a third series, lesioned rats, pretreated with intracisternal 6-hydroxydopamine (250 ugm, twice), showed no post-operative hyperactivity. These observations suggest first that central NE neurons may modulate the appearance and recovery from the post-stroke hyperactivity, secondly that the possible hypofunction of injured NE cells may be overcome by drugs or eventually by axon regeneration or collateral formation, and finally that the appearance of hyperactivity requires that some NE neurons remain viable during the post-stroke period.

476 EFFECT OF LOCUS COERULEUS LESIONS ON MONOAMINES IN THE CNS OF THE CAT. Raymond Marchand*, Marc Fantino* and Louis J. Poirier. Lab. Neurobiol., Sch. Med., Univ. Laval, Québec, QUE. G1K 7P4.

In 4 cats with bilateral lesions of the locus coeruleus NA was significantly decreased in the frontal and occipital cortex of both sides of the brain. The mean concentration of NA in these areas was .16 µg/g (range .06 - .27) compared to .57 (range .46 - .77) and .50 (range .36 - .63) in the same structures of 6 cats with lesions located caudally to the locus coeruleus and of 4 unlesioned cats, respectively. In 3 cats with bilateral lesions involving the area immediately caudal to the locus coeruleus the mean concentration of NA in the spinal cord was .10 µg/g (range .05 - .14) compared to .27 (range .23 - .32) in 2 non-lesioned cats. In the same 3 lesioned animals the mean concentration of NA in the cortical areas of the brain read .54 (range .46 - .68). In one cat with a unilateral lesion of the locus coeruleus the mean concentrations of NA were .02 and .04 µg/g in the ipsilateral cortical areas and spinal cord segments, respectively. In all lesioned animals (survival period of 16-63 days) the concentration of NA in the striata and hypothalamus, of DA in the striata and of 5-HT in the cortex, striata, thalami, hypothalamus and segments of the spinal cord were not significantly different than the values determined in the same areas of non-lesioned cats. NA was also significantly decreased (by 70%) in the thalamus of both sides in 2 of the 4 cats with locus coeruleus lesions and in the ipsilateral thalamus (by 54%) of the cat with a unilateral lesion of the locus coeruleus. These results support the idea that neurons in the area of the locus coeruleus participate in the elaboration of NA in various areas of the cortex and spinal cord and possibly of the thalamus through fibers which predominantly course on the ipsilateral side of the brain stem and spinal cord.
(Supported by the Medical Research Council of Canada).

477 REGIONAL DEPLETION OF FOREBRAIN AMINES BY MESENCEPHALIC KNIFE CUTS: EFFECTS ON LEARNED ESCAPE AND AVOIDANCE RESPONDING, OPEN AND ENCLOSED FIELD ACTIVITY IN LIGHT AND DARK, STEREOTYPY, AND EMOTIONALITY. Ernest W. Kent and Valerie C. Abbott*. Dept. Psychol., U. of Illinois at Chicago, Chicago, Ill. 60680.

To further clarify the factors contributing to the loss of aversively motivated learned behaviors resulting from the interruption of fibers moving laterally from the medial forebrain bundle (Kent and Grossman, 1973), regional depletion of forebrain amines was produced in white rats by mesencephalic cuts made stereotaxically with a wire knife. The depletions were confirmed by fluorometric assay (Welch and Welch, 1969) for dopamine, serotonin, and nor-epinephrine simultaneously in the striatum and in the basal forebrain. Evidence was found supporting the hypothesis (Zis, Fibiger and Phillips, 1974) that the nigro-striatal dopaminergic system is important in avoidance but not escape, while the basal forebrain dopaminergic projections are important in mediating effects on kinetics and goal-directed locomotion, and that the serotonergic projections to both areas play an important role in modulating these effects (frequently in opposition to dopaminergic influences). Data from open vs. closed field activity measures, as well as active vs. passive avoidance measures suggests that the balance between freezing and fleeing as responses to aversive situations is reciprocally affected by these systems, while affective responding is more complexly affected. Other aspects of these manipulations are considered in a companion presentation (Abbott and Kent).

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478 THE EFFECTS OF PARALYZING SPINAL CORD INJURY ON CATECHOLAMINE LEVELS IN GREY AND WHITE THORACIC CORD REGIONS. J.L.Alderman, J.L.Osterholm, J.D.Irvin. Dept. Neurosurgery, Thomas Jefferson Medical College, Philadelphia, Pennsylvania 19107.

It has been proposed that following traumatic paralyzing spinal cord injury (SCI) at the thoracic (T-8) cord level, an excessive local release of norepinephrine (NE) from nerve terminals associated with spinal cord parenchyma and blood vessels may contribute to vasoconstriction of the microvasculature, resulting in local vascular stasis, ischemia and anoxia. Interlaboratory studies on the disposition of cord NE levels following SCI have, to date been equivocal. These studies utilized cord sections without separation of the grey and white cord regions. One possible artifact in these studies may relate to the occurrence of a progressive, necrotising lesion that developes initially in the intermedialateral grey (ILG) cell column, that region postulated to contain the largest thoracic NE concentration. Since the ILG constitutes a relatively small per cent of the cord section, depending on the size of the sample taken for analysis, a dilution artifact may occur. Since spinal cord white matter contains little NE, a separation of white and grey matter should eliminate a substantial amount of this dilution. Cats of mixed breed and either sex were anesthetized with Ketamine and injured at T-8 with a 500g/cm force. After 20 and 60 minutes, samples were taken from the site of impact and immediately adjacent above and below this site, divided into grey and white areas, and analyzed for NE and dopamine (DA). After 20 minutes, levels of NE were significantly decreased from 0.34 ug/g (wet wt.) to 0.21 ug/g (38% $p < .05$) at the injury site. After 60 minutes, NE levels at the injury site, although still reduced 21%, were no longer significantly depressed compared to the control value. After 60 minutes, NE levels adjacent and below the injury site were significantly (59% $p < .01$) reduced. No significant alteration in DA, nor white matter NE, were detected in any area. It is suggested that the decrease in NE levels may represent a significant release in NE, consistent with an extracellular availability of this amine for possible vascular activity.

479 FACILITATION OF PREDATORY AGGRESSION BY LOW DOSES OF PARA-CHLORO-PHENYLALANINE. Judith L. Gibbons* and Gordon A. Barr* (SPON: W. H. Bridger). Department of Psychiatry, Albert Einstein College of Medicine, Bronx, New York 10461.

High doses of para-chlorophenylalanine (PCPA) (316 - 400 mg/kg; 300 mg/kg/day for 3 days) induce killing in spontaneous non-killer rats. However, concomitant with the induction of killing is increased irritability (Sheard, Brain Res., 15: 524, 1969). Moreover, these doses alter the topography of the killing response such that bites occur randomly all over the body of the mouse rather than primarily at the nape of the neck as seen in natural killers (Miczek et al., Pharm. Biochem. Behav., in press). The goal of the present experiments was to determine if PCPA, at low doses, would facilitate mouse killing without the non-specific side effects previously reported.

Ten experienced killer rats were tested at 75, 112.5, and 150 mg/kg of PCPA methylester injected i.p. 48 hours prior to the behavioral tests. Animals were compared to their own vehicle control at each dose and tested in a counterbalanced order. Facilitation was measured by presenting each rat with five mice to kill in rapid succession (60 seconds after the previous kill). This procedure normally results in satiation (as measured by increased kill latencies) of the killing behavior. PCPA inhibited satiation of killing at the 150 mg/kg dose only; there was no effect at the 75 mg/kg dose and but a trend at the 112.5 mg/kg dose.

This suggested that PCPA could facilitate killing but it was possible that PCPA was acting through non-specific disruption of habituation. To test this possibility, a second experiment was conducted with 17 experienced killers. Each was injected with either 150 mg/kg of PCPA methylester or with saline. Two days later, the rat was placed into a novel cage in a separate room and allowed to adapt to that cage for 5 minutes. Then a single mouse was introduced into the cage and the latency to kill was measured. This procedure normally disrupts the short killing latency of experienced killers. Non-specific effects on habituation could thus be ruled out; since presumably it is the novelty of the cage that disrupts killing, the failure to habituate would lengthen, not shorten, the latency to kill. At the dose used, PCPA significantly decreased the killing latency. In neither experiment did PCPA at any dose alter the topography of the killing response, affect irritability to either rough or normal handling, or change open field perambulation.

It is concluded that low doses of PCPA can facilitate killing in experienced killers without affecting other behaviors and that serotonin may play an inhibitory role in predatory aggression.

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480 CHOLINERGIC MOUSE KILLING IN THE RAT. Robert A. Levitt and William Dickinson*. Dept. Psychol. & Sch. Med., South. Ill. Univ., Carbondale, 62901.

In 1970 Smith, King and Hoebel reported converting non-mouse-killer rats to killers by injecting 50 micrograms of carbachol bilaterally into the lateral hypothalamic area (LHA). They interpreted their results as supporting the production of a predatory-type aggressive response. We have replicated the elicitation of killing in nonkiller rats, and have subjected the carbachol-elicited killing to further analysis, which raises questions concerning the credibility of the interpretation of the killing as a predatory response.

In dose-response studies, we have injected 5, 10, 20, 35 or 50 micrograms of carbachol bilaterally into the LHA of adult male or female Long-Evans rats (which were found in preliminary tests not to kill mice). About 25 percent of the rats injected with the four lowest doses begin to kill mice within 2 minutes to 6 hours after the injections. However, the latency to begin killing is the shortest, and the duration of the response is the longest, at the 20 microgram dose. In addition to killing mice, the rats also kill rat pups, and attack adult rats during the period of time in which mouse killing occurs. The appearance of behavioral seizures in all stimulated rats and the nonspecificity of the aggressive responses, suggest a phenomenon more akin to pain-induced aggression or affective attack than to predatory aggression. The absence of these behaviors on posttests one week after brain stimulation confirms the temporary drug-induced nature of the behavioral change.

481 AMPHETAMINE AND MOUSE KILLING: DEMONSTRATION OF TOLERANCE AND A COMPARISON OF D- AND L- ISOMERS. Gordon A. Barr*, Judith L. Gibbons*, David A. Gorelick, and Wagner H. Bridger. Department of Psychiatry, Albert Einstein College of Medicine, Bronx, New York 10461.

Mouse killing (predatory aggression) has a complex relationship to feeding (Polsky, Behav. Biol., 13: 81, 1975). Behavioral studies have shown that manipulations which affect feeding also affect killing (Malick, Physiol. Behav., 14: 171, 1975; Paul, J. Comp. Physiol Psych., 78: 69, 1972). Studies using amphetamine to block killing and eating, however, have shown that predation and feeding are differentially affected by the drug (Gay, East. Psychol. Assoc., 1975). Gay demonstrated that there was a difference in slope of the dose response curves of the inhibition by amphetamine of both behaviors. The present experiments are a further examination of amphetamine's effects on killing.

Tolerance to amphetamine's anorexic effects develops within 7 - 11 days (Gotestam & Lewander, Psychopharm., 42: 41, 1975). The goal of the first experiment was to determine whether tolerance would develop to amphetamine's inhibition of killing. Nineteen experienced killer rats were injected with 1.95 mg/kg (base weight) of d-amphetamine HCl (d-amph) and presented with a mouse; the latency to kill was measured. Then 10 of these animals were given d-amph injections (2.34 mg/kg) twice daily and 9 were given saline injections. On the ninth day all animals were given a challenge dose of 1.95 mg/kg d-amph and the latency to kill was again recorded. The chronically injected amphetamine group showed shorter kill latencies on the last test than did the saline treated group and also showed shorter latency to kill than they did on their own pretest. Pre- and post- tests did not differ for the controls. Thus, like the anorexic effects, tolerance developed to amphetamine's inhibition of killing.

D-amphetamine has been shown to be approximately 10 times as potent in inducing anorexia than l-amphetamine (l-amph) (Mantegazza et al., Inter. Symp. on Amphet. 1970, pp 559). The second experiment compared the relative effects of d-amph (.70, 1.17, 1.56, 1.95 mg/kg base weight) and l-amph succinate (.86, 1.72, 3.45, 5.17 mg/kg base weight) in blocking the killing response. Sixteen experienced killers were treated with one of the two isomers or saline and tested with a mouse 20 minutes later. Each rat was treated with each dose of both drugs. At the lower doses, d-amph and l-amph were equally effective in inhibiting killing; however, d-amph showed a significantly steeper dose response slope than did l-amph. Thus, at the higher doses, d-amph was more effective than l-amph. Moreover, the highest dose of l-amph used (5.12 mg/kg) was not as effective as the almost 100 percent effective dose of d-amph (1.95 mg/kg).

These results indicate a difference in some of the effects of amphetamine on both eating and killing; while the anorexic effect of amphetamine may be partially involved in its inhibition of predation, other mechanisms are likely to be involved.

482 INTRAVENTRICULAR 6-HYDROXYDOPAMINE LOWERS ISOLATION-INDUCED FIGHTING BEHAVIOR IN MALE MICE. J. N. Crawley and J. F. Contrera. Department of Zoology, University of Maryland, College Park, Maryland 20742.

Male mice with high isolation-induced fighting tendencies were administered 200 ug 6-OHDA or vehicle intraventricularly and tested for fighting tendency for up to 10 weeks until sacrifice, and assayed for whole brain norepinephrine levels. A strong correlation was found between norepinephrine depletion and reduced fighting tendencies after 6-OHDA treatment. The depressed fighting by mice with less than 200 ng NE/g persisted throughout a series of test fights, indicating no recovery in fighting behavior throughout the survival time. Other behavioral parameters, including exploration, grooming, feeding, and reproductive behaviors, in the depleted, non-fighting mice were statistically similar to vcontrol values. Neurochemical lesions by 6-OHDA are seen as a useful tool for analyzing the behavioral significance of neurotransmitters in CNS pathways.

483 TURNOVER OF 5-HYDROXYTRYPTAMINE IN BRAIN AREAS AND INTRA-SPECIES AGGRESSION. Jorge H. Daruna and Ernest W. Kent. Dept. Psychol., U. of Ill. Chicago Circle, Chicago, 60680.

Rats matched on body weight and food approach tendency were tested for aggressiveness using a food competition paradigm. Rats winning and those losing all of their encounters were designated as high and low aggressive, respectively. Another group of randomly selected rats was treated identically except that they never fought. They served as a control for the fighting experience. Half of the rats were used to determine 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) steady-state levels in discrete brain areas. The other half received probenecid intraperitoneally (200 mg/kg) and were used to estimate 5-HT turnover in the same brain areas. Multivariate statistics were employed in the data analysis.

High and low aggressive rats were not found to differ in 5-HT and 5-HIAA steady-state levels. In contrast, a faster 5-HT turnover was observed in the high aggressive rats. The turnover differences reached significance in only two of the three brain areas examined. The two areas were: (a) all the structures (minus olfactory bulbs) rostral to the level of the anterior commissure and (b) the brainstem (hypothalamus-midbrain-medulla). These findings cannot be simply attributed to differing behavioral experiences because the high and low aggressive groups did not differ significantly from the non-fighting controls.

484 DOPAMINE: ADAPTIVE UPTAKE CHANGES IN STRIATAL SYNAPTOSOMES AFTER 30 SECONDS OF INTENSE FIGHTING BY ELECTRO-SHOCKED RATS. M. Gary Hadfield and William F. C. Rigby*. Div. Neuropathology, Med. Coll. Va., Richmond, Va. 23298

Dynamic plastic changes in synaptosomal membrane transport have been observed for norepinephrine (NE) following acute fighting in isolated mice (Welch, Hendley and Turek, Science 183:220, 1973) and retired male breeding mice (Hadfield and Weber, Biochem. Pharmacol. in press). In the latter instance, significant changes in both uptake velocity (Vmax) and the affinity constant (Km) were noted.

We wished to see if similar alterations could be observed almost immediately and if they would also apply to a different neurotransmitter, a separate species of animal and another model of aggression. Diphenylhydantoin (DPH) was used in the present study since it has been shown to inhibit fighting in electro-shocked mice as well as to alter the uptake of catecholamines.

The findings essentially paralleled those previously reported for NE. Vmax for dopamine (DA) was strikingly increased in the fighting animals while the membrane affinity was decreased (increased Km). These effects were detected just 30 seconds after combat.

It is presumed that these effects are due either to unmasking or increased utilization of existing DA carrier receptor sites or to creation of new ones. It appears that such plastic changes in catecholamine uptake processes may be wrought almost instantaneously in response to a stressful situation and that this adaptation may help the animal to enhance his performance.

It was also observed that DPH inhibited DA uptake in a non-competitive fashion in both fighting and control animals. This finding was identical to that which we previously reported for NE and may explain the ability of this agent to inhibit fighting in electro-shocked mice.

485 CHARACTERISTICS OF BRAIN ADENYLATE CYCLASES: NOVEL EFFECTS OF ADRENERGIC AGONISTS AND ANTAGONISTS. R. G. Van Inwegen*, M. W. Martin*, G. A. Robison, and S. J. Strada (Spon: A. Suria), The University of Texas Medical School at Houston, Department of Pharmacology, Houston, Texas 77025.

The interactions of various pharmacological agonists and antagonists on dopamine (DA) and norepinephrine (NE) stimulation of adenylate cyclase activities were studied in rat cerebral cortex (CC), caudate nucleus (CN), and pineal gland (PG). GTP (20 μM) stimulated CC adenylate cyclase ~35%. This stimulation was additive to that produced by other agonists, but was inhibited totally by 10 μM haloperidol (HAL) and partially by phentolamine (PHEN) ≥ propranolol (PROP) ≥ chlorpromazine (CPZ) ≥ dobutamine (DOB) ≥ salbutamol (SAL). None of these compounds markedly affected basal adenylate cyclase activity. Activation of CC adenylate cyclase by DA (100 μM) was inhibited completely by HAL, CPZ (2 μM), PHEN (400 μM), and DOB (1 mM), partially inhibited by SAL (1 μM), very weakly inhibited by PRO (400 μM) and unaffected by methoxamine (METH; 1 mM), amphetamine (1 mM) or NE (100 μM). Histamine (1 mM) stimulated CC adenylate cyclase 10-30% and showed additivity to DA stimulation. Activation of CC adenylate cyclase by NE (100 μM) was inhibited completely by HAL, PRO, DOB, SAL and CPZ, but was unaffected by PHEN, METH, and DA. $MnCl_2$ (5 mM) and NaF (5 mM) each stimulated adenylate cyclase (3-5 fold) and their effects were additive. None of the "antagonists" and "agonists" had any apparent affect on $MnCl_2$ or NaF stimulation, except DOB which appeared to decrease the stimulatory effects of Mn through a chemical interaction.

These results suggest that brain contains different receptors for NE and DA, perhaps in association with the same adenylate cyclase system. The data further suggest that the pharmacological classification into α and β receptor systems is not generally applicable to the CNS. Supported by U.S.P.H.S. grants DA 00744 and DA 00926 and the PMA Foundation.

486 THE EFFECTS OF DIPHENYLHYDANTOIN ON ACETYLCHOLINE INDUCED LEVELS OF CYCLIC NUCLEOTIDES IN RAT BRAIN SYNAPTASOMES. M. M. Mader*, R. J. Earley* and N. S. Thampi. Res. Dept.,Norristown State Hospital, Norristown, Pa. 19401

The effects of Diphenylhydantoin (DPH) on the turnover of cyclic AMP and cyclic GMP were studied in rat brain synaptasomes treated with acetylcholine (Ach). Synaptasomes were prepared from whole rat brains by differential centrifugation on sucrose gradients. The cyclic nucleotides were assayed according to the competitive protein binding methods of Gilman (Proc. Nat. Acad. Sci. 67:305-312, 1970) for cyclic AMP and Murad (ibid. 68:736-739, 1971) for cyclic GMP. Levels of c-AMP and c-GMP were measured in aliquots of synaptasomes incubated with 1) DPH (5, 8, 12 & 15 µg/ml), 2) DPH + Ach (1µM) and 3) controls containing no drugs for periods of 0, 5 and 15 min. Optimum activity was shown by 8 and 12 µg/ml doses of DPH at 5 min incubation time. Under these conditions DPH had significant inhibitory effect on the Ach induced increase in c-GMP while there was an apparent relative increase in c-AMP level. This finding further confirms our earlier report that "DPH may exert some of its anticonvulsant action by restoring or modulating the equilibrating effects of the opposing adrenergic/cholinergic mechanisms at the CNS synapse" (Pharmacol. 16:287, 1974).

487 INTERRELATIONSHIP BETWEEN INTRACELLULAR CYCLIC AMP, PHOSPHODIESTERASE AND ADENYLATE CYCLASE ACTIVITIES, AND THE GROWTH AND DIFFERENTIATION OF MYOBLASTS IN TISSUE CULTURE. P. Ravdin and T. Podleski. Dept. Neurobiology and Behavior, Cornell Univ., Ithaca, N.Y. 14853.

Intracellular concentration of cAMP and phosphodiesterase and adenylate cyclase activities were measured at stages of cell growth and differentiation in a rat myoblast cell line, L6. These cells grow exponentially in culture for several days, then cease dividing and fuse to form multinucleated myotubes which develop contractile proteins and receptors for acetylcholine. Intracellular cAMP was high in exponentially growing cells, decreased as the cells ceased dividing and fell even lower in myotubes. The decrease in cAMP was accompanied by an increase in phosphodiesterase and adenylate cyclase activities.

Kinetic analysis of phosphodiesterase activity indicates that at least two enzymes are responsible for cAMP hydrolysis. Both activities increase as the cells age. The ratio of basal to fluoride stimulated adenylate cyclase activities is similar in all stages of growth. Isoproterenol stimulation of cAMP levels and adenylate cyclase activity are slightly greater in myotubes. The subcellular localization of adenylate cyclase and phosphodiesterase was investigated by discontinuous sucrose gradient centrifugation. Adenylate cyclase partitioned similarly to a plasma membrane marker, azide insensitive ATPase. The membrane associated phosphodiesterase did not uniquely partition with any subcellular marker.

The decrease in cAMP is due to a complex interaction between adenylate cyclase and phosphodiesterase activities. These cells do not follow the pattern of most cells where increasing cAMP levels are correlated with a ceasation of cell growth and the appearance of differentiated characteristics. (Supported by a grant from NIH.)

488 CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ACTIVITY IN INDIVIDUAL HYPOTHALAMIC NUCLEI. A. C. Howlett* and B. McL. Breckenridge* (SPON: H. M. Geller). Dept. Pharmacol., CMDNJ-Rutgers Medical School, Piscataway, NJ, 08854.

In order to assess differences in PDE activity among the various hypothalamic nuclei, tissue samples from adult female rabbits were obtained using Lowry histochemical techniques (A Flexible System of Enzymatic Analysis, O. H. Lowry & J. V. Passonneau, 1972), and cAMP hydrolysis was measured. Samples weighing 0.25 - 0.75 μg were dissected from lyophilized sections (20 μ thick) and assayed at substrate levels of 0.6 μM. EGTA was present in the reaction mixture, and no significant activation by addition of Ca^{++} in excess of EGTA was observed.

Greatest activity was found at the interstitial nucleus of the stria terminalis and the medial POA, followed by the DMN, anterior hypothalamus, tuberal region, area lateral to the VMN, and the subformical area. These areas contain catecholaminergic innervation (U. Ungerstedt, Acta Phys. Scand. sup. 367: 1, 1971). Other areas of high activity include the VMN, posterior hypothalamus, and the lateromedial mammillary nucleus. While the SON and PVN receive considerable noradrenergic innervation, cAMP hydrolysis took place at a lower rate than at the previously mentioned regions. Other areas of intermediate activity were at the lateral and dorsal hypothalamus, and the premammillary area. Low activity was observed in the lateral POA, SCH, infundibular nucleus, posterior lateral hypothalamus, and the lateral mammillary nucleus. Negligible activity was found in the optic tracts. It appears that factors in addition to innervation by catecholamine fibers influence the level of cyclic nucleotide phosphodiesterase within the hypothalamus. (Supported by USPHS grant no. NS 10975)

489 β-ADRENERGIC RECEPTOR-MEDIATED CYCLIC AMP SYNTHESIS IN THE RAT-CORPUS STRIATUM. Jane E. Harris. Dept of Pharmacol, Emory Univ, Atlanta, GA 30322.

Employing the prelabeling technique with ^{14}C-adenine, the conversion of newly formed ATP to ^{14}C-cyclic AMP (cAMP) was studied in both slices and synaptosomes of the rat-corpus striatum in the presence of a phosphodiesterase inhibitor(RO 20-1724). The observed order of potency for β-hydroxylated catecholamines in stimulating cAMP synthesis in striatal slices is as follows: (±)isoproterenol(ISO)>(-)epinephrine>(-)norepinephrine(NE) with EC_{50}'s (concentration resulting in 50% of maximum stimulation) of 0.2, 0.7 and 3.0 μM, respectively. In comparison, non-β-hydroxylated catecholamines were much less potent with N-isopropyl-dopamine>dopamine(DA, EC_{50}=60 μM); apomorphine was least active. Stereospecificity was exhibited with the (-) isomers of ISO and NE being more potent than (±)ISO and (+)NE, respectively; dose-response curves for (+)NE and DA were similar. No significant increase of cAMP accumulation was observed when DA was combined with maximum effective concentrations of NE or ISO. β-Adrenoreceptor antagonists, such as: propranolol, sotalol and alprenolol, were more potent in blocking the DA-stimulated cAMP formation than were α-blockers, such as, phentolamine or DA-receptor antagonists, chlorpromazine and trifluoperazine. A similar order of potency for catecholamines was exhibited in a synaptosomal preparation of striatal homogenates with (±)ISO>(-)NE>DA=(+)NE and at maximum effective concentrations, DA was not additive with ISO. Finally, in synaptosomal preparations from brain regions, sparsely innervated by DA terminals, such as, the cerebral cortex and hindbrain, an acceleration of cyclic AMP accumulation was elicited by DA. Our findings in striatal tissue suggest that the DA-induced accumulation of ^{14}C-cAMP may be mediated by a weak agonistic effect of DA on β-adrenergic receptors rather than on specific DA receptors.

490 ELEVATION OF GANGLIONIC CYCLIC AMP BY PGE_2; AND IMPLICATIONS FOR ADRENERGIC TRANSMISSION. Asa C. Black, Jr., Tanemichi Chiba, James K. Wamsley*, and Terence H. Williams. Dept. of Anatomy, University of Iowa, Iowa City, Iowa 52242.

Previously we have studied the effects of dopamine on the bovine superior cervical ganglion incubated in vitro. We now report the effects of prostaglandin E_2 on this ganglion, since prostaglandins of the E series stimulate adenylate cyclase activity in a wide variety of tissues. Slices of bovine superior cervical ganglia were pre-incubated in Eagle's Minimum Essential Medium without glutamine containing 1 mM theophylline for 20 minutes at 37 C. Samples were then incubated in the same medium with and without 50 micromolar PGE_2. Afterwards, samples were frozen in liquid nitrogen and processed for cyclic AMP analysis by the method of Gilman (1972) (Adv. Cyclic Nucl. Res., 2:9). Protein determinations were performed by the Lowry method. Results are shown in the following table:

Incubation Time Minutes	Cyclic AMP (Picomoles per Milligram Protein)*	Percentage Control
1	46.4 ± 3.28 (7)	161%
3	44.5 ± 6.21 (7)	155%
5	41.3 ± 4.41 (7)	143%
10	38.0 ± 1.46 (4)	132%
15	38.1 ± 4.74 (8)	133%
17.5	26.2 ± 3.18 (8)	91.0%
20	21.3 ± 1.47 (7)	74.0%
Control	28.8 ± 2.18 (6)	100%

*Mean ± standard error of the mean; number of samples in parenthesis.

The following points may be made about the above results: (1) PGE_2 and dopamine exhibit different time courses for cyclic AMP production. PGE_2 stimulates maximal cyclic AMP accumulation within one minute, whereas maximal stimulation by dopamine requires approximately ten minutes (Soc. Neurosci., 1974, Abst. No. 72). (2) An equimolar concentration of PGE_2 was less effective than dopamine in producing increased cyclic AMP synthesis. Whereas 50 micromolar dopamine stimulated an increase to 524% of control values, PGE_2 only raised cyclic AMP levels to 161% of control values. (3) The amount of cyclic AMP produced in response to PGE_2 declined below control values by 20 minutes. We suggest that this decrease may be due to increased phosphodiesterase activity.

We conclude that PGE_2 is capable of stimulating cyclic AMP accumulation in bovine superior cervical ganglia. Greengard and his coworkers have shown that dopamine liberated from the ganglionic interneuron (SIF cell) stimulates a dopamine receptor-adenylate cyclase complex on the principal ganglionic neuron. However, the differences noted above make it uncertain whether PGE_2 and dopamine are stimulating the same adenylate cyclase. Further experiments to answer this question are in progress.

491 LOCALIZATION OF ADENYLATE CYCLASE STIMULATED BY EPINEPHRINE IN SPECIFIC REGIONS OF MEDULLA OBLONGATA OF RAT BRAIN.

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Previously neurons containing phenylethanolamine-N-methyl transferase (PNMT) and thus capable of synthesizing epinephrine (Epi) were localized by immunohistological techniques (Hokfelt et al,Acta Physiol.Scand.:89,286,1973) and also PNMT activity was measured directly (Goldstein et al,The Pharmacologist: 16,Abstr.257, 1974)in specific regions of the CNS. One such region, Group C_1 of rat medulla oblongata, contains PNMT cell bodies with nerve terminals located in immediately surrounding specific nuclei. The stimulation of adenylate cyclase (AC) by neurotransmitters affords a possible means of localization of putative postsynaptic receptors. AC activity of homogenates of various regions of brain stem was measured as ATP-dependent formation of cyclic AMP(Mishra et al,PNAS: 71,3883,1974). Epi-stimulated AC activity was present in the C_1 region and to a much greater extent in the immediately surrounding region (C_1-Surround) believed to contain terminals of PNMT neurons. In marked contrast, the remainder of medullary tissue at the level of C_1 had much lower basal AC with no significant stimulation by Epi. Stimulation by Epi appeared to be maximal at 1μM in the C_1-Surround region. Norepinephrine also stimulated AC in the C_1-Surround. Dopamine was entirely without effect at 10μM on AC of C_1 and C_1-Surround. Preliminary experiments indicate that AC-stimulation by Epi is not due to a typical β-receptor interaction; further experiments with other agonists and blocking agents are in progress to define the pharmacological properties of this AC-associated receptor. In summary, these results indicate that at the level of C_1 in the medulla, AC responsive to Epi is selectively localized in the C_1-Surround and to a lesser extent possibly also in C_1 itself. This provides support for a functional role of neuronal Epi in the region of C_1 nerve terminals in the rat brain stem.

492 MEDULLARY PROJECTIONS TO THE SPINAL ADRENERGIC FIBER SYSTEM: A STUDY USING RETROGRADE AXONAL TRANSPORT. J. D. Irvin, M. Kalia, E.T. Angelakos* and J. L. Osterholm. Hahnemann Med. Coll. & Jefferson Med. Coll., Phila. PA.

We have previously described the pattern of parenchymal catecholamine fluorescence (CAF) in the spinal cord of the cat using the Falck-Hillarp formaldehyde condensation technique. This pattern of fluorescence is altered by high thoracic (T_4) cord transection. Proximal to the site, there is an accumulation of CAF in both the gray and white matter (ventral, lateral funiculi). After one week there is no evidence of CAF in the distal segments. These findings support the theory of a descending spinal adrenergic neuron system. In order to identify the neurons (cell bodies) in the medulla, projecting to the intermediolateral gray (ILG) adrenergic column and the previously described CAF commissural band (T_8), the technique of retrograde axonal transport of horseradish peroxidase (HRP) was used. Small amounts (10-60 μl) of 33% HRP in saline were injected unilaterally into the ILG of the Ketamine anesthetized cat, below (T_{10}) and above (T_4) the CAF commissural band. Following a survival time of 24 hours, two distinct columns of labeled cells were found in the medulla. A ventrally located labeled area on the ipsilateral side lateral to the nucleus lateralis reticularis, subnucleus magnocellularis (LrM) was identified. This labeled area extended from the level of the inferior olive rostrally to the medullo-pontine junction. It is possible that this area corresponds to the postulated bulbospinal adrenergic cell system (Nobin and Bjorklund,1973). A second HRP labeled area was identified ipsilaterally in the dorsal medulla, medial to the nucleus cuneatus medialis. This column was less extensive rostro-caudally. There was no difference in the localization of the labeling when the injections were made below or above the commissural band. These results suggest that the spinal adrenergic system (ILG columns) receives direct (monosynaptic) projections from two medullary adrenergic centers. (Supported in part by NIH 00178 and ONR Themis Contr. #NR 108-876)

493 SPINAL CARDIOVASCULAR PATHWAYS: EVIDENCE FOR NOREPINEPHRINE AS THE EXCITATORY TRANSMITTER BETWEEN BULBOSPINAL AND PREGANGLIONIC SYMPATHETIC NEURONS. D.G. Taylor* and M.J. Brody* (SPON: R.K. Bhatnagar). Dept. of Pharmacology, College of Medicine, Univ. of Iowa, Iowa City, IA 52242.

It was previously reported that maximal elevations in femoral vascular resistance were elicited in cats by electrical stimulation of sites in the midcervical spinal cord containing the greatest density of efferent catecholamine-containing axons (Taylor, D.G., Fed. Proc. 34: 420, 1975). If catecholamines function in the spinal transmission of vasopressor responses then α-adrenergic receptor blockade should attenuate stimulus-induced responses. In this study the effects of centrally administered α-receptor antagonists BE-2254:(2-[β-4-hydroxyphenyl)-ethylaminoethyl]-tetralone (HEAT) and phentolamine (P) on cardiovascular responses were examined. Elevations in femoral vascular resistance, arterial blood pressure, and heart rate were elicited by high frequency (50 Hz) stimulation of sites in the dorsolateral white column of the third cervical level of cats with spinal cords sectioned rostral to the level of stimulation. HEAT and P were perfused via a cannula inserted in the subarachnoid space at the fourth cervical segment. HEAT effectively reduced or abolished evoked elevations in femoral resistance, blood pressure and heart rate. Intraspinal P also decreased or abolished the evoked cardiovascular responses. In some instances vasoconstriction was reversed to vasodilation, demonstrating that nonspecific depression of spinal transmission did not occur. Flow responses produced by intra-arterial administration of norepinephrine were unaltered, indicating that the agents did not reach the blood to produce peripheral α-receptor blockade. These observations support the contention that spinal α-adrenergic receptor activation is necessary for the transmission of excitatory impulses from the brain to preganglionic sympathetic neurons subserving a vasoconstrictor function.

494 HISTOFLUORESCENCE USING CRYOSTAT AND GLYOXYLIC ACID IN FRESH FROZEN BRAIN, Stanley J. Watson and Jack D. Barchas. Department of Psychiatry, Stanford University School of Medicine, Stanford, California 94305.

Histofluorescence of monoamines has become an essential biochemical and anatomical tool for the study of the CNS. Recently, glyoxylic acid (GA) has been introduced as a fluorophore-forming agent with a great increase in sensitivity and fineness of anatomical structure revealed (Lindvall & Björklund, *J. Comp. Neurol.*, 1974). Some drawbacks of this technique include difficulty of sectioning with the Vibratome, problems in cooling, and difficulty in demonstrating 5-HT neurons. We have previously shown that cryostat sectioning could offer a valuable tool in increasing the speed and sensitivity of the formaldehyde-induced fluorescence (Watson & Ellison, *Histochemistry*, in press). In the present work, cryostat sectioning was applied to GA histofluorescence. In contrast to the Lindvall & Björklund (1974) method, brains were not perfused with GA, since it appeared harmful to the anatomy after freezing. Instead, fresh blocks of rat brain were placed on cryostat chucks and frozen. Sections (10-30 μ) were immersed in cold phosphate-buffered GA. They were then dried in a stream of warm air for 5 min. A critical step appears to be the subsequent re-exposure to GA gas for less than 10 min and reheating in warm air. The slides were then viewed under the microscope. Very sensitive, well-localized histofluorescence can be produced with this technique. A number of factors, such as pH, duration of incubation, and gasification conditions will be discussed, since they appeared to alter sensitivity of fluorophore formation, preservation of anatomy, and background levels. By using fresh brains, alternate sections can be employed to demonstrate conventional histology, immunofluorescence of proteins, or biochemical analyses on thicker sections.

495 ANALYTICAL ELECTRON MICROSCOPY OF CENTRAL NERVOUS SYSTEM AREAS,
Joe G. Wood, Helena Stuler. Department of Neurostructure and Function, The University of Texas Medical School at Houston, Houston, Texas 77025

Analytical electron microscopy (AEM) is a valuable tool for investigating tissue components. Recent works using AEM in the study of chrome (Cr) deposits from the glutaraldehyde-dichromate-biogenic amine (BA) reaction have been reported. The transmission electron microscope mode (TEM) has shown sites of BA localization via X-Ray determinations for Cr. Such TEM studies show Cr positive norepinephrine sites in adrenal medulla and Cr positive areas in CNS dopaminergic neurons. TEM is a valuable tool; however, with a spot size of approximately 1,000 Å, high resolution analyses are not permitted. The scanning transmission mode (STEM) utilizes a smaller spot, (35-50 Å) and can be used in analyses of small areas. This smaller spot also confines area analysis and presents less background and a more accurate analysis. Current studies show that all electron dense areas are not due to Cr deposition, but many are due to relatively nonspecific osmium deposits. When Cr can be discerned from a dark structure this indicates a positive BA site. In vitro model systems of the BA reaction product show that Cr is present. When such preparations are made by processing the reaction product through routine dehydration and embedding procedures and analyses are done on one micron sections Cr is detectable; however, if the in vitro product is freeze dried, (not subjected to dehydration with organic solvents) a considerably higher amount of Cr is present. Thus the evidence from these AEM in vitro findings show dehydration is responsible for reaction product loss. Processing tissue without organic solvents results in increased reaction product remaining in the tissue and BA-Cr visualization at the light microscopic level. Supported by HEW grant #NS 10326.

496 IMMUNOCYTOCHEMICAL DEMONSTRATION OF A SEROTONERGIC INNERVATION OF CATECHOLAMINE NEURONS IN LOCUS COERULEUS AND SUBSTANTIA NIGRA. Virginia M. Pickel, Tong H. Joh and Donald J. Reis. Laboratory of Neurobiology, Dept. of Neurology, Cornell University Medical College, New York, N.Y. 10021.

It has been proposed, on indirect grounds, that in CNS, serotonergic (5HT) neurons can modulate the activity of catecholamine systems. Anatomical evidence that catecholamine neurons are innervated by 5HT axons is lacking largely because 5HT fibers cannot be definitely distinguished nor synaptic interactions demonstrated by histofluorescence. In this study we have sought to obtain direct evidence for a 5HT innervation of catecholamine neurons by localization of antibodies to the neurotransmitter synthesizing enzymes tyrosine hydroxylase (TH) and tryptophan hydroxylase (TrH), specific enzyme markers for catecholamine and 5HT neurons, respectively, in the noradrenergic neurons of the locus coeruleus (LC) and the dopaminergic neurons of the substantia nigra (SN) of rat brain. For light microscopy, brains were embedded in paraffin and sectioned at 5μ. Adjacent sections through the LC and SN were stained for TH or TrH by the peroxidase-antiperoxidase method. Alternate sections through LC and SN of unembedded brain were cut on a vibrating microtome, comparably stained, and embedded for electron microscopy. In sections through LC and SN stained with TH and examined by light microscopy, peroxidase labelling was seen in the perikarya and processes of intrinsic neurons corresponding to the catecholamine-containing cells. In contrast, in adjacent sections incubated with TrH antiserum, the same cells were unlabeled, but were surrounded by rings of stained fibers which may represent terminal and preterminal axons. Electron microscopy of sections reacted with TH antiserum demonstrated staining of perikarya and proximal processes of intrinsic neurons in LC and SN. In adjacent sections reacted with TrH antiserum, these cells were unstained, but were surrounded by unmyelinated TrH-containing processes. Within the neuronal processes, the label for both TH and TrH was restricted to microtubules. The TrH-containing axons appeared to be primarily associated with dendrites of catecholamine neurons and often formed expanded endings resembling terminals. The association of TrH with microtubules observed in the preterminal axons was not seen in terminal areas. We conclude that both dopaminergic neurons of SN and noradrenergic neurons of the LC are directly innervated by axons arising from 5HT neurons.

(Supported by NIH grants MH 24285, NS 06911, and a grant from the Harris Foundation.)

497 POSTGANGLIONIC CONNECTIONS BETWEEN AUTONOMIC GANGLIA DEMONSTRATED AUTORADIOGRAPHICALLY. Kelts, K. A.*, Shepperdson, F. T.*, Land, L. J., Whitlock, D. G., (Spon: W. S. Worth), Depts. Anat. and Neurol., U. C. M. S., Denver, Colorado, 80220.

Recent histochemical and ultrastructural studies have demonstrated that autonomic ganglia possess a complex anatomical structure. Electrophysiologically, short preganglionic events are converted in ganglia into longer duration, complex postganglionic responses. In the present report, the anatomical substrate for postganglionic interaction between sympathetic ganglia has been examined through the use of radiochemical injected into exposed celiac ganglia.

In anesthetized cats, the left celiac ganglion was isolated by a retroperitoneal approach and injected either electrophoretically or hydraulically with concentrated L-3,4 (n) ^{3}H-proline. Two to twenty days after injection, the celiac and superior mesenteric ganglia, interganglionic connections, adrenals, nearby arteries, and splanchnic nerves were removed from the anesthetized cat, fixed in Bouin's solution, embedded in paraplast, and serially sectioned at 3 u. Sections were prepared for autoradiography with Kodak NTB-2 emulsion and stained with toluidine blue.

Ganglion cell bodies in each injection site showed heavy incorporation of radiochemical with little, if any, local spread to other areas of the ganglion. Occasionally, labelled processes were observed emerging from these cells. Small, radioactively labelled fibers streamed out of the ganglion medially. Small, labelled fibers entered the opposite celiac ganglion and appeared to terminate in the vicinity of ganglion cell bodies. Failure of preganglionic fibers to take up tritiated proline was verified by the absence of labelled fibers entering either adrenal medulla. Light microscopic examination of transverse sections through the greater splanchnic and nerve bundles emerging from the celiac ganglion revealed the presence of three fiber types: large myelinated fibers, small myelinated fibers, and presumed unmyelinated fibers in an area of dense neuropile.

Our data demonstrate anatomical connections between celiac ganglia of opposite sides and suggest that postganglionic sympathetic fibers may influence the complex functions of contralateral celiac ganglia.

This research was supported in part by NIH Grants, #'s NS-08543, and NS-02599.

498 THE DISTRIBUTION OF MONOAMINERGIC NERVE TERMINALS IN CEREBRAL NEOCORTEX OF IMMATURE RAT: A FINE STRUCTURAL STUDY UTILIZING 5-HYDROXYDOPAMINE.

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As part of a study of the development of neuronal connectivity in neocortex cerebri, we have previously analyzed the density and locations of synapses with respect to perikaryal layers of immature rat neocortex. We have now attempted to distinguish between synapses with different pharmacologic properties by means of a fine structural histochemical technique. In particular, we have tried to identify, in immature neocortex, synaptic terminals which possess a mechanism for the uptake and storage of monoamines.

The cytoarchitectonic layers of newborn rat cortex and the distribution of synapses with respect to these layers have already been described (see abstract by Kristt and Molliver in this volume). In summary, during the first post-natal week, the neocortex consists of the marginal zone, the cell-dense cortical plate, and the subplate layer (immediately beneath the cortical plate) that contains relatively mature and more widely spaced neurons. In early stages of development, synapses are concentrated above and below the cortical plate. Thus, at post-natal day 1 (P1), synapses are present in two strata: in the marginal zone and in the subplate layer. At day 6 (P6) there are three strata of high synaptic density: in the marginal zone, in the deep one-third of the cortical plate, and in the subplate layer.

The method used for identification of different synaptic types stems from the finding of a mechanism in nerve terminals for selective uptake and binding of specific neurotransmitters. Tranzer and Thoenen have shown that 5-hydroxydopamine (5-OHDA), a "false" sympathetic transmitter, accumulates in monoaminergic terminals by this uptake mechanism; it forms an electron-opaque precipitate within synaptic vesicles. These small granular (dense-core) vesicles (SGV) can be identified with the electron microscope. In adult animals, 5-OHDA does not cross the blood-brain-barrier and has limited penetration into the brain after intraventricular injection. Based on evidence that there is an incomplete blood-brain-barrier to catecholamines in the newborn rat, we predicted that systemically administered 5-OHDA would penetrate uniformly into the cortex of the neonate.

Accordingly, post-natal rats at P1 and P6 were injected subcutaneously with 5-OHDA (total dose: 5-15 mg) plus an MAO inhibitor and were perfused with a standard aldehyde fixative. Following routine EM preparation, thin sections from somatosensory cortex were examined systematically in the electron microscope. Following treatment with 5-OHDA, 30% of all presynaptic elements in the cortex contain small granular vesicles. At P1 the SGV synapses are distributed both above and below the cortical plate. At day P6, there is a striking change in the distribution of SGV synapses: 30% of the synapses in the marginal zone contain SGV's; in the deep one-third of the cortical plate over 70% of the synapses contain SGV's, whereas, in the subplate layer few SGV synapses were found. Studies of the specificity of uptake of 5-OHDA have revealed that no SGV synapses were found in reserpine or imipramine pre-treated animals.

We have shown that systemically administered 5-OHDA crosses the blood-brain-barrier in the newborn rat and is taken up by a population of cortical synaptic endings. We conclude that synaptic terminals which contain SGV's after 5-OHDA have an uptake-storage mechanism for monoamines. These synaptic terminals presumably arise from cell bodies in specific monoaminergic brain stem nuclei. We propose that, in immature rat, there is a major projection from the brain-stem to both deep and superficial layers of neocortex; at P6 there is an especially dense projection to the deep one-third of the cortical plate, which contains the cell bodies of layer IV. [Support: USPHS NS-08153, NS-10920, NS-11034, NS-02195 & UCP]

499 **HISTOCHEMICAL CHARACTERIZATION OF THE NEOCORTICAL PROJECTION OF THE NUCLEUS LOCUS COERULEUS IN THE SQUIRREL MONKEY.** **Stephen L. Foote, Robert Freedman* and Floyd E. Bloom. Lab. Neuropharmacology, National Institute of Mental Health, St. Elizabeths Hospital, Washington, D.C. 20032.**

We have obtained histochemical evidence for a norepinephrine(NE)-containing projection from the nucleus locus coeruleus(LC) to the squirrel monkey neocortex. Glyoxylic acid-induced fluorescence shows an extensive arborization of fine, catecholamine-containing fibers with prominent varicosities in all layers of the neocortex. LC is identified as a source of these fibers by both ortho- and retrograde histochemical tracing techniques. After injection of 0.5 to 5μl of horseradish peroxidase into frontal, temporal, or occipital cortex, labelled cell bodies are found throughout the major portions of LC. Conversely, after microinjection into LC, tritiated proline is transported into the neocortex where it appears within fibers similar in distribution to those revealed by fluorescence histochemistry. Both transport techniques indicate that the neocortical projections of LC originate from both ipsilateral and contralateral nuclei and are not organized in any simple topographic pattern.

This histochemical demonstration of a moderately dense NE innervation of neocortex complements our previous finding that the spontaneous and stimulus-evoked activity of auditory cortex neurons is substantially altered by small amounts of microiontophoretically applied NE. The combined data suggest that this NE pathway plays a significant role in determining the discharge patterns of neocortical neurons.

500 HIPPOCAMPAL INNERVATION BY NORADRENALINE NEURONS OF THE LOCUS COERULEUS. R.Y. Moore. Dept. Neurosciences, U. Calif., San Diego, La Jolla, CA 92037

The hippocampal formation is innervated principally by related telencephalic regions, the ipsilateral entorhinal cortex and septum and the contralateral hippocampal formation and entorhinal cortex. There are, however, two hippocampal afferents which arise from the brainstem. The first of these is a projection from the serotonin neurons of the midbrain raphe (Moore and Halaris, 1975). The second is from the noradrenaline neurons of the pontine nucleus, locus coeruleus. In the present study the hippocampal noradrenaline innervation was analyzed in the rat using the formaldehyde-Vibratome (Hokfelt and Ljungdahl, 1972) and glyoxylic acid (Lindvall and Bjorklund, 1974) variants of the Falck-Hillarp fluorescent histochemical method.

Axons of locus coeruleus origin innervating the hippocampal formation have a typical appearance in material prepared by the fluorescent histochemical methods. They have very fine preterminal segments with fairly regularly spaced varicosities, 1-3 μ in diameter. The noradrenaline innervation reaches hippocampal formation primarily via the cingulum and fornix with some fibers entering through the entorhinal cortex. In hippocampal zones CA1 and CA2 there is a dense plexus of noradrenaline axons in stratum lacunosum-moleculare with individual fibers turning into stratum radiatum. Stratum oriens is sparsely innervated. In CA3 both stratum radiatum and stratum lacunosum-moleculare are heavily innervated. The dentate fascia receives a dense innervation in the zona limitans region of the polymorph layer but the molecular layer is only moderately innervated. This pattern of innervation by noradrenaline axons from locus coeruleus is quite similar to that of the raphe serotonin projection but dissparate from other hippocampal formation innervation. (Supported by NIH grant NS-12080.)

501 AN AUTORADIOGRAPHIC STUDY OF LOCUS CERULEUS PROJECTIONS IN THE RHESUS MONKEY. Dwight C. German and Douglas M. Bowden, Regional Primate Research Center and Depts. of Physiology & Biophysics and Psychiatry & Behavioral Science, University of Washington, Seattle, Washington 98195.

The projections of neurons in the locus ceruleus (LC) of the rhesus monkey were traced by autoradiography. A volume of 0.7 µl (10-30 µCi/µl concentration) of ^{3}H-amino acid (leucine and/or proline) was stereotaxically injected into the LC region of 3 rhesus monkeys. An ascending bundle of labeled axons was traced into the ventrolateral region adjacent to the central gray, dorsal to the red nucleus, along the medial tip of the internal capsule, into the lateral hypothalamic area, into the preoptic area, and dorsally through the medial septal region. From this ascending bundle labeled axons exited and were observed running in the stria terminalis, cingulum, and internal capsule. Labeled axons were also observed in the superior cerebellar peduncle, anterior spinocerebellar tract, and cerebellar medullary body. Descending axons entered the medulla in the ventrolateral quadrant and coursed toward the spinal cord. The LC projections were primarily ipsilateral to the injection site, however, decussating fibers were observed in the posterior commissure, and contralateral projections were found in the brainstem. The pattern of synaptic termination differed in various brain regions, but was generally not restricted to the soma surface (e.g., in cerebral, hippocampal and cerebellar cortex). These neuronal projections are similar to those observed with histochemical fluorescence, autoradiography, and silver degeneration techniques in the rat, and with histochemical fluorescence in the human fetus.

Supported by NIH Grants RR00166, NS05082 and MH42493.

502 GABA IN HUMAN CSF; THE SIGNIFICANCE OF MEASUREMENT IN NEUROLOGICAL DISEASE. K.M.A. Welch, Eva Chabi*, V.S. Achar*, Katherine Bartosh* and J.S. Meyer. Dept. Neurol., Baylor Coll. Med., Houston, Texas, 77025.

Cerebrospinal fluid of 169 patients undergoing neurological evaluation for suspected CNS disease was analyzed for GABA content by an enzymatic fluorometric assay of sensitivity sufficient to detect GABA at 10^{-12} moles per assay. Control patients without neurological disease showed no evidence of detectable GABA in the CSF. GABA was most frequently detected in patients with recent cerebral hemispheric infarction (0.004-1.36 nM/ml) who exhibited reduced cerebral blood flow and anerobic metabolism and in those patients with vertebrobasilar ischemia (0.13-0.85 nM/ml). GABA was also detected only in those patients with both Parkinson's syndrome (0.03-0.66 nM/ml) or dementia (0.38-0.81 nM/ml) associated with ischemia due to cerebrovascular disease. In patients studied with migraine headache, CSF GABA was detected only during the headache attack (0.004-0.44 nM/ml) when cerebral ischemia is also known to occur. CSF GABA was undetectable in idiopathic Parkinson's syndrome and in patients with dementia associated with Alzheimer's or Huntington's disease.

Results suggest that GABA levels in normal human CSF are of low order and when detected using the present assay may indicate abnormality of CNS GABA metabolism. In the patients studied increase of GABA in CSF appears related to the presence of ischemic conditions and seems best explained by (1) impaired oxidative metabolism with decreased utilization of the GABA shunt causing brain GABA levels to rise and/or (2) GABA release from cells damaged by ischemia. Diseases with associated neuronal abiotrophy such as idiopathic Parkinsonism, Alzheimer's and Huntington's disease, would not be expected to demonstrate detectable CSF GABA since enzymic GABA synthesis may be reduced in these disorders.

503 INCREASE OF 5-HYDROXYINDOLEACETIC ACID AND HOMOVANILLIC ACID IN CISTERNAL CEREBROSPINAL FLUID OF CATS SUBJECTED TO STRESS. Mindaugas Griauzde and Miodrag Radulovački. Dept. Pharmacol., Univ. Ill. Med. Ctr., Chicago, 60612.

Acute forms of stress are known to produce an increase in the rate of 5-hydroxytryptamine (5-HT) and dopamine (DA) metabolism followed by increased concentrations of 5-hydroxyindoleacetic acid (5-HIAA), a 5-HT metabolite, and homovanillic acid (HVA), a DA metabolite, in the brain of several mammalian species. We have devised a model that produces a strong emotionally stressful situation in the cat and measured concentrations of 5-HIAA and HVA in cisternal cerebrospinal fluid (CSF) of cats subjected to such stress. Cisternal CSF was obtained from a cannula in the cisterna magna (Radulovacki, 1974). 5-HIAA and HVA were determined by the method of Korf and Volkenburgh-Sikkema (1969).

One week after implantation, control CSF samples were collected for 3 days at 2-hr intervals starting at 8:30 a.m. and ending at 2:30 p.m. On 3 subsequent experimental days, cats were placed in small transport cages and exposed between 9:30 and 10:00 a.m. to a continuously barking dog confined in a kennel. CSF samples were taken in the same order as controls. The results revealed a significant increase (38.16%, $P < 0.005$) in 5-HIAA 1 hr subsequent to stress which persisted over 4 hr. HVA was significantly increased (37.24%, $P < 0.05$) only for the first 2 hr following stress. These results agree with the findings that both 5-HT and DA metabolism increases in acute stress. They show comparable increases in 5-HIAA and HVA concentrations after stress and confirmed reports of the longer-lasting effect of stress on 5-HT metabolism. This model offers a possibility of studying the effects of psychoactive drugs on brain monoamines in an emotionally stressful situation with each animal serving as its own control. (Supported by PHS Grant NS 10921)

504 ACETYLCHOLINE AND RELATED ENZYMES IN HUMAN VENTRICULAR AND SUBARACHNOID FLUIDS FOLLOWING BRAIN INJURY. R.Grossman,C.Beyer*,P.Kelly* and B.Haber. Div. Neurosurg., Dept. Surg.; Marine Biomed. Inst., UTMB, Galveston 77550.

This study was made to determine if acetylcholine and related enzymes are present in abnormal amounts in cerebrospinal fluid (CSF) and epicortical subarachnoid fluids following traumatic head injury (HI). Ventricular and epicortical fluids were obtained via catheters placed for intracranial pressure monitoring, or during craniotomy for evacuation of hematomas, and CSF via lumbar puncture. All fluids were centrifuged, chilled and aliquots acidified for acetylcholine (Ach) analysis. Samples were concentrated routinely to increase sensitivity of assay procedures and all data are expressed per ml fluid. Ach and choline were measured by radiometric method of Goldberg and McCaman using γ-P^{32} ATP. True and pseudo cholinesterase were determined by microassay using C^{14}-acetyl-B-methylcholine and butyrylcholine as substrates in the presence of appropriate inhibitors (iso-ompa and BW 281). Cholineacetyltransferase (CAT) was measured with C^{14}-acetyl Coenzyme A as substrate. Epicortical fluid averaged 300 picomoles (pM) Ach/ml, whereas ventricular fluid averaged between 100-200 pM, with significantly lower levels in lumbar CSF. The rostral-caudal gradient observed for Ach was seen neither in CAT nor cholinesterase (true or pseudo). CSF fluids averaged 2000pM choline/ml, roughly 70-80% of plasma values. The striking finding is the elevation of plasma choline in all HI fluids tested. No consistent changes were observed in CAT activity in HI fluids, though true cholinesterase seems to be elevated. Additional enzymes monitored in control and HI fluids were MAO, COMT and ARD. Samples contaminated with hemolysed RBC were discarded. Degree of RBC lysis was assessed by hemoglobin determinations. Mechanism of these changes in cholinergic system components and their significance in HI are under investigation. (Supported by NIH Grant NS 07377.)

505 NOREPINEPHRINE IN CEREBROSPINAL FLUID. Michael G. Ziegler*, C. Raymond Lake*, Fredrik H. Foppen*, Irwin J. Kopin and Ira Shoulson. NIMH and NINCDS, NIH, Bethesda, Md. 20014.

Cerebrospinal fluid (CSF) was obtained by lumbar puncture from a group of patients with various neurological disorders. Four ml of CSF was collected in a tube containing 10 mg of ascorbic acid and assayed for norepinephrine (NE) by a modification of the method of Henry et al (Life Sci 16: 375, 1975), employing the radioenzymatic conversion of NE to ^{3}H-epinephrine. In 34 samples of CSF from 22 patients, the concentration of NE ranged from 53 to 907 pg/ml. The limit of detection of CSF NE was approximately 30 pg; blank values were 110 counts per min (cpm) and ^{3}H-epinephrine yielded 8 cpm/pg. The specificity and accuracy of the radioenzymatic method was verified by gas chromatography - mass spectroscopy (GC-MS) on 12 CSF samples. The trifluoroacetate of NE was measured on a Finnegan 1015 chemical ionization mass spectrometer interfaced with a Varian 1400 gas chromatograph. Samples were separated using methane as the reagent gas and D_3-NE or alpha-methyl NE as internal standards. The correlation coefficient between the radioenzymatic and GC-MS results was 0.97. The mean difference between NE determined by the two methods was 11.9%; mean values determined by the radioenzymatic method were 4.6% higher than by GC-MS. We conclude that CSF NE can be reliably determined by either technique, but the radioenzymatic method is more convenient and does not require GC-MS.

506 IDENTIFICATION OF THE MAJOR SITE OF CEREBROSPINAL FLUID EFFLUX IN THE ALBINO RAT. J. Douglas Mann*, Albert B. Butler* and Norman H. Bass. Depts. of Neurol. and Nsurg., U.VA. Sch. Med., Charlottesville, Va., 22901

Arachnoid villi constitute a major site for the drainage of cerebrospinal fluid(CSF). Since such structures are absent in the rat, it has been suggested that major routes of CSF efflux may exist at sites other than the superior saggital sinus. In the present study, the relative importance of CSF drainage through the torcular Herophili was assessed. Pressure was monitored in the subarachnoid space for varying rates of intrathecal infusion (2-153ul/min). At infusion rates below 48ul/min., physiologic compensation was adequate to establish a steady state CSF pressure. Carboxyl ^{14}C-inulin was then infused intrathecally into acutely nephrectomized rats at a rate of 20ul/min for 5 minutes. CSF pressure rose to a steady state of 800 mm H_2O, and fell rapidly to resting levels after infusion was stopped. Blood samples were obtained simultaneously from the torcular Herophili and femoral artery at 30 second intervals for 15 minutes after the start of infusion. Inulin appeared first in torcular blood at 2 1/2 minutes at a time when it could not be detected in the systemic circulation. At 3 minutes, inulin in torcular blood samples was 26 fold greater than in femoral samples. Particulate matter(I-131 labeled 0.5u polystyrene beads), showed a similar efflux profile. The cellular site of efflux in the torcular Herophili was identified by electron microscopy. With increased CSF pressure, these endothelial cells showed(1)no increase in the number of pinocytes;(2)moderate reduction in junctional overlap and(3)a marked increase in vacuoles. Hence, the superior saggital sinus of the rat is a major route for efflux of CSF and particulates under conditions of increased pressure. Specialized endothelial cells exist in the torcular whose mechanism of action appears to be analogous to similar cells found in Schlemm's canal and arachnoid villi.

507 PHARMACOLOGIC AND ENZYMATIC STUDIES IN FRIEDREICH'S ATAXIA

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Friedreich's ataxia is a common, inherited spinocerebellar degeneration. The genetic defect is presumably present from birth but symptoms do not usually appear until age 8 or later and patients typically live 15 to 20 years thereafter.

Four patients with Friedreich's ataxia and 8 with other hereditary ataxias had improved scores on a clinical rating scale for ataxia within 30 to 60 minutes after receiving a single dose of the cholinesterase inhibitor, physostigmine (36 $\pm$ 10% better, mean $\pm$ S.E.M., $p < 0.001$). Five patients including 3 with Friedreich's ataxia underwent a year's randomized, double-blind, cross-over trial. The 3 with Friedreich's ataxia had improved scores while on physostigmine as compared to placebo (average improvement 46%); the 2 with olivopontocerebellar degeneration did not (average improvement 2%). The patients remained ill despite the improved scores.

Abnormalities of pyruvate metabolism have previously been found in the muscle of several patients with Friedreich's ataxia and in intact cultured fibroblasts from 3 (Neurology 24, 964, 1974). Activities of the pyruvate dehydrogenase complex (PDH) and of the 2-oxoglutarate dehydrogenase complex (KGDH) have now been found to be low in disrupted cells of one patient from each of 4 families. Cells were homogenized in 40% (v/v) glycerol in tris buffer, pH 7.4, and assayed radiochemically. Values in picomoles/min per mg protein $\pm$ S.E.M. (and the number of individuals) were:

	Patients	Controls	Significance
PDH	79 + 8 (4)	201 $\pm$ 19 (19)	$p < 0.001$
KGDH	129 $\pm$ 13 (4)	245 $\pm$ 19 (8)	$p < 0.005$

The controls included subjects with other neurological diseases. Mixing experiments gave no evidence for soluble inhibitors or activators. The defects were not reversed by large excesses of substrates or cofactors. Normal activity of cytochrome-c oxidase (another mitochondrial enzyme) was found in the patients' cells. The results are consistent with a mutation affecting a component common to the two dehydrogenase complexes. It is not yet known whether the patients responding to physostigmine have the same biochemical abnormality.

The relationships between these observations and the pathophysiology of Friedreich's ataxia are unknown, but the above data are consistent with the hypothesis that mild deficiencies in the utilization of carbohydrates may affect cholinergic systems.

508 PHENYLALANINE:PYRUVATE TRANSAMINASE (PPT) MIGHT BE SIMILAR TO HISTIDINE:PYRUVATE TRANSAMINASE (HTP) BUT DIFFERENT FROM PHENYLALANINE:α-KETOGLUTARATE TRANSAMINASE (PKT). Jean C. Shih and Robert H.-C Chiu*. School of Pharmacy, Univ. of Southern California, Los Angeles, CA 90033.

In most cases of human disease phenylketonuria, phenylalanine hydroxylase is missing due to a genetic defect. This deficit results in an increase in phenylalanine concentration in plasma and brain and results in mental retardation. Phenylalanine aminotransaminase catalyzed the conversion of phenylalanine to phenylpyruvate, and is inducible by glucagon or cAMP. This enzyme might provide an alternate pathway for the disposition of excess phenylalanine in human phenylketonuria. In our laboratory, we have shown that there were two different phenylalanine transaminases in rat liver. Phenylalanine:pyruvate transaminase (PPT) was heat stable (heat at 60° C, 15 min) and was inducible by chronic glucagon injection (glucagon (1 mg/kg) was injected into rats every other day for 1 week and the animals were sacrificed twenty-four hours after the last injection), whereas phenylalanine:α-ketoglutarate transaminase (PKT) was heat unstable and was not inducible by chronic glucagon injection. These two enzymes could be separated by means of a Sephadex-electrophoresis column, or a DEAE-Sephadex A-50 column. Histidine:pyruvate transaminase (HPT) activity was always found to coincide with PPT activity after column chromatography and it was inducible by chronic glucagon injection, as PPT (the enzyme activity increased about 10 times higher than the controls). Based on their response to chronic glucagon injection *in vivo*, and their heat stability, or chromatographic separation *in vitro* we concluded that PPT might be very similar to HPT, but might be different from PKT. (Supported in part by a grant from Eli Lilly and Company.)

509 INHIBITION OF CNS CHOLESTEROL BIOSYNTHESIS BY TRANS-CLOMIPHENE AND AY9944. R. B. Ramsey, V. W. Fischer, H. J. Nicholas* and M. Fredericks. Inst. Med. Ed. & Res. and Dept's. of Neurology, Anatomy and Biochemistry, St. Louis Univ. School of Medicine, St. Louis, Mo. 63104.

The *trans* isomer of clomiphene has previously been shown to inhibit sterol Δ^{24} reductase, resulting in an accumulation of desmosterol (T. R. Blohm, *et al.*, *Biochem. Pharmacol.* (1970) 19, 2231). Although the nervous system was not examined in the study cited, it has been demonstrated that administration of another sterol Δ^{24} reductase inhibitor, 20,25-diaza-cholesterol, results in a tremendous accumulation of desmosterol in the nervous system. In the present study 50 mg per kg body weight of *trans*-clomiphene citrate was administered to 5 day old rats by intraperitoneal injection. Before the animals were sacrificed at 20 days of age, a total of 5 injections had been given. Analysis of the sterol composition of the CNS of the 20 day old rats indicated that approximately 66% of the brain sterol and 62% of the spinal cord sterol was desmosterol. Another C_{27} sterol, of unknown structure, represented 3% of the total spinal cord sterol. Cholesterol accounted for the remainder of the sterol. Morphological examination of the CNS of these treated animals is presently in progress. A comparison was also made of the effect of *trans*-clomiphene and AY9944, a sterol Δ^{7} reductase inhibitor, on isoprenoid lipid biosynthesis by cell-free preparations of these developing brains incubated with (2-^{14}C)-mevalonic acid. Overall neutral isoprenoid lipid formation was not appreciably affected by AY9944, but a depression of incorporation of labelled mevalonate was seen with the *trans*-clomiphene animals versus controls. Supported in part by NIH Grant NB 06011-09.

510 IDENTIFICATION OF A BRAIN PEPTIDE INDUCED BY BLUE AVOIDANCE TRAINING IN GOLDFISH. D. F. Tate* and G. Ungar. Baylor College of Medicine. Houston, TX, 77025.

It was previously shown (Experientia, 28:1026, 1972) that brain extracts, taken from donor goldfish trained in a divided tank either to avoid the blue compartment and swim into the green (BG) or to avoid the green compartment and swim into the blue (GB), when injected intracranially into naive recipients, can reproduce the respective behaviors of the donors. With this effect used as a bioassay, both substances BG and GB were purified and proved to be peptides (Fed. Proc. 33:481, 1974). - This report deals with the structural elucidation of peptide BG. Because of the small amount of material available, the dansyl method was used for the identification of amino acids and peptides. Amino acid analysis gave the following composition: ala,glu,gly_2,ile,leu,lys_2,phe,pro,ser,tyr,val. After unblocking with NaOH, the N-terminal was found to be glu. Stepwise digestion with COpeptidase A and B, combined with tryptic and chymotryptic digestion, showed the C-terminal fragment to be leu-lys-tyr-gly-ser-lys. The combined results of NHpeptidase M and COpeptidase B digestion suggested that this sequence was preceded by phe-pro. Dipeptides obtained by cathepsin C digestion, characterized by their N-terminal amino acid and comparison with known dipeptides, suggested the following sequence for the N-terminal fragment: pglu-ile-gly-ala-val. The following tentative sequence, therefore, is proposed for peptide BG: pglu-ile-gly-ala-val-phe-pro-leu-lys-tyr-gly-ser-lys. It is subject to confirmation by synthesis and comparison of the chemical properties and behavioral effect of the synthetic and natural compounds. The structure of peptide GB is currently being investigated. (Supported by USPHS grant HE-05435 and studentship of the Medical Research Council of Canada).

511 BRAIN PEPTIDE ASSOCIATED WITH HABITUATION TO A SOUND STIMULUS. S. R. Burzynski and G. Ungar. Baylor College of Medicine. Houston, TX 77025.

It was previously reported that habituation to a sound stimulus in rat induces the formation of a substance in the brain (Nature, 207:301,1965). After isolation and purification, this substance was found to be the hexapeptide pglu-ala-gly-tyr-ser-lys, named ameletin. After confirmation of the structure by synthesis (H. Lackner and N. Tiemann, Naturwissenschaften 61:217,1974), an ultramicroanalytical method of determination was devised based on dansylation of brain extracts partially purified by gel filtration, free-flow electrophoresis and thin-layer chromatography. The spot obtained in the latter procedure was quantitated by densitometry and comparison with known amounts of synthetic ameletin.

The peptide was found to be consistently absent from brains of untrained rats. In animals submitted to the habituating stimulus (the sound of an electric bell), the brain levels of ameletin increased for five days and decreased afterwards, in spite of continuing habituation. Preliminary studies of regional distribution indicate that ameletin is formed throughout the brain without preferential localization. Intraperitoneally administered synthetic ameletin was found in the brain 30 min after injection and persisted there for about 12 h. Habituation to a different type of sound did not induce formation of ameletin.

These results, together with those obtained with another learning-induced peptide, scotophobin (Naturwissenschaften, 60:307,1973), suggest that learning is associated with formation of peptides in the brain. The specificity of this association and its significance for a molecular code of neural information is being further investigated. (Supported by USPHS grants HE-05435 and CA-15056).

512 EVIDENCE FOR THE SYNTHESIS OF THREE SPECIFIC PROTEINS IN GOLDFISH BRAIN AFTER LEARNING. Victor E. Shashoua. McLean Hospital, Biological Research Laboratory, Harvard Medical School, Belmont, MA 02178.

In previous studies we found that specific RNA changes occurred when goldfish acquired a new swimming skill. The possibility that these RNA molecules are templates for the synthesis of specific proteins was therefore investigated using double labelling methods. In each experiment the brains from groups of seven trained animals, labelled with valine ^{3}H and seven controls labelled intracerebrally with valine ^{14}C were pooled, homogenized, and fractionated into their nuclear, cytoplasmic soluble, microsomal, myelin, synaptosomal, and mitochondrial components. Polyacrylamide gel electrophoretic patterns of the labelled proteins were then obtained. The distribution of the labelled proteins was determined in the gels, and the ratio of $^{3}H/^{14}C$ for each of the labelled bands was measured. Methods were developed to give constant ratios of $^{3}H/^{14}C$ for experiments in which control vs. control animals were studied. Three peaks in the $^{3}H/^{14}C$ ratios corresponding to three protein bands (α, β, and γ) were obtained in the polyacrylamide gel patterns of proteins from trained vs. control animals. The pattern of synthesis of the proteins following learning, and in a number of control behavioral situations was also studied. No changes were observed after exhaustive physical exercise, during a stressful situation, and during the performance of a well-known task suggesting that the increased synthesis of the specific proteins may be related to the consolidation process of information storage. The migration properties of the α, β, and γ bands on SDS-acrylamide gels correspond to molecular weights of 37,000, 32,000 and 26,000 daltons. (This research was supported by the Grant Foundation and NINDS).

513 AMINO ACID INCORPORATION INTO BRAIN PROTEINS: THE ROLE OF HORMONES IN BEHAVIOR-RELATED CHANGES. Howard D. Rees* and Adrian J. Dunn. Dept. of Neurosci., Univ. of Florida Coll. of Medicine, Gainesville, 32610.

The incorporation of subcutaneously injected [4,5-^{3}H]lysine into brain and liver and their proteins has been studied in C57Bl/6J male mice following behavioral treatments or hormone injections. Previously we have reported that following shock avoidance training there was an increased uptake of ^{3}H into both brain and liver, accompanied by increases in protein incorporation and relative radioactivity (RR = d.p.m. in protein/d.p.m. in free lysine) in brain (+17%) and liver (+62%) (Brain Res. 68:143, 1974). Behavioral analysis suggested that these changes might be in response to stress. However, the responses were observed in adrenalectomized mice. We have now shown that changes in both brain and liver can be mimicked by the injection of ACTH, although following ACTH the uptake and RR increases in the brain were smaller and more variable. The effects of $ACTH_{1-24}$ on the liver were not mediated by the adrenal glands and were not duplicated by $ACTH_{4-10}$. Saline vehicle injections alone caused some increases, presumably due to the increased release of endogenous ACTH and/or corticosteroids. The effect of corticosterone depended upon the injection vehicle and route. The injection of lysine vasopressin which has some behavioral actions similar to those of ACTH, decreased the uptake and RR in both brain and liver. These results will be discussed in relation to their potential role in stress responses and learning. It will be suggested that stress as an important factor in learning may effect neurochemical changes through the action of adrenal-pituitary hormones, and that these changes may interact with other factors in the formation of memory.

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514 EFFECT OF TRAINING ON THE LYSINE POOL OF MOUSE BRAIN. Moshe Hershkowitz. Dept. of Biochemistry, School of Medicine, UNC, Chapel Hill, N.C. 27514.

The content of lysine in the amino acid pool of the brain of the mouse increases significantly (∿ up to 20%) as a result of training the animal to drink milk from a dipper. The maximum value for free lysine was reached at about 30 min of training and returned to control values at 60 min of training. Control mice were given milk for 30 min before the sacrifice as well as several times during a week before the experiment.

Training of mice did not affect the amount of ^{3}H-Lysine accumulated in the brains of mice 30 min after injection with the labeled amino acid as compared to that accumulated in control animals. Injection time coincided with the beginning of training. The specific activity of ^{3}H-Lysine decreased to about 30% of its value at 10 min after injection during 60 min. The rate of decrease in the specific activity was significantly higher in the trained mice. The same effect of training on the rate of decrease in lysine specific activity was obtained after ^{3}H-Lysine injection into the brain lateral ventricules.

The significance of these observations in relevance to brain protein metabolism during training will be discussed.

515 TIME-DEPENDENT DIFFERENCES IN THE BRAIN RNA POPULATION OF GOLDFISH AFTER BEHAVIORAL TRAINING. J. L. Sirlin. Dept. Anat., Cornell Univ. Med. Coll., New York, NY 10021.

Newly synthesized brain RNAs of fish that acquired a new swimming skill (T) and controls that swam in a whirlpool (C) (Kaplan, Dyer & Sirlin, Brain Res. 56: 239, 1973) were compared. C are more exerted than T as shown by plasma cortisol levels, but the exertion in C is without behavioral effects on subsequent learning (Kaplan & Sirlin, Brain Res. 83: 451, 1975).

Whole brain RNA from T and C was co-extracted 1, 2.5 and 24 h post-intraventricular injection of ^{3}H- and ^{14}C-guanosine, respectively, immediately after a 4-h session. The double-labelled RNA was fractionated by polyacrylamide gel electrophoresis (2.2 % acrylamide) in triplicates for each sample. At 1 h most (>90 %) of the radioactivity is in polydisperse high mol. wt. RNA up to 9 m ('m' represents 10^{6} daltons) and primary ribosomal RNA (rRNA) precursor (2.6 m), and the remainder of the radioactivity is in transfer RNA (tRNA). At 2.5 h there is relatively more activity in primary and secondary (1.6 m) rRNA precursors. At 24 h about half of the activity is in mature rRNAs (0.7 and 1.5 m), and the other half of the activity is distributed about equally between tRNA and polydisperse RNA across the profile.

Radioactivity profiles of ^{3}H- and ^{14}C-RNAs were compared by calculating the normalized ratio of counts, in order to by-pass any differential labelling of precursor nucleotide pools, and analysis of the resulting ratio plots. The results are that relative to C: 1) synthesis and maturation of rRNAs in T is no different during 24 h; 2) incorporation into tRNA is greater in T at all times, i.e. approx. 29 and 16 % excess over C at 1 and 24 h respectively; 3) incorporation into polydisperse RNA is greater in T at all times particularly for the largest RNAs, i.e. up to approx. 72 and 16 % excess over C at 1 and 24 h respectively; 4) two RNA molecular classes (0.5-0.6 and 2.1 m) (collectively, 'class-4 RNAs') are enriched in T at all times. Of these class-4 RNAs in T, 2.1 m RNA is more enriched than 0.5-0.6 m RNA at 1 h (approx. 26 and 14 % excess over C respectively); both RNAs are about equally enriched at 2.5 h (38 and 32 %); and 0.5-0.6 m RNA is more enriched than 2.1 m RNA at 24 h (17 and 10 %). Thus T are enriched in most high mol. wt. RNA species (except rRNA) at 1 h, but mainly in class-4 RNAs at 24 h. Class-4 RNAs are not mitochondrial rRNAs (0.4 and 0.95 m) and comprise approx. 1.7 and 4.8 % respectively of total RNA radioactivity in the profile of T at 24 h.

Content of RNA with 3'-terminal poly A (approx. 50 to 150 A units) was measured in trplicates for each of the previous samples. Poly(+)RNA, as mean % of total precipitable RNA radioactivity, is: 20.0 and 20.6 in C and T respectively at 1 h; 26.9 and 27.3 at 2.5 h; and 11.3 and 9.3 at 24 h (95-% confidence intervals are $<\pm$ 2.0 % of each mean %). By a t-test the mean % poly A(+)RNA in C and T differs significantly at 1 and 24 h ($P < 0.05$ and < 0.001) but not at 2.5 h, which might suggest a slightly faster turn-over of polyadenylation of total DNA-like RNA in T.

The results point to an increased demand for (a) tRNA, (b) polydisperse DNA-like RNA with function(s) still not clear, and (c) long-lived RNA species of a putative messenger-type (class-4) soon after behavioral training. (Supported by USPHS 1 R01 NSMH 10270).

516 BRAIN DOPAMINE AND BEHAVIOR: EVIDENCE FOR AN INVERTED U-SHAPED RELATIONSHIP. Thomas Heffner*, Michael Zigmond, and Edward Stricker. (SPON: Herbert Barry III). Psychobiology Program, University of Pittsburgh, Pittsburgh, PA 15260.

Food intake and general activity were measured in food deprived rats after treatments designed to increase or decrease brain dopaminergic (DA) activity. Decreased feeding associated with increased general activity and stereotopy was seen after systemic administration of DA agonists. These dose-related effects were obtained with the indirect-acting agonists amphetamine, cocaine, and methylphenidate as well as with the direct-acting agonists apomorphine and dihydroxyphenylalanine. Systemic administration of α-methyltyrosine, an inhibitor of dopamine synthesis, or spiroperidol, a dopamine receptor blocking agent, also decreased feeding, as did selective destruction of DA neurons by intraventricular injections of the neurotoxin 6-hydroxydopamine. In contrast to the effects of DA agonists, the anorexic effects of each of the treatments which impaired DA function were accompanied by decreased general activity. Spiroperidol at a dosage that did not alter feeding when given alone antagonized the anorexic and behavioral activating effects of each DA agonist but augmented the anorexic and behavioral depressant effects of α-methyltyrosine. In contrast to the linear relationship between DA function and general behavioral activity, our findings suggest that brain DA activity and feeding may be related by a function that takes the form of an inverted U-shaped curve. That is, DA activity appears to be optimal for feeding in food deprived rats with increases or decreases in this activity resulting in reduced feeding. As such, this relationship differs from the strictly inhibitory or excitatory roles for brain catecholaminergic systems in the control of ingestive behaviors previously proposed. (Supported by grants from Smith Kline and French and U.S.P.H.S. (MH-20620).)

517 NORADRENALINE AND BEHAVIOR-RELATED EEG ACTIVATION OF THE HIPPOCAMPUS AND NEOCORTEX. T.E. Robinson*, B.A. Pappas and C.H. Vanderwolf. Depts. Psychology, University Western Ontario, London, and Carleton University, Ottawa, Ontario, Canada.

Previous studies show that there are at least 2 ascending inputs each of which produce low voltage fast activity (LVF) in the neocortex and rhythmical slow activity (RSA) in the hippocampus. An atropine-resistant input is closely coupled to concurrent Type I behavior while an atropine-sensitive input is unrelated to Type I behavior (Vanderwolf, JCPP, 1975, 88, 300). The hypothesis that the dorsal noradrenergic bundle is responsible for atropine-resistant effects was tested by injecting neonatal rats with 6-hydroxydopamine (50 mg/kg s.c. on days 1-8 after birth). Assays revealed permanent depletion of cerebral noradrenaline (NA) but normal levels of serotonin and 5-hydroxindoleacetic acid. In a 24 hr. time sample test, the behavior of NA-depleted adult rats appeared normal although they ran less in activity wheels than vehicle injected controls. Both atropine-sensitive and atropine-resistant EEG patterns were normal. Atropine-resistant LVF was abolished in both groups by reserpine (2.5 mg/kg) but atropine-resistant RSA was not abolished. The results suggest that the dorsal noradrenergic bundle is probably not essential for EEG activation.

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518 REVERSAL OF PENTOBARBITAL SLEEP BY THYROTROPIN RELEASING HORMONE IN THE RHESUS MONKEY. G.W. Kraemer*, R.A. Mueller, G.R. Breese, B.R. Cooper*, W.B. McKinney* and A.J. Prange, Jr. University of North Carolina, Chapel Hill, N. C. and University of Wisconsin, Madison, Wisconsin.

Previous studies have shown that thryotropin releasing hormone (TRH) can antagonize sedation and hypothermia produced by pentobarbital in rats and mice. The purpose of the present study was to examine the effects of TRH on pentobarbital narcosis in 12 rhesus monkeys. Vital signs monitored included respiration rate, heart rate, temperature, sleeping time, and time of reappearance of certain reflexes. Blood samples were obtained for pentobarbital assay. Two dose schedules for TRH administration were used. One group of six animals received a single dose of 20 mg/kg thirty minutes after barbiturate administration, while the other group received 3 injections of 20 mg/kg spaced at 30,40, and 50 minutes after injection of pentobarbital. Both groups were sex balanced. TRH administration resulted in dramatically increased respiration and heart rates and arrested the progress of barbiturate induced hypothermia. An analysis of variance revealed an effect of the extended dose schedule to prolong increased respiration and a differential effect of TRH on pentobarbital induced hypothermia across sexes. All animals regained reflexes sooner and sleeping time was reduced by 22 percent. Preliminary data showed no differences in pentobarbital blood levels with TRH. These results confirm earlier work in rodents and suggest a possible use of TRH in cases of acute barbiturate intoxication. (Supported by USPHS grants NM-16522; MH-16522; MH-15631 and HD-03110).

519 EFFECTS OF THYROTROPIN RELEASING HORMONE (TRH) ON BEHAVIOR: EVIDENCE FOR AN ANOREXIC-LIKE ACTION. T.S. Barlow‡ B.R. Cooper‡ G.R. Breese, A.J. Prange‡ Jr. and M.A. Lipton. Depts. of Psychiatry and Pharmacology, Univ. North Carolina, School of Med., Chapel Hill, N.C. 27514

The effects of thyrotropin releasing hormone (TRH) on behavior of rats was studied using a fixed-ratio 30 (FR-30) schedule of food reinforcement, shuttle-box avoidance performance, locomotor activity, and electrical self-stimulation of the dorsal brain stem and ventral tegmentum. It was found that 10 or 20 mg/kg (i.p.) of TRH produced a dose related decrease in bar-pressing on the FR-30 schedule of food reinforcement and resulted in a significant increase in locomotor activity at the 20 mg/kg dose. In contrast, self-stimulation measures or active avoidance responding were not affected. No effects on FR-30 responding were seen after administration of 10 or 20 mg/kg of melanocyte stimulating hormone releasing inhibiting factor (MIF), another tripeptide with reported antidepressant effects in man. Studies of ingestive behavior revealed that doses of TRH which reduced operant responding significantly reduced food consumption over the same dose range. This anorexic-like effect did not occur after thyroid stimulating hormone (TSH; 10 IU/kg), or after administration of pyroglutamic acid, histidine, and proline amide, the constituent amino acids of TRH. Destruction of central catecholamine neurons with 6-hydroxydopamine blocked the anorexic effects of a 0.75 mg/kg dose of d-amphetamine but potentiated the anorectic-like actions of TRH. It would appear that TRH has the ability to reduce operant responding through an effect on food consumption. Unlike amphetamine anorexia this effect is not dependent on central catecholamine neurons. These findings provide additional evidence for an extra-pituitary action of TRH. (Supported by USPHS Grants MH-16522, HD-03110, AA-02334 and MH-00013.)

520 Comparative efficacy of various drugs in a T-maze drug discrimination task

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Rats were required to turn right when drugged and left when undrugged in order to escape from 1.6 ma in a T-maze task. Drugs were initially pilot tested to determine the maximum dosage at which performance in the T-maze was feasible. This dosage was used for testing. A separate group of rats was used to test each drug. Each rat received 10 trials per daily training session with drug and no drug conditions alternating during successive sessions. Criterion performance was correct choices on the first trials of 8 out of 10 consecutive training sessions. Experimental procedures are described in more detail in Fed. Proc., 33, 1800-1813, 1974. For each drug, the ip. dosage, number of rats and geometric mean sessions to begin criterion performance were as follows:

Alcohol (3000, 6, 3), Barbital (100-150, 6, 3.2), Glutethimide (100, 6, 9.5), Meprobamate (200, 12, 2.4), Methyprylon(75, 3, 2.3), Pentobarbital (20, 16, 1.4), Phenobarbital (80, 6, 1.3), Urethane (750, 11, 2.3), Flurazepam (100, 9, 4.3), Librium (30, 11, 4.4), Valium (40, 3, 8.6), Carisprodol (75, 9, 8.2), Ketamine (40-60, 15, 5.1), Phencyclidine (10-20, 12, 8.7), Procaine (50, 3, 37), Lidocaine (60, 3, 10), Lobeline (30, 4, 12), Nicotine (4, 8, 2.8), Mecamylamine (30, 3, 9), Arecoline (20, 6, 20), Carbacol (0.6, 9, 52), Physostigmine (1, 6, 22), Scopolamine (4, 6, 4.5),Atropine (50, 9, 2.8), Benactyzine (10, 8, 7.2), Ditran (15-30, 6, 4.5), Cogentin (40, 6, 4.1), Propranolol (35, 5, 16), Amphetamine (8, 5, 13.4), Caffeine (10, 6, 26.8), Cocaine (50, 2, 16), Ritalin (25, 3, 14.9), Bemegride (7.5, 9, 20.3), Metrazol (30, 3, 10.1), Nikethamide (100, 3, 13.9), Strychnine (0.5, 3, 57), Picrotoxin (1.0, 3, 52), Ethosuximide (300, 3, 23.4), Mesantoin (120, 4, 12.2), Trimethadione (300, 3, 37), Codeine (50, 3, 3.4), Levorphanol (50, 4, 6.0), Meperidine (40, 3, 24.6), Morphine (32, 3, 11.1), Pentazocine (40, 5, 18.4), Profadol (40, 3, 14), Ethoheptazine (80, 3, 26), Romilar (50, 10, 9.8), Darvon (30, 5, 22), Levallorphan (75, 3, 14.6), Naloxone (25, 3, 60), Naltrexone (100, 3, 27), Acetominophen (600, 3, 30), Antipyrine (250, 3, 50), Asperin (250, 4, 25), Mescaline (50, 2, 20), Chlorpromazine (4, 3, 41.6), Fluphenazine (2, 3, 41.5), Perphenazine (50, 3, 27.0), Haloperidol (2.5, 6, 18.6), Imipramine (40, 9, 13.8), Parnate (10, 3, 37), Phenelzine (75, 3, 20), Histamine (200, 3, 10), Benadryl (30, 3, 23), Pyrilamine (50, 9, 19.7), Dramamine (75, 3, 16.1), A.M.P.T. (140, 3, 35), Dopamine (15, 3, 45), Serotonin (10, 3, 22), Amyl Nitrate (85, 3, 39), Lithium (80, 6, 56), Piracetam (150, 3, 60), Flaxedil (7.5, 4, 50), Piperidine (200, 6, 14).

The results are relevant to several issues. Only drugs with STC < 3 would be expected to produce obvious state dependency if a 2x2 experiment were conducted in this task. Only drugs with STC < 25 can be easily tested for substitution in this task. The data provide a basis for various theoretical formulations. Discriminability correlates only moderately well with abuse liability in man but correlates more highly with self administration in animals. Many of the drugs have not been previously tested for discriminability.

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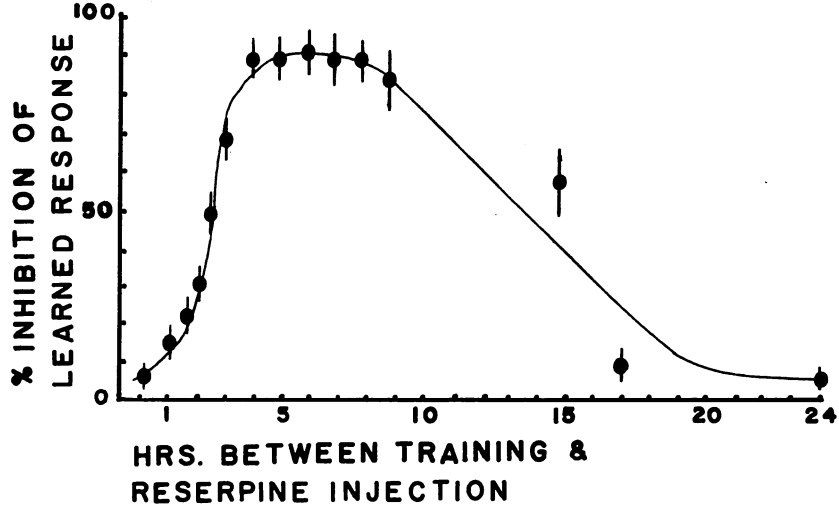
RESERPINE INHIBITION OF PASSIVE AVOIDANCE LEARNING: TIME AND DOSE RESPONSES. Stephen E. Karpiak, Michael Kirchmer*, and Maurice M. Rapport, Div. of Neuroscience, New York State Psychiatric Institute and the Dept. of Biochemistry, Columbia University, College of Physicians and Surgeons, New York, New York 10032.

Several investigators have hypothesized that brain neurotransmitters are critically involved in the acquisition and consolidation phases of learning behavior. It has been reported that the injection (I.P.) of reserpine, and consequent depletion of CNS catecholamines and indoleamines, immediately following training on a passive avoidance learning paradigm blocked the acquisition of the avoidance response. Depletion of the neurotransmitters 24 hours after training did not interfere with the acquisition. The following study was undertaken to investigate the time intervals following training on a passive avoidance task when reserpine injection produces maximal and minimal effects, and the dose response at the time of maximal effect. Male Swiss-Webster mice (20 grams) were trained on a standard passive avoidance task and independent groups (10-15 mice) were injected I.P. with 3 mg/kg of reserpine at various time intervals following training: 5 min., 60 min., 90 min., 120 min., 3,4,5,6,7,8,9, 15,17, and 24 hours. Animals were tested for retention of the avoidance response 7 days later. It was observed that mice injected at 5 to 120 min. after training showed minimal or no inhibition of the learned response as compared to controls. Similarly, mice injected 17 or 24 hours after training also did not show any significant inhibition of the learned response. However, mice injected between 4 and 9 hours after training were significantly inhibited (80-95%). Dose response studies at 4 hours showed that maximal inhibition occurred at a dosage of 0.8-0.9 mg/kg; minimal inhibition was seen at 0.5 mg/kg and no inhibition at 0.3 mg/kg. If the effect of reserpine is mediated by neurotransmitter depletion, then such depletion, in the time periods of the first 2 hrs. or after 17 hrs. following initial learning of a task, does not interfere with the consolidation phase of learning. However, depletion of these neurotransmitters 4-9 hrs. after training significantly interferes with memory consolidation. Since the reserpine effect can be separated into 3 time periods (<2 hrs., 4-9 hrs., >17 hrs.) in this experiment, the effect of biogenic amines in the CNS on learning behavior in the mouse may be better understood by attempting to correlate changes in their concentrations that occur from one of the periods to the next when the minimal dose producing the maximal effect is used. Other mechanisms of reserpine action cannot be excluded.



522 PROTEIN SYNTHESIS OF THE BRAIN AND SPINAL CORD OF THE NORMAL AND SPINAL HEMISECTED MONKEY Michael R. Wells* and Jerald J. Bernstein. Dept. Neurosci., Coll. Med., Univ. of Fla., Gainesville, Fla. 32601

The effects of spinal hemisection on protein synthesis in the spinal cord and in selected brain regions of the Cebus monkey (Cebus apella) were studied by measuring the uptake of 3H-lysine into the trichloroacetic acid precipitable protein one hour after a 1.0 mCi subcutaneous injection. Animals were sacrificed 3, 6, or 13 days after a left hemisection at the lamina interface of the first and second thoracic vertebrae. Compared to normal animals, significant increases in the average uptake of precursor into spinal cord were noted in all postoperative groups including a sham operate. Significant intergroup differences were also observed. In localized regions of spinal cord, significant increases were seen ipsilaterally 20mm rostral and 10mm caudal to the lesion averaged over all hemisected animals. Contralateral increases were restricted to 2mm on either side of the lesion. In brain, significant increases in incorporation of the left over the right side were seen in hindlimb motor cortex occipital cortex and superior colliculus. Left-right differences were noted in primary hindlimb sensory cortex, but they were not significant. Elevations of amino acid uptake were attributed to neuronal response to injury, neuroglial reaction, a nonspecific response to surgical trauma, and the influence of possible trophic substances.

523 PROTEIN ALTERATIONS IN DEGENERATING AND REGENERATING SCIATIC NERVES OF MICE. D. E. Groswald*, M. W. Luttges, P. T. Kelly*, and R. A. Gerren* Dept. Aero. Eng. Sci., Univ Colorado, Boulder, Colo. 80302.

Mouse sciatic nerve proteins were characterized by gradient slab gel electrophoresis using a sodium dodecyl sulfate-polyacrylamide gel electrophoresis system. All characterizations utilized separate samples of proximal, distal and contralateral nerve tissues obtained from mice which had previously sustained experimental, unilateral nerve damage. Changes in more than 40 major migration bands were quantized across post-injury periods of up to 3 months in duration. Gel patterns indicated that distal nerve segments exhibited the most pronounced alterations following experimental nerve damage. Many of these alterations were associated with cellular proliferation and plasma accumulation. The separation of nuclear histone proteins from basic myelin proteins is crucial to this observation. Proximal nerve segments exhibited similar though less pronounced proliferative cellular changes following damage. These proximal changes disappear within about two weeks after nerve damage. Small but reliable changes were also observed in normal nerves contralateral to the damaged nerve. The time course for both degeneration and regeneration is quite reproducible and is suggestive of normal nerve structural dependence on temporal order.

524 REQUIREMENT FOR NON-TUBULIN PROTEINS FOR INITIATION OF IN VITRO BRAIN MICROTUBULE ASSEMBLY. Sandra E. Granett, Roger D. Sloboda* and Joel L. Rosenbaum. Dept. Biol., Yale Univ., New Haven, Ct. 06520

Calf brain microtubule protein purified by two cycles of polymerization-depolymerization contained, in addition to tubulin, two high molecular weight microtubule-associated proteins (MAPs) and a few minor proteins. Fractionation of this microtubule preparation by molecular sieve chromatography yielded one peak (Peak I) eluting with the void volume and another peak (Peak II) included within the column at a molecular weight of approximately 100,000 Daltons (tubulin dimers). SDS-polyacrylamide gel electrophoresis of these peaks revealed that the void volume (Peak I) contained tubulin and the MAPs, whereas Peak II contained primarily tubulin and no MAPs. In the presence of EGTA, $MgSO_4$, GTP and PIPES, pH 6.9, the void volume (Peak I) was capable of polymerizing into microtubules at 37°C at a protein concentration as low as 0.13 mg/ml. Tubulin dimers from Peak II, on the other hand, polymerized only to a very limited extent even at 8-10 mg/ml.

"Ring-like" structures seen in the electron microscope in the unfractionated microtubule preparation were observed only in the void volume peak and not in the tubulin dimer peak. These structures have been implicated in the initiation event in microtubule assembly. Indeed, if the void volume (Peak I) were combined with tubulin dimers (Peak II) which assembled poorly on their own, the resultant polymerization was greater than that obtained with the same amount of void volume. This indicated that the tubulin dimers (Peak II) were polymerization-competent and were entering into the assembly reaction. The few microtubules which were formed in preparations of tubulin dimers (Peak II) were very long, but the addition of increasing amounts of void volume (Peak I) to the tubulin dimers resulted in a decrease in the average microtubule length, suggesting that more initiation sites were being made available.

To determine which of the proteins in the void volume (Peak I) was causing this assembly of tubulin dimers, the void volume was fractionated on DEAE-Sephadex. Two peaks were obtained: one at 0.4M KCl which contained MAPs and a number of other non-tubulin proteins and a second at 0.8M KCl which was pure tubulin. Neither fraction alone formed microtubules, but the 0.4M KCl fraction when added to the purified tubulin dimers obtained either from Peak II of the molecular sieve column or from the 0.8M KCl peak of the DEAE column stimulated microtubule assembly at 37°C. This assembly was preceded by the formation of "ring-like" structures. The void volume (Peak I) was also fractionated on phosphocellulose columns. A peak was obtained at 0.3M KCl which was composed almost entirely of MAPs. This fraction would not form microtubules on its own but stimulated polymerization of the tubulin dimers.

These findings show that tubulin dimers by themselves cannot assemble into microtubules. One or more proteins of high molecular weight which remain associated with the microtubules through cycles of in vitro polymerization and depolymerization are required for assembly. The data also indicate that this stimulation of assembly is due to the induction of initiating centers. (This work was supported by NS Grant 10907 to JLR.)

525 ACTIN AND MYOSIN FROM CHICK BRAIN: ISOLATION, CHARACTERIZATION AND LOCALIZATION. Edward Kuczmarski* and Joel L. Rosenbaum. Dept. of Biology, Yale University, New Haven, Connecticut, 06520.

Actin and myosin have been isolated from chick brain. Brain actin purified from a low ionic strength extract of an acetone powder resembled other actins in several ways: (1) On SDS polyacrylamide gel electrophoresis (SDS-PAGE) a single band which co-migrated with rabbit muscle actin was seen; (2) Addition of KCl and Mg to globular brain actin caused an increase in viscosity; (3) E.M. observations showed the polymerized actin to consist of double helical filaments 6 nm in diameter; (4) Dialysis against 25 mM $MgCl_2$ induced the formation of actin paracrystals; (5) The f-actin from brain bound rabbit HMM to form typical 'arrowheads'; and (6) The Mg-ATPase of chicken breast muscle myosin was stimulated several fold by brain actin.

Brain myosin was prepared from a high ionic strength extract using the method of Pollard et. al. (Anal. Biochem. 60: 258, 1974). The myosin was similar to other cytoplasmic myosins in the following ways: (1) SDS-PAGE revealed a major protein band which co-migrated with muscle myosin (molecular weight, 200,000) as well as two classes of light chains (16,000 and 21,000); (2) In 0.6 M KCl the brain myosin was maximally activated by EDTA (0.37 micromoles P/min/mg protein), partially stimulated by Ca, and inhibited by Mg; (3) Dialysis against 0.1 M KCl, pH 7.0 resulted in the formation of 0.35 micrometer bipolar filaments with a central bare zone of 0.18 micrometers. In addition to tail-to-tail aggregations, head-to-head associations were also seen. The addition of Mg ion induced the formation of highly-regular paracrystals of myosin. When chicken smooth and skeletal muscle myosins were combined under conditions of low ionic strength, hybrid bipolar filaments of intermediate length were formed. Brain myosin, however, failed to form hybrids with skeletal myosin. Furthermore, antibodies against brain myosin showed no cross-reaction with chicken smooth or skeletal muscle myosins; and antibodies to the two muscle myosins showed no cross-reaction with each other or with the brain myosin, suggesting that the three myosins are different gene products.

Two methods are currently being used to localize the myosin and actin within brain neurons: (1) Light and electron microscopic studies employing antibodies specific for these proteins; and (2) Quantitative analysis of the distribution of actin and myosin in purified synaptosomal fractions from both chick brain and squid head ganglion.

526 PROTEIN MICROFRACTIONATION OF MYELIN-FREE AXONS FROM SELECTED NERVES OF THE RABBIT. Robert D. Frankel* and Edward Koenig. Dept. of Physiology, SUNY at Buffalo Sch. of Med., Buffalo, N.Y. 14214.

A method has been developed that permits the fractionation of protein samples in the lower nanogram range (10-50 ngm) by composite polyacrylamide-agarose (2.5-0.6%) gel strip (ca., 3x0.6x0.05 cm) microelectrophoresis. The gel strips were impregnated with an aqueous-glycerol-dextran buffered medium of high viscosity and ionic strength, containing 0.5% SDS, in which major proteins were separated completely over a distance of less than 1 cm. This system has been used to fractionate microscopic samples of myelin-free axons (5-10 cm, cummulative length), solubilized completely by a TRIS-BES buffer, containing 1% SDS and dithiothreitol. Samples of myelin-free axons from rabbit dorsal root, ventral root and peripheral sensory nerve were microfractionated separately. Axons from the three nerve types showed fractionation pattern consisting of the same six major components. Axons from the peripheral sensory nerve, however, contained in addition a major band having a nominal molecular weight of more than 300,000, which was not viable in axon samples from dorsal or ventral roots. These results indicate that the composition of major axonal proteins may not be identical in central as opposed to peripheral branches of the sensory neuron. This research was supported by NINDS grant no. NS-04656 and NIH, NIGMS Training grant no. GM-00341.

527 NUCLEAR PROTEINS FROM RABBIT CEREBRUM, CEREBELLUM AND LIVER: SYNTHESIS AND PHOSPHORYLATION. I. Oh'hara* and T. Yanagihara. Mayo Clinic and Mayo Foundation, Rochester, Minnesota, 55901

Nuclear protein phosphorylation was investigated with an in vitro model by using tissue slices from rabbit cerebrum, cerebellum and liver, and was compared with amino acid incorporation into proteins. Following protein phosphorylation with $NaH_2{}^{32}PO_4$ or protein synthesis with ^{3}H-leucine, nuclei were isolated from tissue slices and NaCl soluble protein, histone and phenol soluble acidic protein were fractionated according to Teng et al (J. Biol. Chem. 246:3597, 1971), and the results were expressed as nmoles/g protein for phosphorylation or d.p.m./μg protein for protein synthesis. ^{3}H-leucine incorporation in the nuclear homogenate was 5 times higher in cerebrum than in cerebellum or liver. In each organ NaCl soluble protein showed high specific radioactivity and histone the lowest, while the incorporation into acidic protein ranged from 1.0 to 1.5 times of the value of the nuclear homogenate. Phosphorylation of the nuclear homogenate was very similar in cerebrum, cerebellum, and liver. In each organ NaCl soluble protein showed highest phosphorylation and histone the lowest, while the value from acidic protein was 60% of the nuclear homogenate. Further fractionation of acidic protein with SDS-acrylamide gel electrophoresis demonstrated considerable heterogeneity among three organs studied. Cyclic GMP or cyclic AMP, added to the incubation medium at the tissue slice stage, did not modify phosphorylation of these nuclear protein fractions. However, the work is underway to investigate the possibility of the effect on a specific protein by further separation of acidic protein with gel electrophoresis. (Supported by grant NS-6663 from NIH and by the George H. Bartel Fund).

528 PHOSPHORYLATED GLYCOPEPTIDES FROM RAT BRAIN GLYCOPROTEINS. Leonard Davis*, Javaid Javaid*, and Eric G. Brunngraber. Dept. Biol. Chem., Sch. Med., Univ. of Illinois, Chicago, Illinois and the Illinois State Psychiatric Institute, Chicago, Illinois 60612.

Rats were injected intraperitoneally 24 hours prior to sacrifice. Glycopeptides, derived from glycoproteins, were released from the defatted tissue by the proteolytic action of papain. The glycopeptides were subjected to affinity chromatography on Con A-Sepharose. Mannose-rich glycopeptides were bound to the concanavalin A-Sepharose column and subsequently eluted with alpha-methylmannoside. After treatment with leucine aminopeptidase, the mannose-rich glycopeptides were subjected to ion exchange chromatography on Dowex 1-chloride and Dowex 50-hydrogen. Approximately 65% of the glycopeptide-carbohydrate applied to the columns was recovered in the effluent. These glycopeptides (MW = 2100) contained 6, 2, and 1 moles of mannose, N-acetylglucosamine and aspartic acid per mole glycopeptide. The mannose-rich glycopeptides that had become labeled with ^{32}P-phosphate were bound to and recovered from the Dowex 1 column. The presence of a phosphate monoester attached to neutral sugar was indicated by NMR analysis. The parent ^{32}P-phosphate labeled glycoproteins were solubilized by treating the defatted tissue residue with sodium dodecyl sulfate. This detergent was exchanged for sodium deoxycholate by dialysis against solutions containing the latter detergent. The solubilized glycoproteins were adsorbed to Concanavalin A-sepharose and eluted with alpha-methylmannoside. Radioactive bands that corresponded to glycoprotein material was revealed by SDS-electrophoresis. Preliminary experiments indicated that cAMP failed to stimulate the enzymatic phosphorylation of these phosphoglycoproteins.

529 REGULATION OF ENDOGENOUS PHOSPHORYLATION OF A SPECIFIC PROTEIN FROM RAT BRAIN HOMOGENATES BY DIPHENYLHYDANTOIN. Robert John DeLorenzo* and Gilbert H. Glaser. Dept. Neurology, Yale Med. Sch., New Haven, Ct. 06510

These investigations have demonstrated that the level of endogenous phosphorylation of a specific protein from rat brain homogenates is affected by the presence of diphenylhydantoin (DPH). DPH caused a marked decrease in this level, while not significantly affecting the phosphorylation of other protein substrates in the homogenate. Homogenates of rat cerebrum were prepared in 0.32 M sucrose and equal aliquots of homogenate were incubated with (γ-^{32}P) adenosine triphosphate (ATP) under standard incubation conditions in the presence and absence of DPH. The reactions were terminated by adding sodium dodecyl sulfate (SDS) and mercaptoethanol and then boiling. Aliquots of each reaction mixture were then subjected to SDS-polyacrylamide slab gel electrophoresis. The gels were stained and dried, and autoradiographs were made by placing the dried gel in contact with x-ray film. DPH caused a substantial decrease in the amount of ^{32}P-phosphate incorporated into a specific radioactive band seen in the autoradiograph. This band was the only band whose phosphorylation was consistently observed to be affected by DPH. The effect of DPH on the amount of ^{32}P-phosphate incorporated into this band was independent of ATP over a wide range of concentrations. This result indicates that the decrease in phosphorylation of this specific band was not due to a competitive effect between DPH and ATP, and further suggests that the effect of DPH on endogenous protein phosphorylation was not attributable to an indirect action, causing a decrease in the concentration of ATP. The ability of DPH to cause a decrease in the net level of endogenous phosphorylation of a specific brain protein could result from either a DPH-induced increase of protein phosphatase activity, from a DPH-induced decrease in protein kinase activity, or both.

The radioactive band affected by DPH was demonstrated to be a phosphoprotein: treatment of homogenate preparations incubated in (γ-^{32}P) ATP with protease caused a complete loss of both the specific protein staining pattern and its associated radioactivity on the autoradiograph. Conversely, treatment of labelled brain homogenates with ribonuclease, deoxyribonuclease, triple lipid extraction with ethanol-ether (3:1), or boiling in 10% trichloroacetic acid had no effect on the presence of the band affected by DPH on the autoradiograph. The minimal molecular weight of the specific protein was estimated by SDS-gel electrophoresis, employing known molecular weight markers, to be approximately 60,000. These initial studies have established an effect of DPH on the endogenous phosphorylation of a specific protein in rat cerebrum. The functional significance of this drug effect on brain protein phosphorylation remains to be elucidated. Current studies are in progress to define the role of this effect in relation to the action of DPH as an anticonvulsant, and to determine the possible role of phosphoproteins in controlling membrane permeability and seizure discharge.

530 PROTEIN PHOSPHORYLATION IN MOLLUSCAN NERVOUS SYSTEM. Irwin B. Levitan and Eric F. Bandle*. Friedrich Miescher-Institut, Postfach 273, CH-4002 Basel, Switzerland.

We have studied protein phosphorylation in broken cell preparations of the circumesophageal ganglia from the gastropod mollusc Helix pomatia. Protein kinase activity, with endogenous protein as substrate, was observed in both the 20,000 g pellet and supernatant prepared from homogenates of Helix nervous system. The kinase activity in the 20,000 g supernatant was 2-3 times higher in the presence of 2 μM 3'-5' cyclic adenosine monophosphate (cAMP), whereas concentrations of the cyclic nucleotide as high as 90 μM did not affect kinase activity in the pellet. These data suggest that the subcellular distribution of cAMP-sensitive protein kinase activity in the Helix nervous system may differ from that in mammalian brain. Analysis of the reaction products on sodium dodecyl sulfate-containing polyacrylamide gels indicated that several polypeptide bands were phosphorylated in the 20,000 g supernatant, and that a large proportion of the radioactivity was incorporated into a single band of approximate molecular weight 30,000 - 35,000 daltons. In preliminary experiments, cAMP appeared to decrease the radioactivity in this band relative to that in other bands in the gel.

531 THE MECHANISM OF RELEASE OF CYCLIC 3',5'-cAMP PHOSPHODIESTERASE ACTIVATOR. Margaret Gnegy, Petko Uzunov, and Erminio Costa*. Laboratory of Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D. C. 20032

Cyclic 3',5'-nucleotide phosphodiesterase (PDE), exists in several molecular forms, which have high or low Km for cAMP. An endogenous protein activator (PDEA), decreases the Km for cAMP of a purified brain PDE from 400 μM to 80 M (Uzunov et al., 1975). In various tissues PDEA activity increases when the cAMP content is enhanced by several fold (Uzunov et al., 1975). Therefore the rise in cAMP triggers a mechanism that facilitates the hydrolysis of cAMP. Since protein kinases are the receptor for cAMP the effect of protein kinase on the release of PDEA from membranes was studied.

The endogenous protein activator (PDEA) has previously been isolated only from 100,000 x g supernatant, however we found that PDEA is present in a membrane fraction of rat brain. The factors that may affect its release from these membranes were studied. The release of PDEA was increased three-fold by incubating the membranes with ATP, protein kinase and cAMP. The two possible substrates for phosphorylation are the PDEA itself or an activator-binding membrane protein which releases the activator upon phosphorylation.

In addition it was found that in the presence of a protein phosphatase the activity of PDEA on a purified activator-deficient PDE is stimulated more than 30%. The phosphatase had no effect on basal PDE activity.

Thus an increased concentration of cAMP could stimulate protein kinase which phosphorylates either the PDEA or its membrane binding protein thus releasing the PDEA. Phosphatase can further increase the activity of PDEA. The endogenous activator then binds to a high Km PDE decreasing the value of this kinetic constant.

532 CHARACTERISTICS AND DIVERSITY OF POLYADENYLATED RNA FROM THE MOUSE BRAIN. John A. Bantle* and William E. Hahn, Dept. Anat., Sch. Med., Univ. of Colo., Denver, 80220.

Genetic transcription in the mouse brain was examined in terms of pre- and "mature" polyA$^+$ messenger RNA populations. Polyadenylated RNAs from whole brain nuclei and polysomes were purified and characterized in a variety of ways. 25% of the total nuclear RNA was found to be polyadenylated. The length of the polyA tract of this RNA was about 210 bases as determined by gel electrophoresis. The mass average size of polyA-nuclear RNA (pan-RNA) was found by sedimentation in DMSO-sucrose gradients to be 7500 nucleotides. The number average (i.e. size of the average individual molecule) measured by electron microscopy was 4800 nucleotides. The size heterogeneity of pan-RNA was extensive with molecules ranging from 6S to over 70S in DMSO-sucrose gradients. Molecules up to 25,000 nucleotides were observed in the electron microscope. DNA/RNA saturation hybridization experiments showed that pan-RNA saturated about 12% of the unique DNA of the mouse genome (equivalent to 24% of the unique sense-strand DNA) as measured by HAP chromatography of S^1 endonuclease-treated hybrids.

Equivalent measurements on polyA-polysomal RNA, which can probably be considered to be polyA$^+$-mRNA, were made. PolyA$^+$-mRNA comprised about 3.0 - 3.5% of the total polysomal RNA. PolyA tracts averaged 130 bases with 90% of the tracts ranging between 50-200 bases. PolyA tracts comprised 7-7.5% of the total molecule and showed more size heterogeneity than tracts from pan-RNA. The mass average size determined by sedimentation was 2000 nucleotides and the number average determined by electron microscopy was 1350 nucleotides. Thus the average polyA$^+$ mRNA is 3-4 times smaller than the average pan-RNA molecule. Saturation hybridization of polyA$^+$ mRNA to labeled-unique sequence DNA yielded a value of 3.5%. This is about 3.5 times less than the saturation value for pan-RNA. If 1350 bases are taken as the size of the average structural gene (i.e. size of an average functional mRNA) a 3.5% saturation value is equal to the transcription of about 160,000 such genes. By plotting saturation curve data on a log scale, 3 transitions were observed. About 1/4 of the DNA sequences were saturated very rapidly by PolyA$^+$ mRNA and the remainder saturated at intermediate and very slow rates. Thus the population of polyA$^+$ mRNA may be roughly described as consisting of 3 copy frequency families.

A portion of the pan-RNA is considered as pre-mRNA or precursor to PolyA$^+$ mRNA. One interpretation of our data is that 3' cleavages of pan-RNA occurs, in which about 1/3-1/4 of the pan-RNA is conserved as mRNA, and that all or most of the various species of pan-RNA molecules are processed in this manner. Two observations support this suggestion; namely that both diversity and average size measurements between pan and polyA$^+$ mRNA differ approximately by a factor of 4 fold. Alternatively, polyA$^+$ mRNA may be processed from only a select portion of the pan-RNA which is more fully conserved.

At present, we conclude that in whole mouse brain that about 160,000 different polyA$^+$ mRNAs exist. The extent to which these messengers are being translated is not known, but potentially an equal number of proteins could be specified from this existing information.

533 PYRENEBUTYRIC ACID:QUANTITATIVE, NON-INVASIVE FLUOROCHROME FOR NEURAL INTRACELLULAR OXYGEN CONCENTRATION IN SITU. Michael H. Mitnick and Frans F. Jöbsis*. Dept. Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina, 27710.

Measurement of intracellular oxygen (O_2) concentration by traditional polarographic techniques has been handicapped by inherent difficulties, such as the invasion of the cells in question and the consumption, however slight, of O_2 by the electrode. Our laboratory has succesfully utilized pyrenebutyric acid(PBA) as an absolute, non-invasive, non-consumptive probe for determining intracellular O_2 concentrations in cat cerebral cortex.

PBA was reported by Weber and Vaughn (1970) to be of potential use as a biological fluorochrome for O_2. They showed that in vitro PBA solutions, when equilibrated with various O_2 concentrations, exhibited typical Stern-Vollmer fluorescent activity. Mathematically,

$\frac{Fo}{F}=1+K[O_2]i$ where: Fo=unquenched fluorescence, F-quenched fluorescence, K=quenching constant, $(O_2)i$=(intracellular) oxygen concentration, in mm Hg.

O_2 acts as a quenching substance for PBA: The more O_2, the more quenching of the fluorescence (excited State-ground State transitions). Therefore, the difference between the two intensities of fluorescence with and without O_2 can be used to calculate the O_2 concentration.

Longmuir and Knopp (1972) extended the technique to in vitro cell suspensions. They showed that PBA has a partition coefficient of over 200 to 1, verified that the Stern-Vollmer relationship was indeed exhibited in living rat liver cell suspensions, and that PBA was non-toxic.

We have extended this procedure to determine in situ values of O_2 concentration in cat brain. The procedure is as follows: cats are supplied with tracheal tube, femoral venous and intraperitoneal cannulae, and a mid-collicular RF electrolytic lesion (bilateral) is performed. The supra sylvian gyrus is exposed and a 3.2 mm diameter field used for optical monitoring after covering with polyethylene wrap (Saran wrap). Two to three hours were allowed for recovery from ether anesthesia and surgical trauma. Optical monitoring was accomplished by compensated differential microfluorometry (Jöbsis and Stainsby, 1968; and Jöbsis, et al, 1971). Excitation of PBA was at 340 nm; monitoring of PBA emission was by means of a secondary filter, 396 nm peak. Baseline autofluorescence was monitored as a control, and control responses of labile autofluorescence at 396 nm noted in response to various periods of anoxia. Anoxia was induced in either of two ways: cats were paralyzed with Flaxedil and mechanically respired with 100% nitrogen, or else spontaneously respiring animals were exposed to 100% nitrogen. After a number of such anoxic episodes, PBA in the form of the Na salt was introduced via an intraperitoneal cannula. After 2-3 minutes fluorescence starts to rise; full equilibration occurring after 40 to 60 minutes. Anoxic episodes were again induced, the resulting cycles of fluorescence being indicative of alterations in cerebral intracellular O_2 concentration. Finally, the animal was terminally exposed to nitrogen. In this way, resting levels of O_2 were calculated, via the Stern-Vollmer relationship. The mean O_2 concentration of the first series of such experiments (n=5) was 45.4 mm Hg., S.D. ± 11.9 with no value falling more than one S.D. away from mean. The effects of anoxic transients, inspired gas mixtures,various physiologic and pharmacologic forcing functions on oxidative metabolism, and ischemia on cerebral oxygen concentration will be discussed.

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534 EFFECTS OF SEIZURES AND SPREADING CORTICAL DEPRESSION ON OXIDATIVE METABOLISM AND HEMOGLOBIN OXYGENATION MEASURED IN SITU. Joseph LaManna and Myron Rosenthal, Duke University, Dept. of Physiology and Pharmacology, Durham, N.C., 27710

The effect of seizures induced by i.v. pentylenetetrazol (Metrazol) or strychnine and spreading depression (SD) produced by suprathreshold surface stimulation was studied in cerveau isole cat preparations.Changes in oxidative metabolism were monitored optically(fluorometric measurements of NAD redox level and dual wavelength reflectance spectrophotometry of cytochrome a, blood volume and hemoglobin oxygenation). Previous studies have shown that seizures (1) and SD (2) are accompanied by increased levels of oxidized NAD. This is consistent with the concept of increased O_2 use, such as required by recovery events,producing a more oxidized mitochondrial state.

Figure 1 shows that SD is also accompanied by an oxidation of cyt a and an increase in the volume of blood in the optical field. Little change occurs in the average oxygenation level of hemoglobin and sometimes a small shift toward a more arterial blood is observed. The effect of a Metrazol seizure is shown in Figure 2. Seizures produced by this drug or by strychnine are accompanied by an increased reduction level of cyt a, a large increase in blood volume and a decrease in the level of hemoglobin oxygenation. When cats were respired on 5% CO_2 in O_2, the increase in cyt a reduction level in a seizure still occurred although it was smaller.

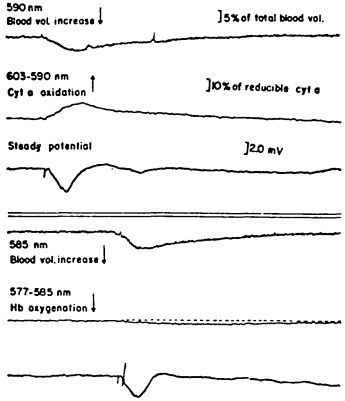

Figure 1

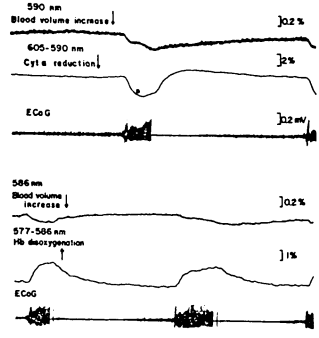

Figure 2

The differential effect on cyt a produced by seizures and SD is, as yet, unexplained. The extent of NAD oxidation that occurs during these events is approximately similar although the rise in extracellular K^+ in SD is 25-80 mM while in seizures, K^+ rose to a maximum of 12 mM (3). Similar K^+ levels were recorded with long trains of direct cortical stimuli which were accompanied by increased oxidation levels of cyt a. The fact that NAD oxidation level increases during seizures indicates that anoxia does not occur. It appears that a loss of regulatory functioning normally associated with changes in ionic gradients or that demands other than ion transport are placed on tissue respiration during seizures.

(This work was supported by PHS grants NS-10384 and NS-06233).

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535 EVOKED POTENTIALS AND OXIDATIVE ENERGY METABOLISM IN SITU DURING AND FOLLOWING SHORT PERIODS OF TRANSIENT CORTICAL ISCHEMIA IN CATS. Myron Rosenthal, David Martel*, Joseph LaManna and Frans Jöbsis*, Dept. of Physiology and Pharmacology, Duke University Medical School, Durham, N.C. 27710.

Cortical ischemia in cats was produced by clamping the innominate artery after previous ligation of all possible alternate ascending circulatory pathways. Oxidative metabolism was recorded noninvasively by means of fluorometric observation of the redox level of intramitochondrial NADH (450-366nm) and by dual wavelength reflectance spectrophotometry of cytochrome a (605-590nm). The latter technique also allowed measurements of oxygenation state of hemoglobin (577-584.5nm) and of blood volume in the optical field. We found that cortical ischemia is accompanied by large and immediate increases in the reduction level of intramitochondrial NAD and in cytochrome a together with a decrease in the volume of blood and in the oxygenation of hemoglobin.

In this study, we sought to establish criteria for complete ischemia based upon the optical monitoring of oxidative events in situ in cerveau isolé cat preparations. Three criteria established the completeness of the ischemic model: 1) an increase in the reduction levels of NAD and cytochrome a and hemoglobin disoxygenation to new steady state values with a new steady level of blood volume. These changes do not begin to return to baseline levels until clamp removal; 2) the extent of NAD or cytochrome a reduction equals that produced by terminal nitrogen respiration where presumably total reduction of these respiratory chain components occurs; and 3) the disappearance of ECoG activity during the ischemic period. Only when these criteria were seen together was the complete nature of the ischemic insult accepted.

We attempted to determine the mitochondrial response to ischemic episodes and to ascertain if changes in mitochondrial activity are produced by this condition independent from changes in circulatory reperfusion. For this reason, we repeated short ischemic episodes and simultaneously monitored mitochondrial and circulatory functioning. The rate of return of NADH and cytochrome a to baseline after successive 1 minute periods of ischemia separated by 1 hour were faster in each successive case. The return of blood volume, however, remained at a constant rate during the successive ischemic episodes while hemoglobin remained at a more disoxygenated level for a greater time period following successive ischemic insults. We consider that this indicates that more oxygen is being consumed per unit time by the tissue after ischemic insult, a circum stance generally accompanying the increased rate of respiration of uncoupled mitochondrial systems.

To clarify this, 2 sec trains of 20 Hz direct cortical stimulation were presented to the cortical surface before and after ischemic insults. Such stimulation is accompanied by a transient increase in the oxidation level of NADH (peak at approximately 3 sec following termination of stimulation) and a recovery to resting levels within approximately 20 sec. Following an ischemic episode, stimulation was accompanied by an oxidation of NADH, the extent of which was initially decreased consistent with the decrease in tissue excitability measured by the size of evoked potentials and the shift in cortical steady potential. The time course of NADH oxidation appeared unaffected by the ischemic insult but the time to recovery to resting NADH levels was markedly slowed, indicating a mitochondrial lesion.

Our data indicate that an effect of short Periods of what has been considered to be functionally reversible ischemia may be the uncoupling of oxidative phosphorylation. This may indicate that the structural integrity of mitochondria, upon which the ability to form ATP is dependent, is the most sensitive cellular component to ischemic injury.

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536 LOCAL CEREBRAL OXYGEN CONSUMPTION RATES. Jack M. Fein. Dept. Neurosurgery, Albert Einstein College of Medicine, New York, N.Y. 10461

In vitro studies have demonstrated the heterogeneity of oxidative consumption rates in the brain. A method has been developed using polarographic principles to determine the quantitative rate of oxygen consumption in small volumes of brain tissue in vivo. Teflon coated platinum electrodes with an exposed tip of exactly 1 mm and 250 microns in diameter were thin coated with cellulose acetate. These electrodes were calibrated against standard NADH solutions, evaluated for stirring and diffusion artifact, and implanted streotactically in cortical and sub-cortical regions in rhesus monkeys. A stable polarographic circuit was utilized to maintain a bias appropriate for the continuous reduction of available oxygen and the simultaneous monitoring of changes in output current.

Three hundred and sixteen measurements were made in eight animals. The cortical oxygen consumption rates in ul/mg/min, assuming a 1 mm^3 electrolysis field, were frontal .040 ± .0037, parietal .037 ± .0051, temporal .042 ± .0081, and occipital .047 ± .0115. Depth electrodes measured the oxygen extraction rates of the putamen and the centrum semiovale which were 0.0312 ± .0049 and .0216 ± .0028 ul/mg/min respectively. Changes in arterial oxygen tension produced no significant change in consumption rate until an aPO_2 or 360 mm. Hg. was achieved. Parietal cortical oxygen extraction then increased ($p<.1$). At an aPO_2 of over 450 mm. Hg. temporal cortical rates also increased ($p<.1$). Below aPO_2 of 40 mm. Hg. a transient increase ($p<.1$) and then decrease ($p<.01$) of cortical rates were noted at all sites. There was no significant effect on the consumption rates of changes in $aPCO_2$ between 18 - 60 mm. Hg. Studies above and below these limits were precluded by hypotension at higher $aPCO_2$ values and cardiac arrhythmias at lower $aPCO_2$ values.

The sensitivity to change induced by metabolic stimulants was estimated using Metrazol 50 mg I.V. Sixty one measurements were made and a significant increase in cortical ($p<.001$), putaminal ($p<.01$) and white matter rates ($p<.01$) was seen.

There are significant differences in the rates of oxygen consumption in cortical grey, deep grey and cerebral white matter locations. The data indicates that despite changing arterial blood gas values which are known to change blood flow rates, there is a large range over which the metabolic rate remains constant. Predictable increases in the oxygen consumption rates were seen when pharmacologic agents which stimulate metabolism directly, were used. The ability to measure oxygen consumption rates in this manner allows for in vivo studies in small volumes of tissue in either an experimental or clinical neurosurgical setting.

537 ALTERATION IN THE ENZYMES INVOLVED IN CYCLIC NUCLEOTIDE METABOLISM FOLLOWING UNILATERAL ISCHEMIA AND RECOVERY IN GERBILS. Joan P. Schwartz, Bogomir B. Mrsulja, Branislava J. Mrsulja*, Janet V. Passonneau, and Igor Klatzo. NIH, Bethesda, Maryland 20014.

The absence of posterior communicative arteries in Mongolian gerbils permits the investigation of ipsilateral ischemia simply by ligation of either of the common carotid arteries; the contralateral hemisphere then serves as a control (Arch. Pathol. 87: 315, 1969). Removal of the clip before freezing the animal in liquid nitrogen or taking out the brain for homogenization allows recovery. For most of the assays the cortex was used for analysis. Cyclic AMP levels were elevated from 30 min. to 2 hrs. of ischemia and then declined slowly. There were no differences in the activities of adenylate cyclase (AC), protein kinase (PK) or cAMP phosphodiesterase (PDE) between ischemic and control sides after 60 min. of ligation. There was also no detectable change in AC after 15 min. ligation, despite the fact that cAMP levels were still increasing at this time. Possible reasons for these results will be discussed. The activities of both cAMP and cGMP PDE's remained constant in four different brain regions (cortex, hippocampus, thalamus and caudate), for as long as 5 hrs. of ischemia. Thus, the elevated cAMP levels did not lead to induction of either enzyme as has been reported for other tissues. Removal of the clip and recovery resulted in a further increase in cAMP in the ischemic side within 5 min. After 5 min. recovery there was a significant depression of both AC and PK on the ischemic side. The time course of these enzyme changes relative to the cAMP changes will be presented.

538 METABOLIC RESPONSIVENESS OF THE ISCHEMIC CEREBRAL CORTEX TO A SECONDARY PERIOD OF ISCHEMIA. B. B. Mrsulja, W. D. Lust, B. J. Mrsulja*, J. V. Passonneau, and I. Klatzo. NIH, Bethesda, Maryland 20014.

The cerebral circulation of the Mongolian gerbil possesses certain characteristics which permit its use as a suitable model for studying various aspects of ischemia. In a previous report, we described the changes which occurred in certain brain metabolites both during and after prolonged ischemia. By 1 hr. of reperfusion following 1 hr. of ischemia, the levels of phosphocreatine (PC), glucose, glycogen, GABA and cyclic AMP had essentially recovered to those of control. To determine if ischemia would affect the responsiveness to a subsequent ischemic insult, the left common carotid artery was occluded for 1 hr. then was released for either 1, 5 or 20 hrs., and finally occluded again for either 5 or 60 min. A comparison of these values to those for a single ischemic insult would indicate any changes in the apparent susceptibility of the cerebral cortex to a second period of ischemia. When the period of reperfusion was 1 hr., the levels of ATP, PC, glycogen and glucose were decreased at 5 min. of a second ischemia to 29, 14, 53 and 14% of control values, respectively. These results contrast to those observed during a primary ischemia where there was no change in these metabolites at 5 min. in 19 out of 20 animals. At 60 min. of a second ischemia, the level of these metabolites decreased to the same degree as observed during 60 min. of a primary ischemia. When the period of reperfusion was extended to 5 hrs., the effect of 5 and 60 min. of ischemia on the cerebral metabolites was minimal. By 20 hrs. of reperfusion, the response had essentially been restored to that seen during a single ischemic episode. Our results suggest that at 1 hr. of recovery the ischemic cortex is perhaps more vulnerable to another ischemic insult, but by 5 hrs. the cortex is somehow protected from another period of ischemia. Finally, the response to repeated ischemia is essentially back to normal by 20 hrs. of recovery.

539 RECOVERY OF ENERGY METABOLITES FOLLOWING PROLONGED ISCHEMIA IN THE GERBIL CEREBRAL CORTEX. W. D. Lust, B. B. Mrsulja, B. J. Mrsulja*, J. V. Passonneau and I. Klatzo. NIH, Bethesda, Maryland 20014.

The ability of the ischemic brain to recover both metabolically and functionally is apparently dependent on the duration of the ischemia. We have previously reported that within 1 hr of unilateral ischemia the levels of ATP, phosphocreatine (PC), glucose and glycogen all declined to a new steady state and then remained constant for up to 6 hrs of ischemia. Other long-term events secondary to the changes in energy metabolites occurred during the 6 hrs of ischemia. Cyclic AMP and GABA increased, whereas cyclic GMP and the biogenic amines decreased. To determine when the ischemia impairs the ability of the cortex to recover, we investigated the levels of certain metabolites at 5 and 60 mins of reperfusion following either 1/2, 1, 3 or 6 hrs of ischemia. Generally, a marked reduction in the rate restoration as indicated by the levels of ATP, PC and glycogen occurred only at 6 hrs of ischemia. At 1 hr of reperfusion following 1/2, 1, 3 and 6 hours of ischemia the relative concentrations of ATP were 92, 73, 74 and 56 percent of the control values, respectively. At the same time of recovery, the PC levels had essentially recovered in all groups. The levels of cyclic AMP increased from 3- to 6-fold over the already elevated levels after 5 min of recovery. This large transient increase in cyclic AMP was evident at 1/2, 1 and 3 hrs but not at 6 hrs. While the greatest impairment to the recovery process appeared to occur during the 3 to 6 hr period of ischemia, other data indicate that loss of recoverability probably starts earlier. After 1 hour of reperfusion, the lactate levels were 1.9-fold greater than those for control in the 1 hr ischemic group and were 5.3-fold greater for the 3 hr ischemic group. This reduced ability of the cortex to restore lactate to the resting level is therefore manifested between 1 and 3 hrs of ischemia.

540 TRANSIENT CHANGES OF REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE LEVEL OF NEURON SOMA IN ANAEROBIC CONDITIONS AFTER PERIPHERAL NERVE STIMULATION. Carlos Rodríguez-Estrada. Cátedra de Fisiología, I.M.E., Facultad de Medicina, Univ. Central de Venezuela, Caracas, Venezuela.

Previous works have shown that neuron soma excitation after a short period of peripheral nerve stimulation produced a transient change of reduced nicotinamide adenine dinucleotide (NADH) level in aerobic conditions. This change was characterized by a transient NADH oxidation followed by a transient increase of NAD reduction. In this work fluorometric determinations of NADH level, were done in aerobic and anaerobic conditions. They were used in vitro preparations of isolated dorsal root ganglia (Rana palmipes spix). It was found that the steady state level of NADH, was larger in anaerobic conditions than in aerobic conditions of resting neuron soma and this NADH level decreased slowly later on. Transient NADH oxidation was observed after neuron excitation in aerobic conditions and it was never observed in anaerobiosis. Transient NAD reduction was not observed after neuron excitation in anaerobic conditions if NADH level was largest. However, transient NAD reduction was observed after neuron excitation in anaerobiosis if NADH level was smaller than the largest NADH level earlier observed. These results indicated that the respiratory chain in anaerobiosis the transient NADH oxidation was bloked after neuron excitation and in contrast, NAD reduction was not blocked after neuron excitation.

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541 TURNOVER OF ^{14}C-ADENINE NUCLEOTIDE POOL IN RAT BRAIN SLICES. P. M. Stanaszek*, R. J. Bull and J. J. O'Neill*. Dept. Pharma., Sch. Med., The Ohio State University, Columbus, Ohio, 43210.

Alterations in adenine nucleotide content have previously been correlated with potassium induced spectral changes and a transient increase in tissue respiration (Bull & O'Neill Psychopharm. Comm. 1: 109, 1975). The apparent contraction of the adenine nucleotide pool was rapidly reversed by lowering potassium ion concentration in a superfused preparation. In the study to be described, rat brain outer cortex slices were pre-incubated with ^{14}C-adenine according to the method of Shimizu et al. J. Neurochem 16: 1609-1619, 1969. Tissues were then superfused with Krebs-Ringer bicarbonate buffer containing normal (3mM), elevated (30mM) potassium or a sequence of 3mM potassium followed by 30mM and a return to 3mM during the recovery period. Following perchloric acid extraction and neutralization, extracts together with marker substances were separated on DEAE Sephadex G-100 according to the method of Caldwell, J. Chromat. 44: 331-341, 1969. Results obtained suggest that the contraction of the adenine nucleotide pool to minimize the decrease in energy charge potential (ECP) probably resulted from increased deamination of the adenine nucleotides by potassium activation of 5' AMP deaminase. The recovery of radioactivity in ATP, ADP and AMP following restoration of normal potassium is further support our previous proposal for the presence of a purine nucleotide cycle operative in brain under certain conditions.

542 REDOX STATES OF BRAIN TISSUE IN THREE DIMENSIONS. B. Chance, B. Quistorff*, S. Eleff*, W. Nadler*, and J. Sorge*. Johnson Research Foundation, School of Medicine, University of Pennsylvania, Philadelphia, PA 19174 USA and Panum Institutet, Copenhagen, Denmark.

The quest for a time- and space-resolved metabolic map of the brain cortex under different physiological conditions is made possible by a combination of rapid trapping of the brain tissue and two-dimensional scanning of the redox state of serial sections of frozen tissues at 77° K. Brain tissues, rapidly frozen and trapped according to the method of Quistorff (J. Neurochem. 21:1345, 1973) are scanned for variations of the ratio of the concentrations of the two components of Site I of mitochondria, the oxidized flavoprotein associated with the lipoate dehydrgenase, and the NADH pool of the mitochondria, as contrasted with scans employing NADH fluorescence only (Chance et al., Soc. Neurosci. Abstr., 1974; Ji et al., Soc. Neurosci. Abstr., 1975). These fluorescences are greatly enhanced at low temperatures, and the ratio of the oxidized to the reduced signal makes the system insensitive to variations of tissue mitochondrial content and to reasonable amounts of screening pigments such as hemoglobin (Chance et al., FEBS Meeting Abstr., Paris, 1975). Initially, data are taken every mm over a raster of 100 points, digitized and contoured according to a computer program. Sequential sections taken every 320 μ are then appropriately registered to indicate the contours of tissue volumes of constant redox state for a particular physiological condition. Preliminary scans of normoxic, anoxic, and phenobarbital-inhibited brain tissues have so far been obtained, and the correlation of isoredox contours with functional or morphological characteristics is in progress. (Supported by USPHS GM-12202 and NS-10939)

543 MEASUREMENT OF NAD(P)H IN ISOLATED BRAIN TISSUES AND THE EFFECT OF DEPOLARIZING AGENTS. Joseph T. Cummins, Addiction Res. Lab., V.A. Hosp., Sepulveda, CA 91343; and Dept. Med. Pharmacol. and Therap., Univ. California, Irvine.

The level of reduced nicotinamide adenine dinucleotides (NAD[P]H) were measured in isolated brain tissues both by dual wavelength absorption spectroscopy and by a laser fluorescence microscope. A He-Cd laser (325.7 nm) was used to excite the fluorescence of tissue NAD(P)H and the emitted light was measured by a photon counting system. A specially designed perfusion chamber under a trioccular stereo zoom microscope allowed the measurement of NAD(P)H fluorescence over a wide range of tissue sizes while allowing the tissue to survive in Ringer solution. 30 mM K^+ depolarization caused a reduction in the level of reduced pyridine nucleotides in slices from the caudate and cortex. Veratridine at 10^{-5} M evoked an decrease in the level of NAD(P)H in tissues from several brain areas within 30 sec. The depolarization responses were similar for tissue from caudate and cortex by either the absorption or fluorescence methods. These studies demonstrate that the laser microscope perfusion technique can measure the steady state level of NAD(P)H and drug induced responses in small areas of surviving brain tissue. This approach should give information on the metabolism of particular brain Loci which have a specific physiological function. (Supported in part by USPHS Grant #DA00624-01.)

544 STUDIES ON PHOSPHATE INTERMEDIATES IN MOUSE BRAIN USING A NEW METHOD. Nandita Pal* and Samuel P. Bessman. Dept. Pharmacol., Univ. South. Calif. Sch. Med., Los Angeles, 90033.

To estimate the in vivo concentration of phosphate intermediates in mouse brain, animals were put in a 1.5 KW microwave oven and radiation was focussed on the head for 1 to 3 sec. The brain was dissected into two halves and one half was processed immediately by homogenization in ice cold 10% TCA, and the other half was kept at room temperature for 1/2 hour prior to processing. TCA extract was neutralized and analyzed for phosphate intermediates according to the method of separation and detection of organic phosphates by Bessman, et al (Anal. Biochem. 59:533, 1974). Of the twenty-four compounds that separate by this method, fourteen have been identified. In order of elution these are creatine phosphate, inorganic phosphate, fructose-6-phosphate, glucose-6-phosphate, NAD, 3PGA, AMP, Fl,6DP, 2,3-DPG, IMP, ADP, GDP, ATP and GTP respectively. The protein content of the brain homogenates was also determined. After 3 seconds exposure to microwave radiation there was no change in the phosphate intermediates whether the brain was processed immediately or kept at room temperature for 1/2 hour, indicating prevention of postmortem changes. The phosphate intermediates were also analyzed in different anatomical parts of the brain.

545 REGIONAL AND DEVELOPMENTAL DIVERSITY OF NON-HISTONE CHROMOSOMAL PROTEINS IN RAT BRAIN. Judith B. Walker, Isaac Bekhor*, and Jung Kim*. Laboratory of Developmental Biology, Gerontology Center, and Department of Biochemistry School of Dentistry, University of Southern California, Los Angeles, California 90007

Electrophoretic gel profiles of non-histone chromosomal proteins (NHCP's) derived from cerebellum, cortex, and liver from male sprague Dawley rats ageing 1, 15, and 30 days were compared. Cerebral cortex is characterized by the presence of very high molecular weight NHCP's, which are not found in cerebellum or in liver. Similarly, NHCP's derived from cerebral cortex demonstrate more developmental changes than do NHCP's from cerebellum or liver.

Analyses of the derivatives plots obtained from melting profiles reveal a reproducible low temperature thermal transition in newborn chromatin that is not found in tissues from older animals. The melting data imply a developmental restriction of template activity, and changes in the physical structure of chromatin are reflected by alterations in the electrophoretic profile of NHCP's.

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Abstract withdrawn by author

547 EFFECT OF pH ON cAMP-ELICITED STIMULATION OF TYROSINE HYDROXYLASE ACTIVITY. R. L. Bronaugh*, T. Kashimoto* and M. Goldstein. Dept. of Psychiatry, Neurochemistry Labs., N.Y.U. Med. Ctr., New York, N.Y. 10016.

The cAMP-elicited stimulation of tyrosine hydroxylase (T.H.) activity has been previously studied by us in striatal slices and synaptosomal preparations. We have now investigated the effects of cAMP on the activity of the soluble striatal T.H. at various pH's. The enzyme activity was assayed in an incubation mixture containing 10^{-5}M ^{3}H-tyrosine and 2.5 x 10^{-4}M $DMPH_4$ (pteridine cofactor). The activity of soluble T.H. was stimulated by cAMP only when ATP and Mg^{++} were added to the incubation mixtures. The addition of exogenous cAMP-dependent protein kinase to the incubation mixture resulted in a marked enhancement of the cAMP-elicited stimulation of T.H. activity. The kinetic analysis revealed that cAMP reduced significantly the K_m for the pteridine cofactor. Although the T.H. activity decreases progressively in the presence as well as in the absence of cAMP as the pH rises from 6.0 to 7.4, the % stimulation elicited by the nucleotide is increased at the higher pH's. To determine whether the increased % stimulation of T.H. activity at higher pH's results from the pH dependency of the activation reaction by the cAMP-dependent protein kinase or of the tyrosine hydroxylation reaction, we have carried out these two enzymatic reactions in separate stages. The enzyme was activated to approximately the same extent at pH 6.0 and 7.0, while the % stimulation of T.H. activity was much higher when the tyrosine hydroxylation reaction was carried out at higher pH's. At the pH range 6.8-7.4 the activated T.H. has much higher catalytic activity (increase of about 250%) than the non-activated enzyme. Thus, the activated enzyme may play an important role in the biosynthesis of catecholamines at physiological pH's *in vivo*. This work was supported by USPHS grant MH-02717 and NSF grant GB-27603.

548 TIME COURSE OF THE CHANGES IN TYROSINE HYDROXYLASE IN CENTRAL NORADRENERGIC CELL BODIES AND TERMINALS AFTER RESERPINE. Richard E. Zigmond, Dept. Pharmacology, Harvard Medical School, Boston, Mass.

Three days after a systemic injection of reserpine in the rat an increase in the activity of tyrosine hydroxylase was found in the locus coeruleus, an area of the brain stem containing a large number of noradrenergic cell bodies (Zigmond, Schon, and Iversen, Brain Res. 70: 547, 1974). The purpose of the current study was to determine the time course of this effect and whether increases in this enzyme also occur in areas of the brain innervated by noradrenergic terminals arising from locus coeruleus neurons. Rats were given a single injection of reserpine (5mg/kg, s.c.) and at various times from 1-42 days later tyrosine hydroxylase activity was measured in the locus coeruleus, cerebellum, hypothalamus and hippocampus. Significant increases in enzyme activity occurred in all areas studied but the delay before the first appearance of significantly increased enzyme activity differed in each brain region. Increased enzyme activity was first detected in the locus coeruleus 3 days after reserpine treatment, in the cerebellum after 5 days, in the hypothalamus after 6 days and in the hippocampus only after 15 days. The time course of these events is consistent with the hypothesis that reserpine stimulates the synthesis of new enzyme molecules in cell bodies of noradrenergic neurons in the locus coeruleus and that the enzyme is then transported to nerve terminals in other parts of the brain by a slow axonal transport mechanism.

549 PARTICIPATION OF PROTEIN KINASE (PK) ACTIVATION AND TRANSLOCATION IN THE TRANSSYNAPTIC INDUCTION OF TYROSINE HYDROXYLASE (TH) OF RAT ADRENAL MEDULLA. Alessandro Guidotti , Atushi Kurosawa and Ermino Costa. Laboratory of Preclinical Pharmacology, NIMH, Saint Elizabeths Hosp., Washington,D.C. 20032

Several stimuli (Reserpine, Carbamylcholine, Aminophylline and cold exposure) that cause a delayed induction of TH in rat adrenal medulla elicit an early increase of cAMP content which is associated co an activation of cytosol protein kinase (PK). The cAMP independent catalitic subunit of the PK is dissociated from the holoenzyme. This PK activation lasts about four hrs. The magnitude of the PK activation and the delayed TH induction elicited by reserpine or carbamylcholine are dose related.

After various stimuli that induce TH the total (cAMP dependent + independent) PK activity in the 2 x 10^4 x g supernatant decreases by about 40% in 3 hrs; this decrease persists for several hours. The decrease of total PK in cytosol was paralleled by a correspondet increase of a particle bound cAMP independent histone kinase. Moreover 4 hrs after reserpine the histone kinase activity in the nuclear fraction of adrenal medulla was doubled. These changes were interpreted as a translocation of PK from cytosol to nuclei or other structures. Using stimuli other than carbamylcholine this translocation occurs only if the adrenal medulla innervation is intact. When monolateral adrenal denervation was performed 1.5 hrs after the stimulus, translocation failed to occur. Since denervation at this time prevented the delayed TH induction, translocation of PK from cytosol to the particulate fractions of chromaffin cells may participate in the transsynaptic induction of TH.

550 A KINETIC ANALYSIS OF THE SYNAPTOSOMAL SYNTHESIS OF DOPAMINE FROM L-TYROSINE AND L-PHENYLALANINE. Gregory Kapatos*and Michael Zigmond. University of Pittsburgh, Pittsburgh, PA 15260.

The synthesis of dopamine (DA) from L-tyrosine (TYR) by rat striatal synaptosomes obeyed Michaelis-Menton kinetics (Km TYR, 0.90μM). L-phenylalanine (PHE) (5-10μM) competitively inhibited DA synthesis from TYR (Ki PHE, 3.86μM). Synaptosomal synthesis of DA from PHE did not obey Michaelis-Menton kinetics, but could be fractionated into a high (Km_1 PHE, 0.83μM) and low (Km_2 PHE, 4.21μM) affinity system. Both kinetic systems were competitively inhibited by 0.5μM TYR (Ki TYR, 1.12μM). At 1.0uM TYR, inhibition of the high affinity system was mixed, while competitive inhibition of the low affinity system continued (Ki TYR, 1.02μM). Exogenous DA (1μM) inhibited synthesis from TYR and from either PHE system in a non-competitive fashion. Assuming that hydroxylation is the rate limiting step in the synthesis of DA from these substrates, the kinetic parameters obtained in this system may reflect the differential affinity of these substrates for the regulatory enzyme tyrosine hydroxylase (TH). The competitive inhibition by PHE of TYR hydroxylation suggests that Ki PHE also represents the Km of PHE for TH (an hypothesis supported by the equality between Ki TYR and Km TYR). That this Km (approx. 4 μM) is equal to Km_2 PHE, suggests that at higher concentrations PHE is directly hydroxylated to dopa, without the dissociation of the intermediate product, TYR. On the other hand, Km_1 PHE is similar to Km TYR, suggesting that at lower concentrations, PHE is converted to TYR which then dissociates and mixes with endogenous TYR before hydroxylation to dopa. This model is further supported by the differential effect of TYR on the high and low affinity system for the synthesis of DA by PHE. (Supported in part by USPHS Grant No. MH-20620 and by a grant from Smith Kline and French Labs.)

551 SPECIFICITY OF TYROSINE HYDROXYLASE REGULATION BY cAMP, CONCOMITANT REGULATION OF DOPAMINE BETA HYDROXYLASE. J. C. Waymire, R. Boehme and K. G. Waymire. (Sponsor: G. Lynch), Univ. of Calif., Irvine, CA.

Addition of cAMP analogues or cyclic nucleotide phosphodiesterase inhibitors to cultures of neuroblastoma clones is known to produce large elevations in the level of tyrosine hydroxylase (TH) (Waymire et al., PNAS 69:2241, 1972). The change of TH is paralled by an increase in the number of neuroblastoma cells extending long neurites. We sought to determine whether the increase in TH produced by cAMP is part of an overall cell differentiation phenomenon produced by the treatment or an example of specific enzyme regulation. Accordingly, all the enzymes believed to participate in norepinephrine metabolism were analyzed in cultured neuroblastoma cells following treatments with either 8Br-cAMP or the phosphodiesterase inhibitors, papaverine and RO-201724. As previously reported for other analogues of cAMP, 8Br-cAMP caused a large increase in TH (14 fold by 48 hr) as did papaverine (35 fold by 120 hr) and RO 201724 (32 fold by 72 hr). However, treatment for up to 172 hr with a wide range of concentrations of the above drugs did not significantly elevate the activities of dopa decarboxylase, monoamine oxidase, or catechol-O-methyltransferase. In contrast, dopamine-β-hydroxylase (DBH) was significantly elevated by all three treatments and at identical dosages as for TH elevation. The time course of the elevation of the enzymes was also similar. These results provide evidence that cAMP may participate in the specific regulation of TH and indicate that DBH may be regulated in these cells by a mechanism coupled to that of TH regulation.

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552 CHOLINERGIC ACTIVATION OF TYROSINE HYDROXYLASE IN NORADRENERGIC NEURONS OF RAT BRAIN. Tommy Lewander,*Tong H. Joh and Donald J. Reis. Lab. Neurobiol., Dept. Neurol., Cornell Univ. Med. Coll., New York, 10021.

In sympathetic ganglia and adrenal medulla, the activity of the enzyme TH can be increased by direct or reflex cholinergic stimulation. We sought to establish if central cholinergic stimulation could also alter the activity and amounts of tyrosine hydroxylase (TH) in the rat brain. Oxotremorine, a centrally active cholinergic agonist, was administered to rats and TH activity was measured in the nucleus locus coeruleus (LC), substantia nigra and adrenal glands. A single injection of oxotremorine (1.5 mg/kg s.c.) caused an increase in TH activity in LC, starting at 24-48h and reaching a maximum of 50% of the control level at 4 days after administration. TH activity was unchanged in the substantia nigra of the same animals. TH activity was increased 60% in the adrenal glands reaching a maximum 24h after the injection. The TH activity returns to control in LC and adrenals at 14d after drug administration. The effect of oxotremorine on TH activity in the LC and adrenal was dose dependent; a maximal effect was obtained with 1.5 mg/kg Increasing the dose to 2.5 mg/kg, the maximal non-lethal dose, failed to further elevate the response. Atropine (10 mg/kg), but not methylatropine (10 mg/kg) administered 30 min before oxotremorine blocked the increase in TH activity in both LC and adrenal.

Additional experiments were conducted in order to determine if the oxotremorine elicited increase in TH activity in LC was a consequence of activation of preexisting enzyme molecules (activation) or increased accumulation of enzyme protein (induction). Animals were treated with oxotremorine for 3d (1.5 mg/d) and killed on day 6 when TH activity was increased twofold. Another group of rats received reserpine (2.5 mg/kg once daily for 3d) which induces TH in LC and adrenal (Reis et al, Brain Res. 81:380-386, 1974). Immunotitration with a specific antibody to TH demonstrated that in oxotremorine treated animals TH was activated in LC and induced in adrenal. In contrast, the comparable increase in TH activity produced by reserpine was entirely attributable in both tissues to induction. Kinetic analysis of oxotremorine activated TH in the LC indicated that the V_{max} increased without change of Km for tyrosine or $DMPH_4$.

We conclude that the centrally active cholino-mimetic drug oxotremorine increases TH activity in the cell bodies of central noradrenergic, but not dopaminergic, neurons through a central muscarinic action of the drug. The induction of the TH activity in the adrenal gland is probably mediated via the central nervous system.

The delayed activation of TH in central noradrenergic neurons may be due to conformational changes of the TH molecule or to removal of some endogenous inhibitor of the enzyme and may represent a new mode of regulation of TH.

Supported by grants from NIMH, MH 24285 and the Scottish Rite Schizophrenia Research Program.

553 ELECTRON-SPIN RESONANCE STUDIES OF MONOAMINE OXIDASE DERIVED FROM HUMAN BLOOD PLATELETS. Rosa Huang* and Samuel Eiduson. Neuropsychiatric and Brain Res. Insts., Depts. Biol. Chem. and Psychiat., Sch. Med., UCLA, Los Angeles, 90024.

We have previously reported that using electron-spin resonance (ESR) techniques we were able to demonstrate that a spin-labeled probe, spin-labeled hydroxyamphetamine (SHA), had different affinities toward the possible multiple forms of brain MAO. We have since used this same probe to monitor and characterize the ligand binding properties of MAO derived from human blood platelets. Although MAO activity derived from human blood platelets differs from that of rat brain MAO activity, they show some similarities in substrate and inhibitor characteristics. Initial results were obtained by measuring the molar concentration of SHA binding to MAO at a concentration of SHA of 2.5×10^{-5} M for four different sets of platelet samples: intact, sonicated, treatment with Triton X-100, and freeze-thawed. It was observed that the results agreed with the corresponding specific activities as measured with ^{14}C-tryptamine and ^{14}C-benzylamine as substrates for the enzyme. Additional studies were made of the effect of temperature on the SHA-MAO binding using 0.2 ml of a sonicated platelet preparation (1ml of the preparation was obtained from 10 to 20 ml of whole blood). The temperature at the sample could be regulated to a stability of + 0.1°C. The molar concentration of SHA-MAO binding was measured as the temperature of the sample was increased from 4° to 56°C by increments of 2°C. When the amount of binding was plotted against temperature (using 0.2 ml of a platelet MAO preparation from "normal" subjects), a curve containing two plateaus was obtained. This strongly suggested that at least two distinct conformations of the platelet enzyme existed. One form appeared to be saturated with the spin-labeled inhibitor at 16°C while the other form was saturated at 54°C. If the concentration of the SHA-MAO complex at saturation levels is assumed to be the total concentration of each of the particular MAO forms, then the affinity constants, at all temperatures, could be calculated. Thus a Gibbs-Helmholtz plot of ln K vs 1/T yielded two intersecting straight lines which further reinforced the notion that the spin-labeled probe was bound to at least two different forms of MAO. Several "non-normal" blood platelet MAO samples showed only one form of MAO when observed at various temperatures indicated above. In these cases, the characteristic temperature at which the binding reached saturation was either at 16°C or 54°C.

With these techniques, using the Gibbs-Helmholtz equation, we have been able to determine the nature, affinity, intrinsic free energy, heat and entropy of the various forms. In short, one is able to obtain unique parameters which may characterize the conformation of each of the possible individual multiple forms of MAO. It should be emphasized additionally, that this ESR method is capable of adding important information about very crude enzyme preparations. Not only has this been accomplished with 0.2 ml of a sonicated platelet preparation but it can also be used with intact brain mitochondria.

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554 THE EFFECT OF MONOAMINE OXIDASE INHIBITORS (MAOI) ON THE IN VIVO UPTAKE OF RADIOLABELLED CALCIUM AND MAGNESIUM IN THE BRAIN AND PITUITARY GLAND. Anatol Costin and Irene Sabbot,* Dept. Anat. and BRI, Sch. Med., UCLA, Los Angeles, 90024

The mechanisms regulating calcium and magnesium transport in the brain and pituitary are not well known. Previously we reported (Experientia, 1975) that reserpine increases the uptake of ^{45}Ca by the pituitary gland and by the cortex and hippocampus. The objective of our present research was to investigate calcium and magnesium movements in vivo by mapping the uptake of these cations by certain areas of the brain and by the pituitary gland of the rat following administration of monoamineoxidase inhibitors (MAOI). Control and test animals were injected i.p with saline, Phenelzine (75 mg/kg) or Pargyline (150 mg/kg). After 1 hr, mixtures containing 1 μCi each of ^{45}Ca and ^{28}Mg were injected into the carotid artery of the rat. Decapitation followed 15 sec later. The brain was quickly dissected and the radioactivity of different areas of the brain and pituitary gland was measured. Both MAOI induced an increase in the uptake of ^{45}Ca and ^{28}Mg by the pituitary gland. Phenelzine increased the uptake of ^{45}Ca by the cortex, hippocampus, thalamus and cerebellum and of ^{28}Mg only by hippocampus. Pargyline increased the uptake of ^{45}Ca only in the cerebellum and had no effect on the incorporation of ^{28}Mg. These results suggest an important role for the biogenic amine transmitters in the regulation of calcium and magnesium movements in the brain and the pituitary gland.

555 SUBCELLULAR LOCALIZATION OF TYPES A AND B MONOAMINE OXIDASE IN RAT BRAIN. Adina K. Student*and David J. Edwards*(SPON: I. Hanin) Western Psychiatric Inst. & Clinic, Dept. Psychiatry, Univ. of Pittsburgh, Sch.Med., Pittsburgh Pa. 15261

The subcellular localization of types A and B monoamine oxidase (MAO) has been studied, using modifications of the procedure of Autilio et al. (Biochemistry,7,2615 [1968]). The myelin, synaptosomal(S) and mitochondrial(M) fractions obtained from a discontinuous Ficoll-sucrose gradient contained (as % of total activity recovered from the gradient) 7,39,and 54% of the MAO activity when serotonin (SER), a type A substrate was used and 4,25, and 71% of the MAO activity when phenylethylamine(PE), a type B substrate, was used. The ratio of SER:PE activity was 2.1-fold greater in the S than in the M fraction. However, the MAO activity in the S fraction using the substrate PE was insensitive to inhibition by clorgyline, indicating that this activity was due to MAO-B. Furthermore, the ratio of SER: PE activity was the same for mitochondria isolated from lysed synaptosomes (9.8) as for intact synaptosomes (9.9). Marker enzymes indicated that the small amounts of both types A and B MAO in microsomal, myelin and synaptic membrane fractions could be accounted for by contamination of other cellular components. Finally, the specific activity of MAO-B, in contrast with MAO-A, varied in subcellular fractions obtained from different brain regions; for example, the specific activity of MAO-B in synaptosomes was lower in cortex than in cerebellum. These data demonstrate that types A and B MAO are differently distributed in subcellular fractions of rat brain. The relatively lower MAO-B activity in synaptosomal mitochondria may be due to either a general difference in the enzymatic content of synaptosomal vs. nonsynaptosomal mitochondria or to certain populations of synaptosomes having little or no MAO-B. Studies with specific brain regions lend support to the latter possibility.

556 POSTNATAL DEVELOPMENT OF MULTIPLE FORMS OF MONOAMINE OXIDASE IN BRAIN. Felor Jourdikian* and Boris Tabakoff. Dept. of Research, National College, Lombard, Illinois and Dept. of Biochemistry, Chgo. Med. Sch., Chgo., Ill.

Monoamine oxidase (MAO) has recently been found to exist in two functional forms in brain and these were designated as form A and B. In attempting to differentiate between these two forms of the enzyme we found that MAO activities in mouse brain responsible for deamination of serotonin (5-HT) and p-dimethylaminobenzylamine (DAB) followed different postnatal developmental patterns. MAO activity which deaminated 5-HT was approximately 25% of adult levels at birth and reached adult levels 15 days after birth. On the other hand, MAO activity deaminating DAB was approximately 16% of adult levels at birth, and did not mature to adult levels until after the 45th postnatal day. Inhibitor studies with deprenyl and clorgyline indicated that the deamination of DAB was due to the action of Type B MAO while deamination of 5-HT was catalyzed by Type A MAO. Differentiation of brain phospholipids may contribute to the development of different forms of MAO. Supported in part by grants from the National Institute of Neurological Disease and Stroke, the National Institute of Alcohol Abuse and Alcoholism and the State of Illinois, Department of Mental Health. BT is a Schweppe Foundation Fellow.

557 Mass Fragmentographic Identification and Quantification of 5-Methoxytryptamine (5MT) in Human Cerebrospinal Fluid (CSF). S. H. Koslow, R., Post, F. Goodwin, C. Gillin, Biological Research Section, CRB, NIMH, Rockville, Md.; Adult Psychiatry Branch and Laboratory of Clinical Science, NIMH/NIH, Bethesda, Md., Clinical Psychopharmacology, NIMH/SMHR, St. Elizabeths Hospital, Washington, D.C.

Animal studies suggest that 5MT is bioactive in mammalian nervous system. This is the first reporting of the presence of 5MT in human tissue. 5MT is isolated from human CSF by precipitating protein with HCl. The supernatant is adjusted to pH 11 with KOH, and extracted twice with 5 volumes of ether. The ether containing the extracted 5MT is dried down in reacti-vials with the internal standard α-methyl-5MT. After reacting with pentafluropropionic anhydride for 2 hours, the acylated derivatives are analyzed by mass fragmentography. 5MT is identified by recording the ion densities of the fragments at m/e 306 and 319 at the gas chromatographic retention time of 5MT. A fragment ratio of 1 confirms the identity of 5MT. Concentrations of 5MT are measured in lumbar CSF sampled from ill and recovered schizophrenics, depressed and manic patients and neurological controls. 5MT concentrations are not altered by probenecid. In neurological controls 5MT was either 0 or less than 5 pmol/100 ul CSF. In drug free schizophrenics and manic patients the concentrations of 5MT were as high as 125 pmol/100 ul CSF. Depressed patients consistently showed lower levels of 5MT than those diagnosed as manic ($p < .03$). The neurochemical role of 5MT is presently undefined. In rodents 5MT is present in pineal, brain and blood and has been shown to alter locomotor activity and calcium metabolism. In man it is implicated in psychiatric illness since it is a precursor to the hallucinogenic substance 5 Methoxydimethyl Tryptamine. Its greater presence in human CSF in the psychoses of mania and schizophrenia suggests an important biological role for 5MT.

558 THE SIMULTANEOUS MEASUREMENT OF ACETYL-CoA, ACETYLCHOLINE, AND CHOLINE IN THE SAME TISSUE EXTRACT FROM RAT BRAIN BY A RADIO-ENZYMATIC METHOD. P.A. Shea* and M.H. Aprison. The Inst. of Psychiatric Research and Depts. of Biochemistry and Psychiatry, Indiana University Med. Ctr., Indianapolis, IN. 46202.

Acetylcholine (ACh), choline (Ch), and acetyl-CoA are extracted into 15% formic acid (1N)-85% acetone (Toru and Aprison, J. Neurochem. 13, 1533, 1966). Any remaining acetyl-CoA is removed by resuspending the tissue in 5% trichloroacetic acid. The two extraction solvents are pooled, lipids removed by a heptane-chloroform wash followed by an ether wash. In the acetyl-CoA assay, endogenous ACh and Ch are removed by extraction into sodium tetraphenylboron-butenenitrile. The acetyl-CoA in the aqueous phase is then converted to labelled ACh in the presence of (^{14}C-methyl)-choline using choline acetyltransferase. After the remaining labelled precursor is converted to choline phosphate by the enzyme choline kinase, the (^{14}C)-ACh formed from acetyl-CoA is extracted into sodium tetraphenylboron-butenenitrile. An aliquot of the organic phase is counted in a scintillation counter. Acetyl-CoA levels in rat whole brain when killed by the near-freezing method (Takahashi and Aprison, J. Neurochem. 11, 887, 1964) were found to be 5.50 ± 0.2 nmole/g. In the same tissue extract, the level of ACh was 20.6 ± 0.7 nmoles/g and Ch 58.2 ± 1.2 nmoles/g as assayed by a modification of the radio-enzymatic assay of Shea and Aprison (Analyt. Biochem. 56, 165, 1973). As noted previously, Ch values in brains of animals killed by our near-freezing method are usually twice the value found in rats killed by total-freezing. The sensitivity of each assay is sufficient to allow the determination of all three compounds in less than 10 mg of tissue. (Supported by Research Grant MH-03225-15,16 and Postdoctoral Training Grant MH 10695, both NIMH).

559 A SCREENING TECHNIQUE FOR TRANSMITTERS IN THE CENTRAL NERVOUS SYSTEM. William R. Woodward* and Sivert Lindstrom* (SPON: E.A.Kravitz). Dept. Neurobiol., Harvard Medical School, Boston, Mass. 02115.

Small amounts of transmitter compounds are transported from cell bodies to synaptic terminals, probably associated with synaptic vesicles. In Aplysia californica, Goldman and Schwartz (J. Physiol. 242: 61, 1974) recently demonstrated by their intracellular pressure injection technique that only the transmitter used by a neuron was transported toward the synaptic terminal. It therefore seemed possible that the specificity of transmitter synthesis and transport might prove useful in screening compounds in the CNS for putative transmitters. The feasibility of using small, localized injections of radioactive precursors of putative transmitters into cell body regions and identifying transmitter compounds in the axons were tested in motor neurons of the cat spinal cord. A 10-100 nl solution of high specific-activity tritiated transmitter precursor (e.g. choline,glutamate) was pressure injected into spinal motor nuclei using a recording electrode with a beveled tip. The ventral roots were removed at various times and divided into small segments. The radioactive compounds were identified by high voltage electrophoresis. Of several possible transmitter compounds examined, only acetylcholine moved centrifugally in motor axons. Acetylcholine was transported at a rate of approximately 1.2 mm/hour. The amount of acetylcholine in the ventral roots increased until 3 days after injection. The transport of acetylcholine was blocked by a colchicine cuff and by ligation of the ventral root. The longitudinal spread of radioactivity from the injection site within the spinal cord was examined by dividing the rootlets rostral and caudal to the injection site into 1 mm bundles and determining the acetylcholine distribution. The spread at half peak was only about 1 mm to either side. We are currently using this technique to screen for transmitters in CNS pathways. (Supported by NIH).

560 SOURCES OF ERROR IN NEUROTRANSMITTER ANALYSIS. Thomas K. Tomosky*, Ian Jardine* and Ernest Bueding*. (SPON: D. H. Rifenberick). Johns Hopkins Univ., Baltimore, Md. 21205

In several invertebrates (Fasciola hepatica, Aplysia californica, Tritonia diomedia and Hirudo medicinalis) a substance is present yielding fluorescence characteristics similar to those of 5-hydroxytryptamine (5-HT) when treated with ninhydrin (Vanable, 1963; Snyder et al, 1965). This substance was reported not to be identical with 5-HT (Andreini et al, 1970; McCaman et al, 1973), although the latter had potent pharmacological effects on Fasciola (Mansour and Stone, 1970). Confirming the findings of Andreini et al (1970) for Fasciola, we also noted that this material is detectable in Spirometra mansonoides and in mouse brain. Using Amberlite CG-50 columns followed by thin layer chromatography, and an amino-acid analyzer, we have identified this substance as lysine. Although 5-HT is a potent stimulator and acetylcholine is a potent inhibitor of the motor activity of Spirometra, lysine had no effect. The presence of 5-HT and acetylcholinesterase activity was demonstrable. The motor activity of Spirometra is also strongly stimulated by dopamine and epinephrine, and these responses are blocked by spiroperidol and propranalol. Using the analytic fluorescence trihydroxyindole technique of Laverty and Taylor (1968) for catecholamines, we noted the apparent presence of dopamine and epinephrine in Spirometra extracts. However, subsequent mass spectrometric analysis revealed neither of these compounds to be present. While tyrosine could be responsible for the apparent presence of dopamine in Spirometra, the identity of the epinephrine - like substance is unknown. Accordingly, lysine, tyrosine, and possibly other substances can be sources of error in studies of the occurrence and role of biogenic amines in invertebrates.

561 GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC ANALYSIS OF BIOGENIC AMINES IN IDENTIFIED NEURONS, GANGLIA AND PIGMENTED TISSUE OF THE MEDICINAL LEECH. David J. McAdoo and Richard E. Coggeshall. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550.

The development of methods sensitive enough to assay neurotransmitters in individual neurons is a prerequisite to the study of the synaptic chemistry of simple nervous systems. We have verified the identity of 5-hydroxytryptamine (5-HT) in the collossal cells of Retzius in the segmental ganglia of the medicinal leech by means of gas chromatography-mass spectrometry. We found a concentration range of 1.3-4.1 pmol/Retzius cell body in individual analyses performed on single, dissected Retzius cell bodies. This value is in agreement with values of about 2.3 pmole of 5-HT/cell body derived by previous workers. The Retzius cell bodies contain about 30% of the 5-HT in each ganglion. An average of 25 pmole of 5-HT/mg tissue, a concentration about 500 times lower than that in the Retzius cell, was found in the fibrous, pigmented tissue surrounding the leech nervous system. We could not detect γ-aminobutyric acid, octopamine, dopamine, or norepinephrine in the Retzius cells, in the pigmented tissue, or, with the possible exception of dopamine ($\leq$ 0.4 pmol/ganglion), in whole ganglia. Furthermore, we could not detect 5-HT in pooled samples of 100 non-chromaffin control neurons. Glutamic acid, glycine and putrescine are also present in high concentrations in the leech nervous system. (Supported by NIH Grants NS 11255 and NS 10161.)

562 CATECHOLAMINE-CONTAINING DENDRITES IN MACAQUE BRAIN. John G. Parnavelas* and John R. Sladek, Jr. (SPON: V. Laties). Dept. Anat., Univ. Coll. London, London, England, and Dept. Anat., Univ. Rochester Sch. Med. & Dent., Rochester, N.Y. 14642.

Falck-Hillarp histofluorescence and rapid Golgi impregnation techniques were used to examine catecholamine-containing perikarya in rhesus monkey brain stem. Individual neurons within the locus coeruleus, subcoeruleus, and an area dorsal to the locus coeruleus (i.e., M_6, M_5, and M_4 respectively - Garver & Sladek, 1975, J. Comp. Neurol. 159:289-304) often possessed catecholamine fluorescence within many processes, some of which could be traced to varicose structures. Rapid Golgi impregnation of this area in additional macaques revealed a similar morphology to those neurons of the coeruleal region which contained fluorescent processes. These processes were positively identified as dendrites with the rapid Golgi technique. Catecholamine-containing dendrites also were seen within the zona compacta and pars lateralis of the substantia nigra, and in and around the lateral reticular nucleus.

Microspectrofluorometric analysis of fluorescent dendrites revealed excitation (410 nm) and emission (475-480 nm) peaks characteristic of catecholamines.

The presence of catecholamines within dendrites and dendritic varicosities suggests that neuronal processes other than axons may assume a pre-synaptic role in neurotransmission. (Supported by USPHS Program Project Grant NS 11642.)

563 QUANTIFICATION OF CATECHOLAMINE-CONTAINING CELL GROUPS IN THE BRAINSTEM OF THE RHESUS MONKEY. Bang-Hsiung Hwang*, Tanemichi Chiba, Asa C. Black, Jr. and Terence H. Williams. Dept. Anatomy, University of Iowa, Iowa City, Iowa 52242.

A quantitative analysis of the catecholamine-containing cells in the brainstem has not been reported, except for the work of Anden, et al. (1966) (Acta physiol. scand., 67:306, 1966) on dopaminergic neurons in the rat. We have studied the size and distribution of the catecholamine-containing cell groups in the brainstem of the Rhesus monkey (Macaca mulatta), using a modification of the glyoxylic acid method of Lindvall and Björklund (1974). The hemisected brainstem was divided into 20 micron sections, and the number of catecholamine-containing cells with nuclei was determined for every third section. The catecholamine-containing cell groups of the brainstem were divided into 9 groups. The mean number of cells with nuclei $\pm$ the standard error of the mean for each group is given in the following table:

Cell Group	Number Cells	Cell Group	Number Cells
C1	1866 $\pm$ 416	C6	7316 $\pm$ 930
C2	209 $\pm$ 57	C7	906 $\pm$ 168
C4	749 $\pm$ 113	C8 + C9 + C10	65232 $\pm$ 3210
C5	999 $\pm$ 301		

In the present study the number of mesencephalic dopaminergic neurons (C8, C9, and C10) was six times more than the other six pontine and medullary catecholamine-containing neurons. These data are expected to provide useful information for further physiological and morphological experiments on the catecholamine-containing systems of the central nervous system of the Rhesus monkey.

564 DEMONSTRATION OF CONJUGATED DOPAC IN RAT BRAIN. Mary A. Elchisak*, Robert H. Roth, and James W. Maas. Yale Univ. Sch. of Med., New Haven, Ct. 06510.

Our recent synthesis of ^{14}C-3,4-dihydroxyphenylacetic acid (DOPAC)-conjugate has enabled us to quantitatively determine the occurrence of conjugated DOPAC in animal tissues. Free DOPAC was extracted into butyl acetate from acidified rat brain homogenate supernatants; the conjugated DOPAC remaining in the acid phase was then hydrolyzed at 100°C and the free DOPAC thus released was separately extracted into butyl acetate. Both naturally-occurring free DOPAC and the DOPAC freed by hydrolysis were then estimated fluorometrically. Levels of free DOPAC (μg/g±SEM) were 0.96±0.05 in rat corpus striatum, 0.86±0.10 in tuberculum olfactorium, and 0.05±0.01 in frontal cortex. Conjugated DOPAC levels (μg of freed DOPAC, corrected for percent hydrolysis, per g of tissue) were 0.46±0.02, 1.04±0.07, and 0.06±0.01 in the same three brain areas, respectively. The levels of conjugated DOPAC were affected similarly to the levels of free DOPAC by most psychoactive drugs studied thus far. However, in all three rat brain areas, probenecid treatment resulted in an accumulation of conjugated DOPAC while having little or no effect on the levels of free DOPAC. This effect was most marked in the frontal cortex. Since probenecid inhibits the removal of many monoamine metabolites from rat brain, it appears that DOPAC may first be conjugated and then removed from the rat brain by a probenecid-sensitive transport mechanism. Since the relative concentrations of free and conjugated DOPAC are area-specific and since the magnitude of the response to probenecid also varies with the brain area, studies concerned with alterations in dopamine metabolism in response to pharmacological manipulations which include measurement of both free and conjugated DOPAC should be of value in the assessment of the dopaminergic theory of schizophrenia. (Supported by U.S.P.H.S. Grant MH 07144 and NIMH Grant MH 24607).

565 SIMULTANEOUS QUANTITATION OF GLYCINE AND γ-AMINOBUTYRIC ACID IN NEURAL TISSUE USING CHEMICAL IONIZATION MASS FRAGMENTOGRAPHY. Frederick Petty, John G. Wood, Hugh N. Tucker*, Samuel V. Molinary, Joseph D. Wander, and Norman Flynn*, Stout Neuroscience Lab.and Depts. of Anat. and Biochem., Univ. Tenn. Cent. Health Sci., Memphis, 38163, USA.

A sensitive and specific procedure has been developed for the simultaneous quantitative analysis of the putative amino acid neurotransmitters glycine (GLY) and γ-aminobutyric acid (GABA) by chemical ionization (CI) mass fragmentography (MF) using deuterated analogues as interal standards and methane (Me) as carrier/reagent gas. *n*-Butyl *N*-trifluoroacetyl derivatives of the amino acids are prepared from deproteinized supernatant of tissue homogenates. The CI spectra of these derivatives exhibit characteristic "pseudomolecular" (M+1-56,GLY) and m/e 182 (M+1-74, GABA), which account for 60% of the total ion current and are thus eminently suitable for MF. Gas chromatography (GC) is accomplished with a 45cm (2mm ID) .25% EGA column, temperature-programmed from 70-100° at 6°/min, with Me flow 18cm^3/min, resulting in elution times for GLY and GABA of 2.13 and 6.41 min, respectively (Fig 1). Analyses were performed with a Finnigan 1015D gas chromatograph/mass spectrometer, using the Finnigan 6000 Interactive Data System MF program. Calibration curves obtained with mixtures of pure GLY and GABA immediately before and after the tissue assays were linear over a hundred-fold dilution range (r=.999).

Using this technique, concentrations of GLY and GABA in rat spinal cord were found to be:

	Ventral	Dorsal (nmole/mg wet tissue ±SEM, N=3)
GLY	8.94 ± 0.79	8.29 ± 0.30
GABA	0.475 ± 0.01	1.10 ± 0.13

These values agree with those obtained using considerably more tedious procedures. Peak ratios (protium/deuterium) are replicable within 5%.

Sensitivity of the procedure permits its extension to the femtomole range with unsurpassable specificity. The derivatives employed are relatively stable, and the entire MF analysis, including calculation of peak areas, requires less than 6 min. Use of CI provides relatively high-molecular-weight ions for MF and minimizes interference from column bleed and "biological background", as ions produced are few and characteristic, permitting use of short GC columns.

This technique may be easily extended to the determination of asparatate and glutamate (Fig. 2) and is of sufficient sensitivity to permit simultaneous quantitation of all four compounds in discrete regions of the CNS.

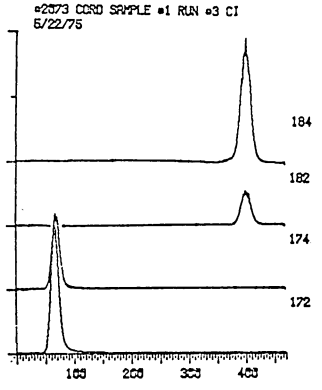

FIG. 1 MASS FRAGMENTOGRAM OF CORD EXTRACT

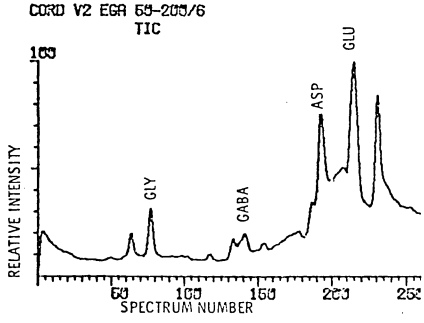

FIG. 2 TOTAL ION CURRENT (GAS CHROMATOGRAM) OF VENTRAL CORD EXTRACT

566 NEURONAL VS. GLIAL ORIGIN OF RELEASABLE POOL OF ^{3}H-GAMMA AMINOBUTYRIC ACID IN RAT CORTICAL SLICES. John P. Hammerstad. Dept. of Neurology U. of Oregon Health Sciences Center, Portland, Oregon 97201.

Depolarization of neural membranes in brain slices by electrical field stimulation and high K^+ solutions has been used to study the release of neurotransmitter candidates, including gamma-aminobutyric acid (GABA). Under the assumption that it mixes with endogenous pools, release of exogenously administered radiolabeled GABA is taken as evidence of a neurotransmitter function for GABA. Because of evidence for metabolic compartmentation and glial uptake of radiolabeled GABA a glial pool and possibly two neuronal pools of GABA have been postulated (Balazs et. al., Biochem. J. 116: 445, 1970). The source of the releasable pool of radiolabeled GABA in cortical slices is uncertain. Marked reduction of electrically stimulated release of ^{3}H-GABA after exchange diffusion with structurally related amino acids might also imply the releasable pool is bound to cell membranes rather than in an intracellular neuronal or glial compartment (Hammerstad and Cutler, Eur. J. Pharmacol. 20: 118, 1972).

Recently, β -alanine has been found to inhibit uptake and stimulate the efflux of ^{3}H-GABA in glial cells of rat sensory ganglia, whereas 2,4 diaminobutyric acid (DABA) had little effect. (Schon and Kelly, Brain Res. 66: 289, 1974, Minchin, J. Neurochem. 24: 571, 1975). This is the opposite of the effect of these two amino acids on the uptake and efflux of ^{3}H-GABA in rat cortical slices (Iversen and Johnston, J. Neurochem. 18: 1939, 1971., Crnic et. al. J. Neurochem. 20: 203, 1973). Because the affinity of structurally related amino acids for the GABA uptake system parallels their ability to stimulate carrier mediated efflux of ^{3}H-GABA from cortical slices and sensory ganglia, and because of the differential effect of DABA and β -alanine on the uptake and efflux of ^{3}H-GABA in cortical slices and sensory ganglia, we have examined the effect of these two amino acids on the K^+ stimulated release of ^{3}H-GABA in order to define further the nature of the releasable pool.

Superfusion of rat cortical slices preloaded with ^{3}H-GABA produced a slow exponential rate of efflux (0.4 $\pm$ .1%/min.) after an initial faster washout phase. Addition of 5 mM. DABA or 5 mM. β -alanine to the superfusion medium stimulated a more rapid efflux of ^{3}H-GABA (DABA = 3.6 $\pm$ .5%/min., β -alanine = 2.3 $\pm$ .5%/min.) lasting 10 minutes followed by a slower rate that was similar for both amino acids (DABA = 1.9 $\pm$ .2%/min. β -alanine = 1.1 $\pm$ .2%/min.). Exposure of the preloaded slices for ten minutes to a 40 mM. K^+ medium produced a marked increase in the release of ^{3}H-GABA (25 $\pm$ 6% of the radioactivity present in the slice above background efflux at the start of the K^+ stimulation). Superfusion with 5 mM. DABA or 5 mM. β -alanine produced no change in the release of ^{3}H-GABA (DABA = 22 $\pm$ 2%, β -alanine = 26 $\pm$ 4%) when the slices were exposed to 40 mM. K^+ during the rapid phase of stimulated efflux (?exchange with external membrane bound ^{3}H-GABA). K^+ stimulation during the second, slower phase of efflux stimulated by 5 mM. DABA or β -alanine (?exchange with intracellular ^{3}H-GABA) resulted in a marked difference in the release of ^{3}H-GABA (DABA = 2 $\pm$ .5%, β -alanine = 16 $\pm$ 2%. $P < .001$). These results suggest that carrier mediated efflux produced by structurally related amino acids may be used to differentiate two pools of ^{3}H-GABA in cortical slices. The releasable pool is influenced by exchange with DABA and to a much lesser degree by β -alanine suggesting that the major component of the releasable pool is neuronal rather than glial in origin.

567 A COMPARATIVE DETERMINATION OF GABA IN APLYSIA GANGLIA BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND AN ENZYMATIC METHOD. B. Haber, C.B. Beyer,* R.P. Collins* and D.J. McAdoo. The Marine Biomedical Institute, Univ. of Texas Medical Branch, Galveston, Texas 77550.

Possible discrepancies have been found in the measurement by different methods of glutamic acid decarboxylase (GAD) activity, and possibly in the concentration of its product, γ-aminobutyric acid (GABA). To help clarify this situation, GABA levels were determined in Aplysia ganglia by gas chromatography-mass spectrometry (GC-MS) and by an enzymatic-fluorometric method. Trimethylsilyl derivatives were employed in the GC-MS procedure. This permitted the quantitations of glycine, glutamic acid and aspartic acid, as well as that of GABA, by GC-MS. Individual ganglia of A. californica were excised, irradiated in a microwave oven, homogenized in 75% ethanol and centrifuged to remove insoluble matter. Aliquots of the supernatant were analyzed by the two procedures. GABA concentrations derived from GC-MS measurements agreed well with those from the fluorometric method when deuterated GABA was used as a carrier and internal standard in the GC-MS procedure, but were substantially lower otherwise. GABA concentrations ranged from 15 μg/g wet weight in the left pedal ganglion to 30 μg/g wet weight in the abdominal ganglion. Average concentrations for the other amino acids determined were 190 μg/g wet weight glycine, 260 μg/g wet weight aspartate, and 280 μg/g wet weight glutamate. Thus GC-MS gives results that are in good agreement with the enzymatic determinations of GABA, and can be used to simultaneously quantitate a number of other possible neurotransmitter substances in invertebrate nervous systems. (Supported by NIH Grant NS 11255, Welch Foundation Grant H-504 and M.D.A.A. Grant.)

568 INTERACTION OF GLUTAMATE AND NEUROTROPIC COMPOUNDS WITH AN EXCITABLE MEMBRANE. J. E. Heavner and R. H. Haschke. Anesthesiology and Biochemistry University of Washington, Seattle, Washington, 98195.

Substantial evidence indicates that glutamate is an excitatory neurotransmitter in the mammalian CNS and at invertebrate neuromuscular junctions. Recent experiments (Comp. Gen. Pharmacol. 5:91-99, 1974) have also shown that glutamate will induce contraction of the non-innervated crayfish vas deferens. We have used the crayfish vas deferens preparation to elucidate the interaction of glutamate and various neuroactive compounds. l-Glutamate. causes a dose-dependent contraction of the muscle with half-maximal response occuring at 2×10^{-4}M. Similar concentrations of d-glutamate had no agonistic or antagonistic action. Glutamate diethyl and dimethyl esters (1.2×10^{-3}M) each reduced maximum contraction to approximately 40% of control, but produced no response in the absence of glutamate. Strychnine (10^{-4}M) and picrotoxin (1.7×10^{-5}M) which are antagonists of glycine and γ-aminobutyric acid, respectively; as well as d-amphetamine, impramine and cocaine (each 1×10^{-3}M) were without effect. The CNS depressants halothane (0.25%) and pentobarbital (1×10^{-4}M) inhibited the glutamate induced contraction approximately 50%. The action of these agents was localized at the membrane and not the contractile elements of the muscle since they did not affect potassium induced contraction. These results indicate that the crayfish vas deferens provides a convenient preparation to study the interaction of glutamate and neurotropic compounds with an excitable membrane.

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569 GLYCINE LEVELS IN THE NORMAL AND THE DEGENERATED HUMAN SPINAL CORD. D.H. Boehme, M.D.*, Neville Marks, Ph.D. and Michael W. Fordice, Ph.D.*, VA Hospital, East Orange, N.J. 07019

The amino acid, glycine, is found in spinal cord and brain of man in concentrations comparable to those of higher mammals (cats) and shows a similar anatomical distribution with maximum levels found in the lumbar grey (3.3 ± 0.9 mol/gm wet weight) and lowest values encountered in the thoracic dorsal white and grey (2.44 ± 0.87 mol/gm wet weight). Anatomical and physiological evidence favors the assumption that glycine may play a role as an inhibitor of a motoneuron in strychnine sensitive synapses of feline spinal cord as shown by Aprison (Comp. Biochem. Physiol. 1969, 28, 1345-1355). It has been postulated that transection of the spinal cord may lead to an increased glycine content only if this transmitter would be produced by supraspinal structures. In cats, such a rise was not observed. (Rizzoli, Brain Res. 1968, 11, 11-18).

We examined 18 human cords who had undergone bilateral or unilateral degeneration following vascular accidents, metastasising tumor to the brain, Amyotrophic Lateral Sclerosis or transverse myelitis and were removed within 6 hours post mortem. These were compared to the normal side of the same cord or to wholly normal cords. An increase in glycine was not observed (degenerated lumbar lateral columns 3.07 ± 0.85, normal lumbar lateral column 3.5 ± 1.17 mol/gm wet weight), lending credence to the fact that glycine may be locally (segmentally) produced. Glycine concentration at cervical, thoracic and lumbar levels of degenerated cords did not differ significantly from those of healthy cords. The results suggest that, in man, glycine is supplied at the segmental rather than at the supraspinal level. (Supported by the VA, Washington, D.C.)

570 BIOCHEMICAL AND MORPHOLOGICAL ANALYSIS OF SUBCELLULAR FRACTIONS FROM RABBIT RETINA. Dianna A. Redburn. Department of Neurostructure and Function, University of Texas Medical School, Houston, Texas 77025

Subcellular fractions were prepared from isolated rabbit retina using techniques previously developed for whole brain. The P_1 (debris pellet) fraction contained outer rod segments, nuclei, mitochondria, and aggregations of cytoplasm. Of special interest, was the presence of large synaptic endings 2-5μ in diameter, having a general appearance similar to the synaptic endings of rod cells. In several cases synaptic invaginations were seen which contain post-synaptic processes, possibly from horizontal and bipolar cells. The general content of the P_2 (crude mitochondrial pellet) fractions was similar to P_2 fractions from whole brain and contained many small "conventional" synaptosomes, free mitochondria, empty vesicular membrane sacs, but few large synaptosomes. These results suggest a partial separation of two morphologically distinct synaptosomal populations can be achieved, i.e., large receptor cell synaptosomes in P_1 and smaller, "conventional" synaptosomes presumably from amacrine, bipolar, and horizontal cells, in P_2. ^{14}C-GABA was bound by homogenate, P_1, and P_2 fractions, however, the P_2 fraction bound 3 times the amount bound by other fractions. Ca-dependent depolarization-stimulated release of bound GABA was also more pronounced in the P_2 pellet. The Ca-dependent release was blocked by Mn and thus mimics the release characteristics described for in situ, electrophysiological measurements of transmitter release. These data support the proposed role of GABA as a neurotransmitter in retina.

571 GLUTAMINE TRANSAMINASE AND ω-AMIDASE ACTIVITIES IN BRAIN. A.H. Lockwood* and T.E. Duffy. Dept. Neurol., Cornell Univ. Med. Col., N.Y., N.Y. 10021.

Homogenates of brain, liver and kidney carry out the transamination-deamidation of glutamine to α-ketoglutarate and ammonia, involving α-ketoglutaramate (α-KGM) as an intermediate. Detailed studies of the enzymes that catalyze this conversion have been confined to liver and kidney. We measured the activities (μmol/h/g tissue ± SE) of the isozymes of glutamine transaminase (glutamine-glyoxylate and glutamine-phenylpyruvate transaminases, GGT & GPPT; Cooper & Meister, 1974) and of ω-amidase (ω-Am) in adult brain of four species, and GGT and GPPT in developing rat brain. Transamination was assayed by the appearance of ^{14}C-α-KGM from L-^{14}C-glutamine (glycylglycine pH 8.4, 20mM substrates, 37°), and deamidation by the formation of α-ketoglutarate (Tris-HCl pH 8.4, 20mM α-KGM, 37°) coupled in a two-step reaction to NADH-dependent glutamic dehydrogenase.

	Mouse brain	Rabbit brain	Guinea Pig brain	Rat brain	Rat liver	Rat kidney	Rat brain newborn
GGT	<0.5	1.78 ±0.15	5.24 ±0.11	1.84 ±0.37	39.3 ±7.4	19.8 ±3.0	1.26 ±0.06
GPPT	<0.5	0.70 ±0.02	2.71 ±0.08	1.61 ±0.49	10.1 ±2.2	29.3 ±4.7	0.75 ±0.03
ω-Am	20.2 ±0.8	53.3 ±4.2	38 ±1	19 ±2	1310 ±94	726 ±48	

The activity of GGT, the predominant isozyme in brain, always exceeded GPPT; the activities of both isozymes were lower in newborn brain but reached adult levels by age 30 days. Portacaval shunting in adult rats for 8 weeks produced chronic hyperammonemia and tripled brain glutamine, but had no effect on the activity of these enzymes and ω-amidase in brain, liver or kidney. The uniformly high ratio of ω-amidase:GGT+GPPT activity probably explains the low level of α-KGM in normal tissues (Duffy et al., 1974). (Supported by USPHS Grants NS02149 and AM16739.)

572 EXCITOTOXIC AMINO ACIDS. John W. Olney, Lawrence G. Sharpe and Taisija de Gubareff.* Dept. Psychiatry, Sch. Med., Wash. Univ., St. Louis, Mo. 63110

Glutamate (GLU) and certain of its structural analogues, including both straight-chain and heterocyclic compounds, excite central neurons when iontophoresed onto their dendritic and somal surfaces. Some analogues are equal to GLU in excitatory potency whereas others such as DL-homocysteic acid (DLH), N-methyl-DL-aspartic acid (NMA) and kainic acid (KA) are substantially more powerful than GLU.

Various researchers have demonstrated that neurons in the inner retina and arcuate nucleus of the hypothalamus undergo toxic degeneration within hours following the subcutaneous administration of GLU to experimental animals. Having found that the same neurotoxic syndrome can be produced by subcutaneous administration of the various excitatory analogues of GLU, Olney et al. (Exp. Br. Res. 14, 61, 1971) postulated that the excitatory and toxic properties of these amino acids are linked by a common mechanism acting at a common receptor locus on dendritic and somal membranes. Consistent with this hypothesis, the order of potencies was noted to be the same (KA > NMA > DLH > GLU) for the excitatory and toxic activities of these compounds and their toxic action on retinal and hypothalamic neurons, as analyzed by electron microscopy, was characterized by early changes in dendritic and somal constituents.

Van Harreveld and Fifkova (Exp. Molec. Path., 15, 61, 1971) induced Glu-type lesions in the rat cerebral cortex by microelectrophoretic application of GLU at higher current or longer duration than is required to reversibly depolarize nerve membranes. The present experiments were undertaken to explore the effects of the more potent excitatory analogues of GLU (DLH, NMA, KA) when introduced by microinjection directly into brain.

When administered into the diencephalon in 0.5 µl volume as an isotonic solution (total dose = 77 nmoles), DLH induced an acute reaction which destroyed neurons in an area of > 1 mm diameter. Isotonic NaCl produced no such effect. NMA and KA were substantially more powerful in that diencephalic lesions roughly comparable in size and appearance to the DLH lesion resulted from only 15-30 nmoles of NMA or 3.5-7 nmoles of KA. Ultrastructural analysis revealed 1) a primary effect on dendritic and somal (post-synaptic) constituents, progressing rapidly to neuronal necrosis. 2) Little or no primary effect on axonal (pre-synaptic) elements. 3) A total sparing of select neurons within the lesioned area, the basis for which warrants further study.

It is tentatively concluded that the group of compounds known as neuroexcitatory amino acids have structural characteristics which allow them to act on the receptive surfaces (at synaptic loci?) of central neurons to depolarize these cells reversibly if stimulation is intermittent or, to kill them if stimulation is continuous. In the latter case, a sustained increase in plasma membrane permeability is postulated as the pathogenic mechanism leading to acute neuronal necrosis. These observations have both theoretical and practical implications as they suggest that central neurons can be excited to death when stimulated extracellularly by a species of molecule found abundantly in the intracellular compartment of brain. It is also suggested that these potent analogues of GLU might serve as useful chemical lesioning agents, particularly where it is desired that axons passing to or through an area be spared while neuronal perikaya in the area are destroyed. The term "excitotoxic" amino acid is proposed as an appropriate generic for the structural analogues of GLU which mimick both the neuroexcitatory and neurotoxic properties of this putative CNS transmitter.

573 SEROTONERGIC NEURONS IN THE MAMMALIAN PERIPHERAL NERVOUS SYSTEM. M. D. Gershon, C.F. Dreyfus*, V.M. Pickel, T.H. Joh, and D.J. Reis. Depts. of Anatomy and Neurology, Cornell University Medical College, New York, N.Y.

In mammals, serotonergic (5-HT) neurons have never been demonstrated outside of the central nervous system. However, on the basis of indirect evidence we have proposed that these neurons exist in the myenteric plexus. In the present study we have sought to identify 5-HT neurons in gut directly by the demonstration of neurons which contain the enzyme tryptophan hydroxylase (TrH) and store 5-HT. Three preparations were studied: (a) the longitudinal muscle and adherent myenteric plexus dissected from guinea pig intestine; (b) organotypic tissue cultures of smooth muscle and myenteric plexus from 17-18 day fetal mice (only intrinsic neurons survive in these cultures); and (c) rat intestine. Incubation of preparations (a) and (b) in-vitro with ^{3}H-tryptophan led to accumulation of ^{3}H-5-HT. In both, fluorescence with the spectral characteristics of the 5-HT formaldehyde fluorophore was seen in intrinsic neurons providing histochemical evidence of synthesis in these neurons of 5-HT from tryptophan. TrH was visualized immunohistochemically in neurons after incubation of gut with a specific antibody to TrH purified from the raphe nuclei of rat brain and localized by the peroxidase-anti-peroxidase method. TrH was exclusively cytoplasmic. The small neuronal cell bodies (20-30 um diameter) corresponded in size, shape, and location in the myenteric plexus to those showing 5-HT fluorescence after tryptophan loading. In the rat, TrH was also found in neurons of the submucosal plexus. We conclude that serotonergic neurons are present in the mammalian peripheral nervous system within the myenteric plexus.

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574 SEROTONERGIC AXONS IN THE DEVELOPING MYENTERIC PLEXUS: LOCALIZATION BY ELECTRON MICROSCOPIC RADIOAUTOGRAPHY. T. Rothman*, L.L. Ross, and M.D. Gershon. Dept. of Anatomy, Cornell Med. Coll., New York, N.Y.

Serotonin (5-HT) may be a neurotransmitter in the myenteric plexus. However, the study of the presumed 5-HT innervation has been complicated by the presence of adrenergic axons in the tissue and would be facilitated if the myenteric plexus could be studied when it did not contain adrenergic axons. It has previously been shown that the uptake of 3H-5HT develops during the ontogeny of the ileum of the rabbit prior to the uptake of norepinephrine (NE). The elements responsible for the uptake of the two amines have not been previously identified. 3 cm lengths of ileum, dissected from fetal rabbits of 16 to 28 days gestation, were incubated for 30 minutes with either 3H-5HT or 3H-NE. On the 16th day of gestation radiographic label appears over axons in the myenteric plexus in those segments which had been incubated with 3H-5HT. Statistical analysis of EM autoradiographs indicates that on the 20th day of gestation label due to 3H-5HT is associated with axons, most of which contain large dense core vesicles (DCV). An unusual expanded axonal process containing very large DCV and flattened cisternae is particularly heavily labeled. Silver grains do not appear over axons of tissues incubated with 3H-NE until the 24th day of gestation. The expanded axonal process is never labeled with 3H-NE at any stage of development. These data provide further evidence that a distinct and separate 5-HT innervation exists in the myenteric plexus which develops during ontogeny prior to the innervation of the gut by adrenergic axons.

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575 SEROTONIN BINDING PROTEIN IN THE PERIPHERAL NERVOUS SYSTEM (MYENTERIC PLEXUS). G.M. Jonakait*, H. Tamir, M. Rapport, and M.D. Gershon. Dept. of Anatomy, Cornell Med. Coll. and Div. of Neuroscience, N.Y. State Psychiatric Inst. and Dept. of Biochem., Columbia Univ., New York, N.Y. Sponsored by B. Grafstein.

Serotonin appears to be a neurotransmitter in the mammalian myenteric plexus where intrinsic neurons have recently been shown to have both tryptophan hydroxylase and a specific uptake mechanism for serotonin. We now wish to report the presence in myenteric plexus of both rabbit and guinea pig of a soluble protein with a high affinity for serotonin, similar to that previously found in rat brain by Tamir et al. Partial purification by $(NH_4)_2SO_4$ fractionation increased the ratio of specific (measured at 10^{-7}M) to nonspecific binding (at 10^{-4}M) to 9:1. Two dissociation constants were obtained by equilibrium dialysis (7.5×10^{-10}M and 1×10^{-7}M). The specific binding is saturable and enhanced by Fe^{++} (10^{-4}M). Indole derivatives such as 5,6- and 5,7-dihydroxytryptamine, and 6-hydroxytryptamine inhibited binding by 50% at 10^{-7}M. Norepinephrine was a poor inhibitor (50% at 10^{-6}M). Most of the complex (serotonin-protein-Fe^{++}) had a very high M.W. and did not penetrate a 6.5% acrylamide gel. A small, faster migrating band could be detected. 48 hours after injection of 5,6-dihydroxytryptamine (50mg/kg, x2, s.c.) into guinea pigs the specific binding capacity of the myenteric plexus increased 11-fold. 6-Hydroxydopamine administration (50mg/kg, x3, s.c.) had no effect. The time of appearance of the binding protein in fetal rabbit intestine correlated closely with the development of the 5-HT uptake mechanism (no specific binding at 15 days of gestation; 1/3 of adult binding capacity at 21 days of gestation). This precedes development of adrenergic innervation. It is concluded that the serotonin binding protein is associated with serotonergic neurons in peripheral as well as central nervous system. Supported by grants GM00895, NS07436 (NIH) and Ben. Found. Scot. Rite Freemas.,N. Jur. U.S.A.

576 STRUCTURE AND LOCALIZATION OF DENSE CORE VESICLES IN THE STOMATOGASTRIC GANGLION OF THE SPINY LOBSTER. Brenda Friend* and Edith Maynard. Biol. Dept., Univ. of Oregon, Eugene, Oregon 97403.

As part of a collaborative effort studying transmitter chemistry, monoamine fluorescence histochemistry and fine structure in the crustacean stomatogastric ganglion (SGG), we have identified two morphologically distinct types of electron-dense vesicles in neural elements and have mapped their distribution at 28 representative points in the SGG and adjacent 1mm lengths of the principle input nerve, the stomatogastric nerve (SGN) and the largest output nerve, the dorsal ventricular nerve (DVN). In material prepared for electron microscopy by a modified Woods' technique, the smaller of the two types of dense core vesicles has an average diameter of 85nm and a discrete core which often is separated from the bounding membrane. The larger vesicles' average diameter is 120nm and the dense contents lie close against the vesicle membrane. The smaller type of vesicle occurs in certain nerve fibers in the SGN and in numerous synaptic-type processes within the ganglionic neuropil. The larger vesicle type is present in some other fibers of the SGN, in fibers lying in the ganglion's peripheral cell body zone, in the DVN, and in nerve elements running in the connective tissue sheaths of the SGN, the ganglion, and the DVN. Semi-serial sections show these larger vesicles in fiber bundles that interconnect sheath and neural regions of both SGG and DVN. Release sites for the larger vesicles have not been observed; their presence in large numbers in the sheaths suggests a neurohemal function. The distribution of the smaller vesicles corresponds to the fluorescence observed with the Falck method (Kushner and Maynard) and thus at least some of them must represent monoamines, possibly dopamine in view of the results from biochemical studies (Barker and Hooper). (Supported by USPHS Grants NS-09474 and NS-09614.)

577 MONOAMINE HISTOCHEMISTRY OF THE CRUSTACEAN STOMATOGASTRIC NERVOUS SYSTEM. P. D. Kushner* and E. Maynard. (SPON: Eve Marder). Dept. of Biology, Univ. of Oregon, Eugene, Oregon 97403.

Formaldehyde-induced fluorescence studies were performed on the nervous system innervating the foregut of the spiny lobster, Panulirus interruptus. In whole mount preparations fluorescent fibers occur in the superior oesophageal nerve (SON), in the stomatogastric nerve (SGN) and appear to enter the stomatogastric ganglion (SGG) and branch in the peripheral cell body zone. There are intensely fluorescent blebs throughout the neuropil of the SGG. To increase specific fluorescence tissues were incubated in .1mM dopamine, washed thoroughly, and then freeze-dried. This procedure has demonstrated 1 large and 2 small fluorescent somata in each commissural ganglion (CG). The pattern of fluorescence in the SGG and in the SGN corresponds to the distribution of the smaller of two types of dense core vesicle seen in the EM work of Friend and Maynard. The nerves and ganglia which show specific fluorescence synthesize dopamine from ^{3}H-tyrosine (Barker and Hooper). The large cell of the CG, plucked and analyzed separately, has also shown synthesis of dopamine from ^{3}H-tyrosine. There is no fluorescence in the dorsal ventricular nerve (DVN) or in the sheath of the SGG, regions containing the larger type of dense core vesicle (Friend and Maynard). Fluorescence has not been observed in somata of the SGG, suggesting that the somata of fluorescing processes in the SGG neuropil are extrinsic to the ganglion. These somata may be in the CG since neural elements in the CG synapse in the SGG (David Russell, per. comm.).

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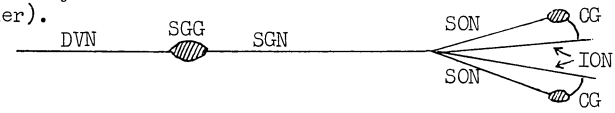

578 EFFECTS OF LSD ON DOPAMINE-INDUCED RESPONSES OF THE EXCITATORY AND INHIBITORY TYPES, OBSERVED IN APLYSIA GANGLION CELLS. Makoto Sato and Masashi Sawada*. Neuroscience Lab, Division of Neurosurgery, University of Oregon Health Sciences Center, Portland, Oregon 97201.

Dopamine-induced postsynaptic responses of both the excitatory and inhibitory types were recorded from identified neurons of Aplysia ganglion. Responses were evaluated by the change in membrane potential (ΔE) as well as the increase in membrane conductance (ΔG). The inhibitory response was significantly depressed by a 30 sec. exposure to 0.1 μM LSD-25 whereas the excitatory response was not affected by a 60 sec. exposure to 10 μM LSD. The time course of the above depression indicated that it takes place rather gradually over a period of 20 - 30 min. before reaching the maximum plateau. The blockade of the inhibitory response lasted more than 2 hours while continuously washing with normal Ringer. The recovery of the inhibitory response took 5 - 8 hours although this depended on the concentration of LSD-25. The dose-response curves showed a decrease in slope and a fall in the maximum responses as LSD-25 concentration increased. This fact indicated that the mode of LSD-25 interaction with the dopamine receptor was of the non-competitive type. Neither the resting potential nor the resting conductance was appreciably altered by exposure to 10 μM LSD-25.

BOL (2-Brom LSD) was also found to depress dopamine-induced hyperpolarization but the blocking potency was approximately one-tenth that of LSD-25. BOL did not show a distinct selective action between the excitatory and inhibitory responses.

We concluded that LSD-25 of dilute concentration selectively blocks inhibitory dopamine-responses of Aplysia ganglion cells and the blockade is due to non-competitive interaction with the inhibitory receptor.

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579 NEUROTRANSMITTER DEVELOPMENT IN AN INSECT SENSORY SYSTEM. Joshua R. Sanes*, David J. Prescott*, and John G. Hildebrand. Dept. of Neurobiology, Harvard Medical School, Boston, Mass. 02115.

Antennal flagella of the moth, Manduca sexta, contain $2x10^5$ primary sensory neurons and no other neural elements. These neurons develop synchronously from undifferentiated cells during the 18 days of metamorphosis from pupa to adult (Soc. Neurosci. 1974 Abstract no. 596). This system permits correlation of biochemical with morphological and physiological aspects of neural development. Mature antennae in vitro synthesize and accumulate [^{14}C]acetylcholine (ACh) from precursor [^{14}C]choline, whereas none of 8 other transmitter candidates was produced detectably from appropriate precursors. Antennae contain choline acetyltransferase (ChAc) and specific acetylcholinesterase (AChE). These results support the hypothesis that the sensory neurons are cholinergic, and we have studied the ontogeny of ACh, ChAc, and AChE in developing antennae. ChAc is first detectable about day 3 of metamorphosis, one day after the developing neurons send out axons, and peaks about day 13, or 5 days after axonal growth is complete. The increase in ChAc specific activity is at least 10^4-fold. The developmental profile of ACh closely parallels that of its biosynthetic enzyme, ChAc. AChE also begins to increase about day 3, but it peaks later than ChAc, about day 16. The major synaptic target of antennal neurons is the antennal lobe of the brain. ChAc, ACh, and AChE increases in the lobe roughly parallel those in the antenna. Morphological and neurochemical differentiation of the antennal lobe is attenuated by deafferentation (removal of developing antennae). Removal of the target (brain including antennal lobes) early in metamorphosis has retrograde effects on antennal differentiation. (Supported by USPHS Grant 11010, by a Research Fellowship of the A. P. Sloan Foundation, and by an Established Investigatorship of the American Heart Association to JGH.)

580 BEHAVIORAL EFFECTS OF INTRACEREBRAL GLUTAMIC ACID INJECTIONS. W. J. Freed* and E. K. Michaelis (SPON: R.N. Adams). Dept. of Human Development, Univ. of Kansas, Lawrence, 66045.

It has been postulated that glutamic acid (GA) release is involved in memory formation (Van Harreveld & Fifkova, Brain Res., 81, 455). There exists some support for this hypothesis in that GA antagonists interfere with learning (Van Harreveld & Fifkova, op.cit., Desi, et al., Acta Physiol. Acad. Sci. Hung., 32, 323). Other studies have demonstrated that peripherally administered GA improves or has no effect on learning (Wincze & Vogel, J. Genet. Psychol., 115, 97; Vogel, et al., Psychol. Bull., 65, 367), increases general activity (Wincze & Vogel, op.cit.), and suppresses operant behavior (Tadokoro, et al., Pharmacol. Biochem. Behav., 2, 619). This study was designed to investigate the effects of GA when injected intracerebrally.

Nineteen adult male albino rats bearing cannulae implanted in the right lateral ventricle served as subjects. The animals were food deprived and maintained at 80% of their ad lib weights. Immediately prior to each testing session each animal was injected with 10 ul of either saline (control, or C group) or 100 mM GA in saline (GA group). Bar pressing was investigated in LVE operant conditioning chambers for 30 min. per day, 3-4 days per week. Each chamber contained two bars and a food cup.

First, the operant level of bar pressing was measured and no significant differences were found. Next, both bars were programmed so that each bar press resulted in the delivery of one 45 mg. food pellet. For the first 5 sessions, additional pellets were delivered noncontingently on a fixed-time interval schedule. The number of bar presses per animal per session was recorded to determine when acquisition of the bar press response had occurred. Testing was terminated when each animal had earned 600 food pellets. Any significant deviation by the GA group from the control group learning curve was considered to be due to the effect of the drug. For the first session, and after the seventh session, the groups did not differ significantly. Acquisition of the response by the GA group was, however, retarded as evidenced by a significant decrease in the bar pressing of the GA group from the second through the seventh session. The median responses per session for the two groups are presented in the table below.

session	op. level	1	2	3	4	5	6	7	8	9	10	// 15
controls	13	3	81	163	185	174	222	209	213	213	213	-
GA	12	4	2	2	4	1	38	100	130	119	148	189

The GA group, when tested on a fixed-interval schedule, showed a suppression of bar pressing, but only for the first 10 min. of each session. This effect disappeared after six sessions. This 10 minute effect was apparently not related to metabolism of the drug, since similar effects were observed in other animals given freehand intraventricular injections of homocysteic acid, a GA agonist, 30 min. prior to testing. When the animals were then tested for extinction of the bar press response, and for general activity in a LVE photocell activity cage, no significant differences between the GA and C groups were found.

Intracerebrally injected glutamic acid interfered with learning of a bar press response for food and performance on a fixed-interval schedule. The possibility that this was due to nonspecific behavioral suppression was ruled out. (Supported by Univ. of Kansas General Research Support Grant 3568-5038, and NICHHD Grant HD-02528.)

581 EFFECTS OF AMINO ACID TRANSMITTERS ON SYNAPTOSOMAL Cl^- FLUXES. A.T. Tan*. (SPON: R. Chase). Dept. Research in Anaesthesia, McGill Univ., Montreal, Canada.

Synaptosomes isolated from guinea pig cortex were incubated at room temperature in Krebs-Ringer phosphate buffer, pH 7.4, containing 10 mM D-glucose and trace amounts of $Na^{36}Cl$ for 10 min. The suspension was then passed through a Sephadex G-50 column and eluted either with 0.32 M sucrose or Krebs-Ringer phosphate buffer. A ^{36}Cl peak which coincides with the synaptosomal protein peak appeared in the void volume. This ^{36}Cl peak was less than 1% of the amount of the second ^{36}Cl peak, representing the free Cl^- in the suspending medium. When the synaptosomes were ruptured by eluting with 20 mM phosphate buffer, only the second ^{36}Cl peak was observed. When γ-aminobutyric acid (GABA, final concentration 0.1 - 1.0 mM) was added to the synaptosome suspension, 1 minute before passing through the column, the chromatogram was unchanged. However, the synaptosomal ^{36}Cl to protein ratio was consistently greater than that of control (with 1 mM GABA, the mean increase was 45 $\pm$ 10%, n = 5). Glutamate and glycine (1 mM) were ineffective. The synaptosome ^{42}K content was not affected by the three amino acids. These results suggest that Cl^- is present in the osmotically sensitive compartment (presumably intrasynaptosomal) and that GABA increases the Cl^- permeability of cortical nerve endings.

Supported by the Medical Research Council of Canada.

582 THE ACTION OF PRESUMED BLOCKERS OF CHLORIDE TRANSPORT ON SYNAPTIC AND AMINO ACID RESPONSES IN THE FROG SPINAL CORD. R. A. Nicoll. Dept. of Physiol., State Univer. of New York at Buffalo 14226.

Recent evidence suggests that the hyperpolarizing action of the postsynaptic inhibitory transmitters (GABA and glycine) are due to a selective influx of chloride ions, while the depolarizing action of the presynaptic inhibitory transmitter (GABA) on dorsal root ganglion cells is due to a selective efflux of chloride ions. Implicit in the above proposals is the presence of an outwardly directed chloride pump in motoneurons (MNs) and an inwardly directed pump in primary afferents (PAs). To examine these possibilities the action of ammonium ions (NH_4^+), acetazolamide, and furosemide, agents which are thought to block the transport of chloride ions, has been examined in the isolated frog spinal cord. Responses were recorded either with sucrose gap recording from spinal roots or with intracellular microelectrodes. Acetazolamide had no effect on amino acid (AA) responses of MNs or PAs. NH_4^+ and furosemide completely blocked the hyperpolarizing action of neutral AA on MNs but had no effect on glutamate depolarizations. A conductance increase could still be elicited by the neutral AAs in the presence of NH_4^+. Furosemide, but not NH_4^+, blocked presynaptic inhibition and the GABA depolarization of PAs. The peak of the GABA dose-response curve was depressed by furosemide. The antagonism of the GABA depolarization was not associated with a shift in the equilibrium potential for GABA. These results suggest that 1) the sensitivity of the outward chloride pump in MNs to NH_4^+ does not apply for the inward chloride pump in PAs and 2) the block of GABA depolarizations by furosemide is not due to a block of the inward chloride pump.

583 EFFECTS OF PENICILLIN ON GAMMA-AMINOBUTYRIC ACID INDUCED MEMBRANE CONDUCTANCE AT THE CRAYFISH NEUROMUSCULAR JUNCTION. L.F. Eastman*, W.H. Evoy, and R.A. Davidoff. Univ. of Miami, Miami, Fla., 33124

The epileptogenic action of penicillin on mammalian cerebral cortex is a well documented phenomena. Recently penicillin has been shown to antagonize the effects of the inhibitory transmitter GABA; a finding which has been suggested to be the basis of its neurotoxicity. In order to investigate this hypothesis further the superficial abdominal flexor NMJ of the crayfish Procambarus clarkii was used as a model system of GABA-mediated inhibition. Changes in actual membrane conductance (g) were measured by impaling single muscle fibers with three microelectrodes (one current and two recording) and using the corrected equations for short cables. Addition of GABA (10^{-5} to 10^{-3}M) to the perfusing saline induced increases in g which produced typical sigmoid log dose-response (d-r) curves. When $5x10^{-4}$, $5x10^{-3}$, or $5x10^{-2}$M penicillin were premixed with GABA the resulting g changes were less than those due to GABA alone and yielded d-r curves significantly different from the GABA control curve. Penicillin shifted the GABA d-r curve to the right in a non-parallel manner and depressed the maximum g change. Both of these phenomena were concentration dependent. Use of Lineweaver-Burk double reciprocal plots yielded straight lines which intersected the abscissa at the same point. Thus, these data indicate that penicillin acts as a non-competitive GABA antagonist at this NMJ. Non-competitive antagonism was evident with or without penicillin preincubation. Application of penicillin by itself caused no changes in membrane g.

584 EFFECTS OF IONTOPHORESIS OF NEUROTRANSMITTERS AND CYCLIC NUCLEOTIDES AND OF STIMULATION OF THE LOCUS COERULEUS (LC) ON PURKINJE NEURONS (PNs) OF THE WEAVER MUTANT MOUSE. G. R. Siggins, S. J. Henriksen and S. C. Landis, Lab. of Neuropharmacology, NIMH, St. Elizabeths Hosp., Washington, D.C. 20032 and Dept. Neuroscience, CHMC, Boston, Mass. 02115

Despite absence of granule cells in weaver cerebellum, weaver PNs morphologically resemble normal PNs, possessing dendritic spines with "postsynapsynaptic" thickenings. We have compared PNs of adult C57BL normal mice and adult C57BLxCBA weaver mutant mice electrophysiologically. Superficially, weaver PNs discharge spontaneously like normal mouse PNs (mean rates of 39 Hz vs 40 Hz, respectively). However, several abnormalities are seen: high frequency bursts of single (simple) spikes occur in 5-10 sec episodes in 38% of weaver cells, compared to 8% in normal mice; spontaneous complex spikes (climbing fiber-like responses) occur in several different forms in a given weaver PN. As in normal mice and rats, the spontaneous simple spike activity is depressed in weaver by stimulation of LC, the source of an abnormally dense norepinephrine (NE) plexus in weaver cerebellar cortex. The anti-adrenergic agent, fluphenazine, antagonizes responses to LC stimulation. Iontophoresis of NE, GABA and 5-HT also uniformly depressed simple spike activity in all normal and weaver PNs; cyclic AMP depressed 55% of normal and 70% of weaver PNs. Glutamate is always excitatory. The only qualitative difference is seen with acetylcholine, which slows most normal PNs, but speeds 42% of weaver PNs. Cyclic GMP is predominantly excitatory in both. Thus, despite absence of parallel fiber input, weaver mutant PNs resemble normal PNs electrophysiologically as well as morphologically. These findings in weaver, in which several sites of indirect, presynaptic action are eliminated, also further substantiate the postsynaptic inhibitory nature of GABA, 5-HT, NE, cyclic AMP and the NE pathway from LC to PNs, and the previously hypothesized involvement of cyclic AMP in the postsynaptic response to NE.

585 RETENTION IMPAIRMENT OF PASSIVE AVOIDANCE BY POST-TRIAL INJECTION OF PICROTOXIN INTO THE SUBSTANTIA NIGRA IN RATS. Haing-Ja Kim*, David Miskit* and Aryeh Routtenberg. Cresap Neuroscience Laboratory, Northwestern University, Evanston, Illinois 60201.

Low-level unilateral electrical stimulation of the substantia nigra (SN), pars compacta disrupts retention of passive avoidance (Routtenberg and Holzman, Sci. 181, 1973). We now report that blockade of the γ-amino-butyric acid (GABA)-mediated caudatonigral synaptic inhibition (Precht and Yoshida, Br. Res. 32, 1971) with intranigral injection of picrotoxin, a GABA-specific antagonist, produced a significant memory disruptive effect in the same learning paradigm as used by Routtenberg and Holzman. Picrotoxin (0.05, 0.1 or 0.2 μg) dissolved in 0.5 μl of physiological saline was injected unilaterally into SN, 5 or 30 min post-learning. Intranigral cannula implantation had no effect on original learning. Neither a saline-injected control group nor an implanted control group showed retention impairment. Two to 10 min after intranigral injection of picrotoxin, motoric responses, such as rotation and seizures (at high dosage only), were noted. No particular pattern of motoric response predicted retention impairment. We suggest that this post-trial intranigral injection of picrotoxin may have caused a memory disruption by interfering with memory storage processes which continue after learning. The sites producing an effective memory impairment coincide with the rostral nigral region rich in dopaminergic cell bodies and dendrites. The nigral dopaminergic cells send their axon terminals to the head of the caudate nucleus, which has also been implicated in memory storage processes. The memory impairment obtained in the present study may, therefore, be attributed to the disturbance of physiological activities of this dopaminergic nigrocaudatal fiber pathway. Supported by MH 25281-02 and The Alfred P. Sloan Foundation.

586 EFFECTS OF STRYCHNINE, BICUCULLINE AND PICROTOXIN ON INHIBITION OF HYPOGLOSSAL MOTONEURONS OF THE CAT. L. Felpel. Dept. Pharmacology, Univ. of Texas Health Science Center at San Antonio, Texas 78284.

Inhibitory postsynaptic potentials (IPSPs) can be elicited in hypoglossal (XII) motoneurons of the cat by stimulation of either the lingual nerve (Morimoto et al., Exp. Neurol. 22:174-190, 1968) or the XII nerve (Morimoto and Kawamura, Exp. Neurol. 37:188-198, 1972). The present study was designed to determine if these IPSPs were differentially sensitive to the glycine antagonist, strychnine, or the gamma amino butyric acid (GABA) antagonists, bicuculline and picrotoxin in the chloralose-urethane anesthetized cat.

Postsynaptic potentials (PSPs), evoked by lingual or XII nerve stimulation, were recorded from XII motoneurons with glass microelectrodes filled with 1M potassium acetate. Before drugs, lingual nerve-evoked IPSPs (LIPSPs) were recorded in 97%, and XII nerve-evoked IPSPs (XII IPSPs) in 80% of XII motoneurons. Following the intravenous administration of strychnine (0.08-0.6 mg/kg), LIPSPs were recorded in only 63% and XII IPSPs in 78% of XII motoneurons. Following i.v. bicuculline (0.2-0.4 mg/kg), LIPSPs were recorded in 94% and XII IPSPs in 73% of XII motoneurons. After picrotoxin (1.0-4.0 mg/kg), LIPSPs were recorded in 86% and XII IPSPs in 80% of XII motoneurons.

Recently, it has been suggested that the XII IPSP in XII motoneurons of the rat is an after-potential rather than an IPSP (Lodge et al., Exp. Neurol. 41:63-75, 1973). The results of the present study would support this in that hyperpolarizing current decreased and depolarizing current increased the LIPSP but had no effect on the XII IPSP. The results also suggest that glycine is a more likely candidate for the inhibitory transmitter in this linguo-hypoglossal reflex than GABA. (Supported in part by NIH Grant No. 5 SO1 RR05654-05).

587 BEHAVIORAL AND ELECTROPHYSIOLOGIC CONSEQUENCES OF "KINDLING" IN MESOLIMBIC DOPAMINE SYSTEM. Janice R. Stevens and Arthur Livermore, Jr.*, Depts. of Neurol. & Psychiat., Univ. of Oregon Med. Sch., Portland, 97201.

In previous experiments we have shown that 15-40μg of the putative GABA blocking agent bicuculline applied to the ventral tegmental area of Tsai (A-10), origin of the mesolimbic dopamine system, produces an acute state of fear, staring, searching, sniffing and hiding behaviors in the cat, accompanied by stereotyped spike activity in the nucleus accumbens septi (Stevens, Wilson, Foote, Psychopharm. 39:105, 1974). These behaviors were enhanced by catecholamine agonists and inhibited by catecholamine receptor blocking agents or by prior local application of 6-hydroxydopamine. Utilizing the "kindling" model of Goddard (Nature 214:1020, 1967), we have attempted to induce chronic hyperexcitability in the mesolimbic pathway by daily electrical stimulation of A-10 in cats through chronically implanted insulated cannulas through which the specified behavioral response to bicuculline has been ascertained. Following 25 days of electrical stimulation with 2 sec. trains of 90 cps 1ms bipolar square wave pulses of 200-800μa, a chronic behavior syndrome strikingly similar to the acute response to bicuculline application to A-10 developed. Systematic observations of free behavior, social interaction, conditioned bar press for food and EEG activity from A-10, nucleus accumbens, medial and lateral geniculate nuclei, amygdala and Sylvian cortex were accomplished. Power spectra were computed from serial EEGs from the cortical and subcortical recording sites. Following "Kindling" of the abnormal behavior, effects of local application of GABA to the A-10 area and of systemic Baclofen (Lioresal, beta-chlor-phenyl gamma-amino butyric acid), atropine and dopamine antagonists on the behavior and the EEG were examined.

588 REGULATION OF ACETYLCHOLINE SYNTHESIS BY SODIUM-DEPENDENT HIGH AFFINITY CHOLINE UPTAKE. Jay R. Simon*, Samir Atweh* and Michael J. Kuhar. Dept. Pharmacol., Johns Hopkins Univ. Sch. Med., Balto., Md. 21205.

Acetylcholine (Ach) synthesis is coupled, in some unknown way, to Ach release caused by neuronal impulse flow. Recently, we have reported a coupling between high affinity choline uptake and impulse flow which could be a major factor in the regulation of Ach synthesis (Nature 255: 162, 1975). In subsequent experiments we found that the changes in choline uptake following changes in impulse-flow were restricted to the sodium-dependent portion of the uptake. Accordingly, we have examined in vitro, the sodium dependent high affinity choline uptake (SDHACU) into synaptosomes from rat brain after in vivo treatments which would alter the activity of cholinergic neurons.

We utilized a number of various treatments to reduce the activity of cholinergic neurons in the brain. Administration of pentobarbital (65 mg/kg), chloral hydrate (40 mg/kg) and gammabutyro-lactone (750 mg/kg) caused a 50-80% reduction in SDHACU in several brain regions (30 min). This depression was not found 24 hrs after injection, and direct addition of the anesthetics (0.1-1 mM) to uptake samples did not result in a decreased uptake. Interruption of the cholinergic septal-hippocampal or habenulo-interpeduncular tracts by lesions (10 min-1 hr) also caused a similar, large reduction in SDHACU in the hippocampus and the interpeduncular nucleus respectively. This reduction in choline uptake appeared to be selective in that the uptake of serotonin, dopamine, norepinephrine, GABA, proline and glycine were unaffected.

We reversed the inactivity after pentobarbital administration by direct electrical stimulation of the cholinergic septal-hippocampal tract. Stimulation (40 Hz) for 10 or 15 min completely reversed the depression in SDHACU. Stimulation at lower frequencies or for shorter times caused a partial reversal.

Administration of pentylenetetrazol (75 mg/kg), a convulsant, was utilized to increase the activity of central cholinergic neurons. After drug administration, we found a large (60%) increase in SDHACU. This increase was not found in the hippocampus when cholinergic afferents were interrupted by septal lesion prior to drug administration. Subconvulsant doses of the drug, or direct addition to uptake samples, did not alter SDHACU.

We also examined the uptake after administration of cholinergic drugs. Oxotremorine (0.75 mg/kg), a muscarinic agonist which reduces Ach release and turnover, caused a 30-50% reduction in uptake. On the other hand, administration of scopolamine (5 mg/kg), a cholinergic antagonist which increases Ach turnover, caused an increase in SDHACU. Low doses of these drugs or direct addition to uptake samples did not alter uptake.

We examined the conversion of ^{3}H-choline to ^{3}H-Ach in hippocampal synaptosomes after septal lesion, pentylenetetrazol administration and in untreated controls. In all cases, 60-70% of the total sodium-dependent tritium content was present as ^{3}H-Ach.

A kinetic analysis of SDHACU was performed after all treatments. We found a 30% decrease in the apparent Km only after treatments which would cause a reduction in neuronal activity. However, we found changes in Vmax after all treatments, and these changes were consistently in the same direction as the alterations in activity. Since the concentration of choline in CSF, brain and the synaptic cleft has been measured and estimated to be about 10 μM and higher, the SDHACU would be saturated, and the Vmax changes would alter choline entry. Thus, the SDHACU appears to be coupled to cholinergic activity in such a way as to regulate the entry of choline for the maintenance of Ach synthesis. These studies were supported by USPHS grants MH 25951 and MH 00053.

589 IMPAIRED SYNTHESIS OF ACETYLCHOLINE ACCOMPANYING MILD HYPOXIA. G.E. Gibson and J.P. Blass. UCLA Medical School, Los Angeles, California 90024.

Reduced synthesis of acetylcholine (ACh) accompanied impaired oxidation of pyruvate in minced rat brains *in vitro* (Biochem. J. 148, 17). Impaired incorporation of $^2H_4$-choline into ACh accompanied hypoglycemia *in vivo*; incubating minced rat brains with reduced concentrations of [U-^{14}C]glucose reduced ACh synthesis *in vitro*; and pretreating mice with the cholinesterase inhibitor physostigmine reduced the number of mice dying within 3 hours after insulin overdose (Trans. Amer. Soc. Neurochem. 6, 139). These findings are now extended to the effects of hypoxia *in vivo* and *in vitro*.

In vivo, hypoxia was induced by injecting mice with KCN (i.p.) or with $NaNO_2$ (s.c. to oxidize hemoglobin to methemoglobin). The animals were then injected with $^2H_4$-choline i.v. 1 minute before sacrifice by microwave irradiation. KCN treatment reduced the incorporation of $^2H_4$-choline into ACh: values were 784 ± 46 pmoles/gm brain for controls (± SEM), and 526 ± 51 and 99 ± 79 for 3 and 6 mg of KCN, respectively. Treatment with $NaNO_2$ also reduced incorporation into ACh: to 508 ± 73, 465 ± 69, and 228 ± 52 pmoles/gm brain at 34%, 59%, and 81% methemoglobin, respectively. Total unlabelled ACh declined only with the high dose of KCN. With both KCN and $NaNO_2$, both $^2H_4$-choline and unlabelled choline increased. At the highest doses of KCN and $NaNO_2$, $^2H_4$-choline increased from 3.57 ± 0.38 to 9.26 ± 3.93 and 10.17 ± 1.92 picomoles/gm brain respectively, while unlabelled choline increased from 29.2 ± 1.5 to 58.3 ± 5.7 and 65.1 ± 8.1 nmoles/gm, respectively.

In parallel experiments, animals were frozen in liquid N_2 and the brain's cytoplasmic redox state determined from [pyruvate]/[lactate], the mitochondrial redox state from [2-oxoglutarate][NH_3]/[glutamate], and the adenylate energy charge as (ATP + 0.5 ADP)/(ATP + ADP + AMP). The energy charge changed only after treatment with the high concentration of KCN (from 0.915 ± 0.01 to 0.863 ± 0.017). The mitochondrial NAD^+/NADH increased with $NaNO_2$ and decreased with KCN. Cytoplasmic NAD^+/NADH decreased with both KCN and $NaNO_2$. The relationship between $^2H_4$-ACh and cytoplasmic NAD^+/NADH followed the equation (with r = 0.85)

$$(^2H_4\text{-ACh}) = 0.0076\ (\text{cytoplasmic } NAD^+/NADH) - 0.068.$$

Also, $(^2H_4\text{-ACh}) = 18.7\ (E_c - E_m) - 2.66$, with r = 0.85,

where $E_c = -0.272\ v + (RT/nF)\log(\text{cytoplasmic } NAD^+/NADH)$ and

where $E_m = -0.272\ v + (RT/nF)\log(\text{mitochondrial } NAD^+/NADH)$.

In vitro, with minces of whole rat brain incubated in buffer containing 31 mM-K^+, the synthesis of ACh decreased when glucose was reduced from 5 to 0.5 mM, when KCN was added, or N_2 replaced O_2. Synthesis of ACh then fit the equations

$$(\text{ACh}) = 228\ (\text{cytoplasmic } NAD^+/NADH) - 32.3 \quad (r = 0.82) \text{ and}$$

$$(\text{ACh}) = 15.4\ (E_c - E_m) - 1.3 \quad (r = 0.95).$$

Although the mechanisms relating redox potentials and ACh synthesis are unknown, it is clear that even relatively mild hypoxia can impair the synthesis of ACh; the changes in ACh metabolism occur as early as the changes in cytoplasmic redox state.

590 BRAIN ACETYLCHOLINE: INCREASE AFTER DIETARY CHOLINE INGESTION. Edith L. Cohen, Marianne Unger*, Nicole Verbiese*, Richard J. Wurtman.
Massachusetts Institute of Technology, Cambridge, MA 02139.

Precursor availability influences the synthesis of brain serotonin (Fernstrom and Wurtman, Sci. Amer. 230:84, 1974) and catecholamines (Wurtman et al., Science 185:183, 1974). Recently we have shown that brain acetylcholine (ACh) levels increase after i.p. choline injection (Cohen and Wurtman, Life Sci. 16:1095, 1975). Now we have extended these studies to include the effects of chronic oral consumption of various amounts of choline per day. Male 100-120 g. rats were kept for 11 days on a choline-deficient diet which, for some animals was supplemented with choline chloride - either in their drinking water or in their food. Rats were killed by microwave irradiation of the head. In rats on a choline intake of 23 mg/day (an intake comparable to that found in rats eating standard laboratory chow), brain [choline] rose to 134±3% ($p<.001$) and brain [ACh] to 105±1% ($p<.05$) of levels found in control rats consuming no choline. On a choline intake of 170 mg/day, brain [choline] rose to 368±8% ($p<.001$) and brain [ACh] to 123±1% ($p<.001$) of control levels. [ACh] in the caudate nuclei rose to 128±4% ($p<.05$) of control levels in rats consuming 20 mg choline/day and to 140±3% ($p<.01$) of control levels in rats consuming 130 mg choline/day. Serum choline increased linearly with dietary choline content ($r=.91$).

Thus, physiological modulation of precursor availability (by dietary means) can control brain ACh levels. This finding is consistent with the hypothesis (previously based on studies of serotonin and catecholamine synthesis), that nutritional state can modulate neuronal composition and, perhaps, function.

591 ACETYLCHOLINE TURNOVER IN WHOLE MOUSE BRAIN FOLLOWING LITHIUM AND PHENOBARBITAL ADMINISTRATION. K. A. Cosgrove*, C. L. Scudder*, A. G. Karczmar, and G. Kindel* (SPON: D. R. Haubrich). Dept. of Pharmacology, Loyola University Medical Center, Maywood, IL 60153

Effects of lithium (5meq/kg) and phenobarbital (60mg/kg) on acetylcholine (ACh) and choline (Ch) levels and on ACh turnover were examined in male CF-1 mice. Transmitter levels were determined by the enzymatic radioassay of Haubrich, (Ch and ACh:Hndbk. of Chem. Assay Methods, I. Hanin, ed., 1974), and turnover rates were evaluated by the method of Jenden et al. (Life Sci., Vol. 22, 1974). Lithium increased ACh levels significantly (17.7±0.63 nmoles/g) compared to controls (12.2±0.68 nmoles/g), while Ch levels did not change. Phenobarbital was found to increase ACh levels significantly (18.80±1.39 nmoles/g) compared to controls, and to increase Ch levels. Lithium was found to increase ACh turnover in contrast to phenobarbital which inhibited ACh turnover with respect to controls. Following drug pretreatment, animals were subjected to stress, whereupon levels and turnover rates were measured. ACh levels did not change from untreated controls after combined lithium and stress treatments, and the ACh turnover rate was augmented in stress-lithium treated animals. ACh levels were slightly elevated and turnover rates were unchanged in the stress-phenobarbital treated group. Supported in part by NIH Grant NS06455.

592 THE PARTIAL CHARACTERIZATION OF ESTERASES IN LIMULUS POLYPHEMUS. James G. Townsel, Henry E. Baker* and Thyckla T. Gray*, Division of Molecular Medicine and Department of Physiology, Meharry Medical College, Nashville, Tennessee 37208

Acetylcholine (ACh) has been suggested as a cardioexcitatory transmitter in Limulus. Recently, the ACh biosynthetic enzyme choline acetyltransferase has been purified from extracts of Limulus nervous tissue {Emson, P.C. et al., J. Neurochem, 22:1089 ('74)}. Histochemical studies suggest the presence of acetylcholinesterase (AChE; E.C. 3.1.1.7) in Limulus cardioregulatory nervous tissue and butyrylcholinesterase (BuChE., Ch.E., E.C. 3.1.1.8) in cardiac muscle {Stephens, L.B. and Greenberg, M.J., Histochem, Cytochem. 21:923 ('73)}. This study was initiated to provide a biochemical characterization of the Limulus esterases as a necessary prelude to distribution studies within single identified cells in the cardioregulatory system. Kinetic studies of esteratic activity in homogenates of the ventral nerve cord (VNC) and that of cardiac muscle (CM) corroborates at least two species of esterase in Limulus. Acetylthiocholine (AcThCh) as a substrate gives an apparent K_m of 0.08±0.02 mM with the VNC preparation while for the CM preparation the apparent K_m is 1.32±0.52 mM. With butyrylthiocholine the apparent K_m's are quite similar for the two preparations (≈3.0 mM). These results are compatible with the suggestion of an AChE in the VNC and ChE in CM. However, the results of studies with two definitive inhibitors, iso-OMPA (tetraisopropylpyrophosphoramide) and BW284C51 {1,5 - bis (4 - allydimethyl - ammoniumphenyl) pentan-3-one} dibromide, caution against quick conclusions. Polyacrylamide gel electrophoresis studies of Limulus esterases are currently in progress.

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593 TWO ISO-ENZYME FORMS OF CHOLINE ACETYLTRANSFERASES IN HUMAN NEOSTRIATAL NUCLEI. Vijendra K. Singh, Edith G. McGeer and Patrick L. McGeer. Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, Univ. of B. C., Vancouver, B. C., Canada.

Choline acetyltransferase (CAT), which catalyzes the synthesis of neurotransmitter acetylcholine in cholinergic neurons, was isolated and purified from human neostriatal nuclei. The fractionation of partially purified material on a phosphocellulose column resolved two peaks of CAT activity. The protein peak eluted with 0.15 M potassium phosphate contained most of the enzyme activity (Enzyme A), and a smaller fraction of CAT activity (Enzyme B) was eluted around 0.3 M potassium phosphate. Each of the enzymes appeared to be a single polypeptide on SDS-polyaerylamide gels, having similar molecular weights (about 67,000). Enzyme A, the major component of the total acetylcholine synthetic activity, was highly antigenic producing specific anti-CAT in rabbit serum, whereas enzyme B appeared to be devoid of any antigenicity when injected into rabbit. The rabbit anti-human brain CAT cross-reacted with the CAT preparations from other mammalian species (including human placenta), but not from non-mammalian species. The activity of enzyme B was markedly inhibited by acetylcholine, the reaction product, whereas the activity of enzyme A was unaffected. Such a differential effect may be important in the regulation of Ach levels in brain.

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594 SELECTIVE LOCALIZATION OF A HIGH AFFINITY CHOLINE UPTAKE SYSTEM IN CHOLINERGIC NEURONS. J. Suszkiw* and G. Pilar. (SPON: W. Chapple). Biol. Sci. Group, Univ. Conn., Storrs, Conn. 06268.

The kinetics of choline (Ch) uptake and its conversion to acetylcholine (ACh) were determined in ganglion cell somas and their nerve terminals. Normal and denervated ganglia and irises isolated from 10-day-old chicks were incubated in different H^3Ch concentrations from $10^{-7}M$ to $10^{-4}M$ in normal oxygenated Tyrode and sodium-free solution at 37°C. In the iris preparation (ciliary nerve terminals and iris muscle cells), low ($K_m=10^{-4}M$) and high ($K_m=2x10^{-6}M$) affinity Ch uptake systems were determined. The high affinity uptake obeys Michaelis-Menten kinetics with a V_{max} of approx. .5 pmoles per minute per preparation. This high affinity uptake was not present when the preparation was incubated in sodium-free Tyrode or in preparations denervated 3 days prior to the experiments. Therefore, this high affinity system is localized in the nerve terminals. The low affinity system was unchanged under similar experimental conditions and is localized only in the muscle. In nerve terminals preincubated at the avian plasma Ch concentrations (5μM), about 50% of the H^3Ch taken up was converted to ACh. In ganglia denervated 3 days prior to the experiments (no preganglionic terminals present), Ch uptake was not changed in sodium-free solutions and a small fraction of the Ch uptake (approx. 10%) was converted to ACh. This synthesis of ACh was also not sodium-dependent. It is concluded that the high affinity sodium-dependent Ch transport--ACh synthesis system is present predominantly in nerve terminals and is either negligible or absent in the cell somas of the same cholinergic cells. This suggests a membrane specialization in nerve terminals related to the synthesis of neurotransmitter. (Supported by NIH grant NS 10338 and the Univ. of Connecticut Research Foundation).

595 ACETYLCHOLINE, CHOLINE AND CHOLINE ACETYLTRANSFERASE ACTIVITY IN VARIOUS NUCLEI OF THE DEVELOPING RAT BRAIN. Gabriella Zsilla, Darwin L. Cheney*, Gorgio Racagni and Erminio Costa. Laboratory of Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D. C. 20032

Choline acetyltransferase (CAT) was measured after decapitation, choline (Ch) and acetylcholine (ACh) after focused microwave irradiation. Brain nuclei were dissected stereomicroscopically and Ch or ACh measured mass fragmentographically. Increments of the two substrates and of CAT activity after birth were estimated as a percent taking 28 days values as 100. The pattern of value changes was monophasic for ACh and CAT, and biomodal for Ch. The highest values of Ch were present at birth (about 200), the trough was reached at day 5 and the value steadily increased to reach adult values at 14 days. The values of ACh at day 1 were 25 for N. interpeduncularis, 50 for N. accumbens, caudate, dorsal raphe and locus coeruleus. In all structures adult values were reached between 14 and 28 days. The values of CAT at day 1 were 1.7 for N. caudatus, 3.6 for N. accumbens, 2.9 for N. interpeduncularis, 5.2 for N. dorsal raphe and 7.6 for N. locus coeruleus. The rate of increment of CAT was greater than that of ACh indicating that CAT was never a rate limiting event for the control of ACh. Since acetylcholinesterase (McCaman and Aprison; in Prog. Br. Res. 9: 220. Ed. by Himwich (1964) develops at a rate comparable to that of CAT the ACh content reflects an equilibrium between these two enzyme activities.

596 THE DEVELOPMENTAL PATTERNS OF CHOLINE ACETYLTRANSFERASE, CHOLINE KINASE, AND ACETYLCHOLINESTERASE ACTIVITY IN THE CHICK CILLIARY GANGLION. Alvin M. Burt and C. H. Narayanan. Dept. of Anat. Vanderbilt Univ., Nashville, TN, 37232 and Dept. of Anat., LSU, New Orleans, LA 70119.

Cilliary ganglia were removed from chick embryos of from 9 to 21 days' incubation, rapidly frozen, lyophilized, and stored at -76°C until assayed. Individual ganglia were weighed on a Cahn electrobalance, homogenized and immediately assayed for choline acetyltransferase (ChAc), choline kinase (CK) and acetylcholinesterase (AChE) activities. Values are expressed as μmoles choline acetylated (ChAc) or phosphorylated (CK)/g dry weight/hr at 37°C or as μmoles acetylthiocholine hydrolysed (AChE)/g dry weight/min. at 20°C.

ChAc activity increased slowly from day 9 through 13(from less than 4 to 28), then rapidly to 108 by day 17 and peaked at 148 by day 19. The pattern for CK was similar with maximal values of from 20 to 28 for the period of day 17-21. The pattern for AChE was quite different: maximal levels (100-150) were observed from days 9-13 and again from days 17-21 with a significant drop in activity at day 15 (41). Although the general aspects of the ontogenetic patterns for ChAc and AChE agree with Sorimachi and Kataoka (Brain Res., 70:123), there are marked quantitative differences.

The well defined choroid and cilliary cell regions were dissected from other ganglia, weighed on a quartz-fiber balance and assayed for enzyme activity. Cilliary neurotransmission is mediated both by direct electrical coupling and acetylcholine; choroid transmission is mediated only by acetylcholine. The specific activity of ChAc in the choroid region, however, was only 70% that of the cilliary region.

This work was supported by a grant from the Dysautonomia Foundation and grants NS-07441 and FR-05424, U.S.P.H.S.

597 REGIONAL N-ACETYLTRANSFERASE IN THE DEVELOPING RAT BRAIN. Hsu, L. L.* and Mandell, A. J. Dept. Psychiatry. Sch. Med. UCSD, La Jolla, CA 92037

We have reported N-acetyltransferase activity in whole rat brain and determined the specific activity of the enzyme in 15 regions of the brain; the highest specific activity appeared in cerebellum (Paul et al., Life Sci. 15:2135, 1974; Hsu et al., Fed. Proc. 1975, Abstract No. 147). At seven weeks of age, the partially purified enzyme from whole rat brain showed biphasic kinetics with respect to acetyl-CoA in the presence of either tryptamine or β-phenylethylamine. In order to determine whether or not the regional enzyme activity changes with brain development, we examined the enzyme activity in several regions of the rat brain at various times after birth. We also studied the enzyme kinetics using the progressively purified enzyme from newborn rat brain (3 and 5 days after birth). Our data indicate that the NAT activity in the brain regions as well as in the whole brain peaked 36 days after birth. The enzyme in 3 or 5 day old rats' brains did not show a biphasic kinetics as the mature brain had done.

598 MICROIONTOPHORETIC STUDY OF THE CHOLINERGIC PHARMACOLOGY OF HIPPOCAMPAL PYRAMIDAL CELLS. Stephanie J. Bird and George K. Aghajanian. Yale Univ. School of Medicine, New Haven, Ct. 06510

The purpose of this study was to examine the pharmacological properties of an identified cholinergic system in the CNS of the rat. By extracellular recording the responses of hippocampal pyramidal cells to the microiontophoretic application of various nicotinic and muscarinic agents were monitored. In addition, interactions between muscarinic and nicotinic compounds were investigated.

Acetylcholine (ACh) and carbachol, agents effective at both nicotinic and muscarinic sites, readily produced excitation of hippocampal pyramidal cells. The muscarinic agonists muscarine, acetyl-β-methylcholine (MCh) and bethanechol also excited these cells at low iontophoretic currents. The muscarinic antagonists scopolamine and quinuclidinyl benzilate (QNB) totally blocked ACh excitation of pyramidal cells without altering their excitation by glutamate. Hippocampal pyramidal cells were also responsive to nicotinic compounds. The nicotinic agonist phenyltrimethylammonium (PTMA) caused excitation of pyramidal cells. The nicotinic agonist tetramethylammonium (TMA), and the nicotinic antagonists dihydro-β-erythroidine (DHβE) and gallamine specifically blocked ACh excitation of these cells. However, other nicotinic agents investigated produced a nonspecific block of ACh excitation and the classical nicotinic antagonist d-tubocurarine produced no effect in the amounts tested. The nicotinic antagonist DHβE completely blocked the excitation of pyramidal cells by the muscarinic agonists muscarine and MCh. It was concluded: 1) hippocampal pyramidal cells are responsive to muscarinic and some nicotinic agents and 2) these agents are not acting at two independent receptors since there is crossover between nicotinic antagonists and muscarinic agonists. (Supported in part by USPHS grants MH 17871 and GM 1113-13).

599 MICROIONTOPHORETIC APPLICATION OF SOME BIOGENIC AMINES AND THEIR ANTAGONISTS TO SINGLE VESTIBULAR NEURONS IN THE CAT. J.N. Sharma* and E.B. Kirsten. Department of Pharmacology, Columbia College of P. & S., New York, N.Y., 10032.

Both adrenergic and cholinergic mechanisms have been suggested as playing significant roles in the synaptic integration of vestibular neuronal activity. Microiontophoresis of norepinephrine (NE; 50-100 nA) and d-amphetamine (A; 50-100 nA) was employed to investigate the possibility of adrenergic synapses on vestibular neurons in decerebrate, cerebellectomized cats. Five barrel micropipettes were positioned in the vestibular nucleus (VN) stereotaxically and confirmed later by histology. It was observed that neurons located in the medial VN were inhibited by both NE (75%; n=94 cells) and A (76%; n=29). Neurons located in the lateral VN were predominantly excited by these agents (NE - 64%; n=42 and A - 50%; n=21). Acetylcholine (50-100 nA) excited neurons (81%; n=47) in both areas. While the α-adrenergic blocking agent phentolamine (5-20 nA) blocked the NE excitatory responses (n=10), the inhibitory responses were uneffected (n=12). The β-adrenergic blocking agents MJ-1999 (Sotalol; 50-100 nA) and propranolol (50-100 nA) had no effect on either the excitatory (n=12) or inhibitory (n=23) responses to NE. These observations suggest the involvement of adrenergic neurotransmission in the VN and also the possibility of α-adrenergic receptors on neurons located in the lateral VN.
(Supported by NINDS Grant NS-11858).

600 LSD-25 ENHANCEMENT OF DORSAL LATERAL GENICULATE UNIT REPONSES TO RETICULAR STIMULATION IN CAT. W.E. Foote, R.J. Maciewicz*, J. Mordes*, and C. Seitz*. Dept. Psychiatry, Harvard Medical School, Boston, Mass.

Extracellular recordings were made from neural elements in cat dorsal lateral geniculate nucleus (LGN). Cells were identified on the basis of their receptive fields, reponse to optic tract stimulation and response to antidromic stimulation of optic radiation fibers. Records of spontaneous activity, optic tract driving, response to spots of light placed in the center of the receptive field and response to electrical stimulation of the mesencephalic reticular formation were obtained for each unit. Animals were then given 25 to 30 microgram/kg doses of LSD-25 intravenously and the above observations of unit activity repeated over time, in most cases until the effects of the drug appeared to have dissipated.

We found no observable changes in receptive field size or shape and no uniform change in spontaneous activity. All units, however, displayed a uniform decrease in the efficacy of optic tract driving and most displayed an enhancement of either the facilitation or the inhibition which occurred after reticular stimulation. In order to determine whether this enhancement of the reticular effect was correlated with decreased optic tract driving, a series of cats had both eyes enucleated and geniculate activity was recorded four to six days later. The effect of LSD-25 on reticular stimulation was still present, suggesting that this compound affects both retinal and extra-retinal afferents to the LGN.

601 SEROTONERGIC INFLUENCE ON SINGLE UNIT ACTIVITY IN THE AMYGDALA. Rex Y. Wang* and George K. Aghajanian. Depts. Psychiat. and Pharmacol. Yale Univ. Sch. Med., New Haven, Ct. 06508

A serotonergic (5HT) input to the amygdala (AMYG) from the midbrain raphé has been previously demonstrated by histofluorescence methods. By use of the horseradish peroxidase retrograde tracing technique, we found that the serotonergic input to the AMYG is primarily derived from dorsal raphé nucleus (DRN). Extracellular single unit activity from AMYG neurons was recorded from cerveau isolé rats during DRN stimulation and during iontophoretic administration of 5HT (ionto-5HT). Both ionto-5HT and electrical stimulation of the DRN markedly inhibited spontaneous firing of AMYG cells; in many cases, inhibition was total at relatively low frequencies or currents. Concurrent iontophoresis of chlorimipramine (C), which blocks 5HT reuptake, potentiated both the inhibition produced by DRN stimulation and ionto-5HT. 5,7-Dihydroxytryptamine (5,7DHT), which is relatively selective in destroying 5HT axons, injected into the ventral tegmental 5HT pathway (VTP) resulted in the disappearance of 5HT terminals in the AMYG. In 5,7DHT-treated animals, DRN stimulation no longer inhibited AMYG units. In these animals, concurrent ionto-C failed to enhance inhibition produced by ionto-5HT. In control rats (injected with vehicle into the VTP or 6-hydroxydopamine into the dorsal norepinephrine bundle) DRN stimulation and ionto-C were still able to exert their influences on AMYG cells. Stimulation of the DRN was much less effective in producing inhibition 24-48 hrs after i.p. injection of p-chlorophenylalanine (PCPA, a selective 5HT depletor). In these animals, administration of RO4-4602 (50mg/kg) and 5HTP (the immediate 5HT precursor 5-20mg/kg) reversed the effect produced by PCPA. We conclude: 1) stimulation of the DRN has a marked inhibitory influence upon AMYG neurons; 2) this inhibitory effect is mediated by a direct DRN-AMYG serotonergic pathway. (Supported in part by USPH grants MH 17871 and MH 14459).

602 EFFECT OF ANTIPSYCHOTIC DRUGS AND ADRENERGIC BLOCKING AGENTS ON THE FIRING RATE OF SEROTONERGIC (RAPHE) CELLS IN THE RAT BRAIN. D.W. Gallager and G.K. Aghajanian. Depts. of Psychiat. and Pharmacol. Yale Univ. Sch. of Med., New Haven, Ct. 06508

Previous studies have shown that serotonergic (5HT) neurons in the dorsal raphe nucleus are inhibited by low i.v. doses of the antipsychotic drug methiothepin (MTT). However, another antipsychotic agent, chlorpromazine (CPZ), had little effect on raphe cell firing. Therefore, a larger series of antipsychotic drugs were tested for their effects on raphe single unit activity. In these studies relatively low doses of MTT (0.1 to 0.25 mg/kg,i.v.), clozapine (1.0 to 1.5 mg/kg,i.v.) and thioridazine (4.5 to 7.5 mg/kg,i.v.) totally inhibited raphe cell firing. In contrast, CPZ inhibited only some raphe cells (>8 mg/kg,i.v.); other raphe cells seemed resistant to this drug. Large doses of pimozide (30 mg/kg,i.p.) and near lethal doses of haloperidol (up to 8 mg/kg,i.v.) were virtually ineffective. The potency of the above series of antipsychotic drugs in depressing raphe cell firing correlates well with presumed central noradrenergic blocking efficacy, based on the studies of Keller et al (Eur. J. Pharm.23:183,1973). To investigate a possible connection between noradrenergic blockade and raphe neuronal activity, the effect of the α-adrenergic blocking agent, piperoxane, was examined. This drug totally inhibited raphe firing (5 to 9 mg/kg,i.v.). In contrast, the β-adrenergic blocking agent, propranolol, had only slight effects on raphe firing rates except at near lethal doses (>19 mg/kg,i.v.). These data are consistent with the hypothesis suggesting a correlation between impairment of noradrenergic transmission in the CNS and depression of raphe neuronal firing (Svensson, Bunney and Aghajanian, Brain Res.91:in press,1975). In addition, the data suggest the latter effect may be mediated via an α-adrenergic receptor. (USPH grants MH 17871, MH 14459 and MH 07114).

603 DEPRESSION OF RAPHE UNIT ACTIVITY BY SYSTEMIC ADMINISTRATION OF L-5HYDROXYTRYPTOPHAN IN THE RAT. Michael E. Trulson and Barry L. Jacobs. Dept. Psychol., Princeton Univ., Princeton, N.J. 08540.

5-Hydroxytryptophan (5HTP) is the immediate precursor of serotonin in vivo. However, the ability of exogenously administered 5HTP to serve as an effective serotonin precursor in central serotonergic neurons has been questioned. Recent reports indicate that 5HTP is ineffective in depressing raphe unit activity and does not increase raphe neuron histofluorescence, while tryptophan, monoamine oxidase inhibitors and serotonin reuptake blocking agents are all effective in both of these situations. We report, however, that exogenously administered 5HTP does depress raphe unit activity, and does so in a dose-dependent manner. Unit activity in the dorsal and median raphe nuclei was recorded with tungsten microelectrodes in chloral-hydrate anesthetized male rats. L-5HTP or L-tryptophan was injected i.p. in a dosage of 10, 25, 50, or 100 mg/kg. 5HTP and tryptophan consistently produced similar decreases in raphe unit activity, which ranged between 5-10% for 10 mg/kg and 60-95% for 100 mg/kg dosages. Pretreatment with a peripheral decarboxylase inhibitor (MK486) had no effect on the response of raphe units to 5HTP. The activity of non-raphe units was not affected by 5HTP or tryptophan administration. The increase in cerebral serotonin is proportional to the doses of 5HTP and tryptophan, reaching a 360% increase after 100 mg/kg 5HTP, while administration of 100 mg/kg tryptophan produced only a 40% increase in cerebral serotonin content. These data demonstrate that 5HTP is as effective as tryptophan in depressing raphe unit activity and therefore argue in favor of its action as an effective serotonin precursor in central serotonergic neurons.

604 SUBSTANCE P EXCITATION OF SPINAL NOCICEPTIVE NEURONES. James L. Henry, Dept. Anaesthesia Research, McGill Univ., Montreal, Canada.

Immunohistochemical evidence showing a concentration of substance P (sP) in the dorsal horn of rat spinal cord (Nilsson et al., Med. Biol. 52: 424, 1974) prompted an extension of an earlier study (Henry et al., Can. J. Physiol. Pharmacol. 53: 423, 1975) to determine the effects of sP on histologically and functionally identified single units in spinal segments L_6-L_7 of cats (intercollicular decerebration or chloralose anaesthesia). Multibarrelled micropipettes (tips 5-10 μm) were used for recording and for iontophoresis of synthetic sP (Beckman), putative transmitters and Pontamine blue for marking electrode tip positions. Units were identified functionally by their responses to mechanical stimuli and to noxious radiant heat applied to the skin. Clear results were obtained from 33 units; 25 were classified as nociceptive, 8 as non-nociceptive. Most nociceptive neurones also responded to non-noxious stimuli. Excitation with sP (n = 19 units) consisted of a very slow increase in discharge rate after a delay of 10-30 sec, and lasted up to several minutes after termination of the iontophoretic current. Inhibition by sP was not observed. All units excited by sP were among those classified as nociceptive, and were found in laminae I and IV-VI. sP had no consistent effect on units responding only to non-noxious stimuli. Similarly, there was no obvious correlation between responsiveness to sP and the actions of ACh, noradrenaline and 5-HT. The results suggest that sP is probably not a quickly acting transmitter but may play a role in regulating the level of excitability of spinal neurones in nociceptive pathways.

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605 MODIFICATION OF β-PHENYLETHYLAMINE-INDUCED STEREOTYPED BEHAVIOR BY NEUROLEPTIC AGENTS. Egidio A. Moja*, David M. Stoff, J. Christian Gillin, and Richard J. Wyatt. Laboratory of Clinical Psychopharmacology, NIMH, SMR, IRP, St. Eliz. Hosp., Wash., D.C. 20032.

We report data on the induction of stereotyped behavior (SB), associated with increased motor activity, by β-Phenylethylamine (PEA) in pargyline pretreated rats (Sprague-Dawley/Zivic Miller, 150-250 g, male) and modification of this behavior by neuroleptic agents. (i) SB consisted of head bobbing, visual checking and sniffing, consistently elicited by 16 mg/kg PEA (ip) in rats treated 2 hrs earlier with 2 mg/kg pargyline (iv). It was quantified by two blind judges (inter-rater reliability, r=0.88) on a four point scale over a 30-sec observation period: (0) no SB, (1) SB < 10 sec, (2) SB < 20 sec, (3) SB > 20 sec, at time intervals of 5, 10, 20, 30, 40, 50 and 60 mins after PEA or saline treatment. The onset of SB was 5 mins after injection, peaking at 10-20 mins and disappearing completely by 60 mins. (ii) Alteration of these effects was studied by administration of the following drugs (ip) 30 mins before PEA or saline treatment: Haloperidol (0.125, 0.50 mg/kg), Pimozide (0.125, 0.50 mg/kg), Chlorpromazine (1.0, 2.0, 3.0 mg/kg), Clozapine (5.0, 10.0 mg/kg) and Diazepam (1.0, 2.0, 4.0 mg/kg). Data was analyzed by Mann-Whitney U tests for each drug group at the different time intervals. In the following order of potency, there was dose-response reduction of PEA-induced SB from complete blockade to marked attenuation: Haloperidol 0.5 mg/kg > Haloperidol 0.125 mg/kg, Pimozide 0.5 mg/kg, Chlorpromazine 3.0 mg/kg, Clozapine 10 mg/kg > Chlorpromazine 2.0 mg/kg > Chlorpromazine 1.0 mg/kg. Pimozide 0.125 mg/kg, Clozapine 5.0 mg/kg and all doses of Diazepam did not influence SB. There was a direct relationship between motor activity scores and SB both with and without neuroleptic drugs. Therefore, all the neuroleptic drugs produced dose-response reduction of SB, whereas Diazepam, which reportedly does not have antipsychotic effects, did not modify this behavior. (iii) Preliminary work suggests that chronic administration of pargyline and PEA potentiates the duration of SB which can also be attenuated by neuroleptics. Because PEA is found naturally in human brain (Inwang et al., J. Neurochem, 20, 1469, 1973) and is a good substrate for Type B monoamine oxidase (Yang and Neff, J. Pharmacol. Exp. Ther., 187, 365, 1973), then (i) induction of SB by PEA with an MAOI reported here and elsewhere (e.g., Mantegazza and Riva, J. Pharm. Pharmacol., 15, 472, 1963), (ii) reversal of this effect by neuroleptics, and (iii) potentiation after chronic administration may have relevance to psychotic illnesses, consistent with the report of low platelet MAO in schizophrenics (Wyatt et al., Science 179, 916, 1973).

606 PHENYLACETIC ACID: LEVELS AND BIOSYNTHESIS IN RABBIT BRAIN; POSSIBLE IMPLICATIONS IN PHENYLKETONURIA. U. P. Madubuike* and A. D. Mosnaim (SPON: W. A. Pedemonte). Dept. Pharm., Chicago Med. Sch./Univ. Hth. Sci., Chicago, 60612

Recent work from several laboratories has stressed the possible physiological role of 2-phenylethylamine (PEA), an endogenous neuroamine, and some of its metabolites, in the modulation of synaptic transmission as well as in certain psychopathologies (Sabelli and Mosnaim, 1974). This amine is highly sensitive to MAO_B and therefore it may be expected to be a major source of brain phenylacetic acid (PAAc). We have now isolated and identified PAAc from rabbit brain using a specific extraction procedure followed by TLC and mass spectroscopy. Its tissue levels were estimated by GLC to be 0.275±0.043 µg/g of wet brain. Labeled PAAc was extracted from brain ten minutes after the intraventricular injection of radioactive PEA or L-phenylalanine. Pretreatment of the rabbits with pargyline (50mg/Kg., ip, 72, 48 and 24 hour before sacrifice) markedly reduced the recovery of (^{14}C) PAAc following the injection of either labeled precursor thus suggesting that in vivo brain synthesis of PAAc from L-phenylalanine proceeds mainly via PEA. We will discuss the possible use of PAAc to estimate brain PEA turnover as well as the potential implications of abnormal brain PAAc levels (e.g. excess or deficit due to impairment in PEA biosynthesis and/or activity of MAO or aldehyde dehydrogenase, or to a defect in the system involved in disposing of brain PAAc) in brain damage and mental retardation such as seen in phenylketonuria.
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607 MAJOR UPTAKE OF PHENYLETHYLAMINE (PEA) INTO THE SECRETORY VESICLES OF SYNAPTOSOMES. Christopher Whalley* and Ruven Greenberg. Dept. of Physiology, U. of Ill. Med. Sch., Chicago, Ill. 60680.

Previously, we reported (Physiologist 17:357, 1974) that roughly 30% of the radio-labeled PEA-^{14}C content of the brain was in the synaptosomes at 15 sec subsequent to the in vivo i.a. injection by the Oldendorf procedure (Brain Res. 24: 372, 1970). We also reported the lack of a Na^+ dependent active transport system by rat brain synaptosomes from whole brain and from various brain regions. Rather, the PEA-^{14}C uptake into synaptosomes is by passive diffusion. We now report that, subsequent to in vitro incubation (method of Kuhar et al (JPET 181: 36, 1972), 60-80% of the PEA-^{3}H of the synaptosomes is associated with secretory vesicles subsequent to their release by hypotonic shock and 20-40% of the synaptosome content is associated with the cytosol. One-half of the PEA-^{3}H content of the secretory vesicles is released and one-half is associated with the membranes after lysis of the secretory vesicles by repeated (8x) freezing and thawing. In other experiments, PEA-^{3}H displaced norepinephrine-^{14}C previously incorporated into the secretory vesicles. This indicates that circulating PEA, which readily crosses the blood-brain barrier, may play an important role in displacing norepinephrine, and probably other biogenic amines, from storage sites in secretory vesicles. (Supported in part by GRSG, U. of Illinois.)

608 INFLUENCE OF CATECHOLAMINE DEPLETERS AND BLOCKERS ON 2-PHENYLETHYLAMINE EFFECTS AND DISPOSITION. R. L. Borison* and H. C. Sabelli. Department of Pharmacology, The Chicago Medical School, Chicago, Illinois 60612.

In view of the possible role of endogenous catecholamines and of 2-phenylethylamine (PEA) on behavior (Sabelli et al., in Neurohumoral Coding of Brain Function, Myers and Drucker-Colin, Plenum Press, 1974), and the close metabolic and structural relationships between these compounds, we are conducting pharmacological studies designed to test the hypothesis that PEA is a modulator of brain catecholaminergic synapses.

Metabolic studies were conducted in rabbits. The disposition of intraventricularly injected C^{14}-PEA was delayed by the inhibitor of catecholamine synthesis α-methyl-p-tyrosine (αMPT) (100 mg/Kg, 3 hrs prior) but not by reserpine (0.5 mg/Kg, 24 hrs prior plus 3 mg/Kg, 4 hrs prior). D-amphetamine (10 mg/Kg) accelerated PEA disposition (probably via PEA release) in control animals and delayed PEA disposition (possibly via MAO inhibition) in rabbits pretreated with αMPT or with reserpine.

Physiological studies were conducted in mice. In the maximal electroshock seizure test, PEA (100 mg/Kg) or PEA (20 to 50 mg/Kg, 24 hrs after isocarboxazid 50 mg/Kg) was weakly anticonvulsant. This effect of PEA was reduced by the α blocker phentolamine (20 mg/Kg); however, this PEA effect was not prevented by αMPT or by reserpine and it was enhanced by chlorpromazine (1 mg/Kg).

Exploratory behavior was augmented by PEA (an effect not prevented by αMPT or by reserpine), and it was initially reduced (30 min) and subsequently (120 min) augmented by L-DOPA (200 mg/Kg); this delayed hyperactivity induced by L-DOPA was absent in mice pretreated with MK-486 (L-α-methyldopa hydrazine, 200 mg/Kg) a brain PEA depleter (Borison et al., Life Sci., 1974) suggesting that DOPA and its metabolites increase activity via endogenous PEA.

Regarding extrapyramidal function, in which dopaminergic synapses are involved, the administration of PEA [in mice pretreated with monoamine oxidase inhibitors (MAOI)] relieved the "parkinsonism" induced by reserpine, induced choreic-like movements, and markedly potentiated the similar neurological effects of L-DOPA. Droperidol, which is presumed to block dopaminergic receptors, effectively prevented most of these neurological effects of PEA; chlorpromazine was weaker, and specific blockers of norepinephrine receptors (phentolamine and propranolol) were totally ineffective as PEA antagonists. In mice pretreated with the MAOI isocarboxazid (100 mg/Kg, 24 hrs prior, 50 mg/Kg 2 hrs prior), the dopamine-β-hydroxylase inhibitor bis(4-methyl-1-homopiperacinylthiocarbonyl) disulfide (FLA-63) (50 mg/Kg, 4 hrs prior) potentiated the behavioral stimulation induced by PEA (6 mg/Kg), by Δ^9-tetrahydrocannabinol (5 mg/Kg) [which increases the brain content and effects of PEA probably via inhibition of its disposition (Sabelli et al., Life Sci., 1974; Nature, 1974)] or by their combined administration.

These results confirm previous conclusions that β-hydroxylated phenylethylamines do not mediate the stimulant effects of PEA (Sabelli et al., Psychopharmacologia, in press).

These and other results suggest the following working hypothesis: alerting stimuli and drugs (e.g., amphetamine) release brain catecholamines thereby causing arousal; catecholamine release may in turn release PEA which would sustain behavioral excitement. This sequence of events is suggested by the ability of αMPT and MK-486 to block the central effects of amphetamine (Sabelli and Borison, ASPET, Fall Meeting, 1975) but not those of PEA, while PEA depletion by MK-486 blocks some of the stimulant effects of DOPA. Further, catecholamine depletion reversed the influence of amphetamine upon PEA disposition, and αMPT per se delayed PEA disposition, suggesting that endogenous newly formed catecholamines might physiologically release brain PEA. In turn, the central effects of PEA appear to be due in part to catecholamine release and in part to direct effects on non-catecholamine receptors. Supported by NIMH (MH-14110) and St. Ill. (510-11)

609 OCTOPAMINE IN THE BRAINS OF CHILDREN WITH ACUTE HEPATIC ENCEPHALOPATHY (REYE'S SYNDROME). K.G. Lloyd, L. Davidson*, D.G. Gall* and H.J. McClung*. Clarke Institute of Psychiatry (K.G.L. and L.D.) and Hospital for Sick Children (D.G.G.), Toronto, Ont., Canada and Children's Hospital, Columbus, Ohio, U.S.A. (H.J.M.).

Previously we have shown a large deficit of noradrenaline (NA) in the hypothalamus taken post-mortem from children dying of Reye's syndrome (RS). Other brain areas were less affected, and in the hypothalamus HVA, 5-HT, 5-HIAA and choline acetyltransferase levels were similar to those of age-matched controls. The false transmitter hypothesis of hepatic coma suggests that the lowered NA levels are due to displacement by the accumulation of octopamine (OCT). In the present study hypothalamic OCT was found to be much higher in RS patients than in age-matched controls (12 fold) or in adults (8 fold). However the RS patients were maintained on a respirator before death; in 3 age-matched controls (2 with severe liver complications) also maintained on a respirator, hypothalamic OCT levels were within the RS range (10 fold control). Whether this was due to the liver complications or the respirator is unknown, but the former is suggested as in 2 adults who were maintained on a respirator the OCT levels were not elevated. In other areas (e.g. caudate, septum, hippocampus) the OCT levels were not different between RS and age-matched controls. The present results of elevated hypothalamic OCT in RS lends direct support to the hypothesis of a NA deficit and false transmitter involvement in hepatic coma. However, a note of warning should be made as to the significance of OCT levels in patients maintained on respirators. Supported by the Clarke Institute of Psychiatry and the Hospital for Sick Children Foundation.

610 OCTOPAMINE INCREASES CYCLIC AMP CONTENT OF CRUSTACEAN GANGLIA AND CARDIAC MUSCLE. R. E. Sullivan* and D. L. Barker. (SPON: C. Kimmel). Biology Department, University of Oregon, Eugene, Oregon 97403.

Although octopamine has been found in several invertebrate nervous systems, little is known about its physiological roles. We have studied the effects of octopamine on cyclic AMP content and physiological activity in neural and cardiac tissue from the spiny lobster Panulirus interruptus and the crab Cancer magister. Isolated ganglia or hearts were incubated for various times at 12°C in aerated saline containing 100μM d,l-octopamine, and assayed for cyclic AMP by the protein binding method of Brown et al. (Biochem. J. 121, 561 (1971). Average values for unincubated controls were (pmoles cyclic AMP/mg protein): lobster thoracic ganglia, 17.5±6.5; lobster heart, 6.3±1.1; crab thoracic ganglia, 28.3±2.7; crab heart, 10.1± 2.9. The cyclic AMP content of lobster thoracic ganglia increased to 1.7 and 3.8 times the control value after 5 and 15 min. exposure to octopamine. For lobster heart, cyclic AMP was 2.4 and 11.8 times control after 1 and 5 min. incubation. Similar increases were found for crab tissues. Values were reduced by 30% when sample aliquots were passed through alumina columns before assay, suggesting significant amounts of cross-reacting material in unpurified samples. The cyclic AMP content of lobster stomatogastric ganglia was also measured, and found to increase about 5 fold after 5 min. exposure to octopamine. Extracellular recordings of the motor output from this ganglion show characteristic changes in the firing patterns of identified cells which develop 2 to 5 min. after exposure to octopamine. Isolated, perfused crab hearts gave dose-dependent, positive ionotrophic and chronotrophic responses to octopamine, which were maximal at 5 to 10μM. These observations may have physiological significance, since octopamine is synthesized in the lobster stomatogastric nervous system and in the crab pericardial organ (Barker and Hooper). Supported by USPHS Grant NS-10614.

611 SYNTHESIS OF DOPAMINE AND OCTOPAMINE IN THE CRUSTACEAN STOMATOGASTRIC NERVOUS SYSTEM. D. L. Barker and N. K. Hooper*. Biol. Dept., Univ. of Oregon, Eugene, Oregon 97403.

Modulation of the motor patterns intrinsic to the crustacean stomatogastric ganglion (SGG) may occur in at least two ways: 1) synaptic input from extrinsic neurons whose axons reach the SGG through the stomatogastric nerve (SGN), and 2) hormonal control by factors flowing in the dorsal aorta, in which the SGG lies. We have studied tyrosine metabolism in stomatogastric nerves and ganglia from Panulirus interruptus to identify candidates for synaptic transmitters and other modulators. Isolated nerve-ganglion preparations were incubated in saline containing 40μM ^{3}H-tyrosine for 4 hours. After washing with cold saline, nerves and ganglia were extracted at pH 1.9 and labeled metabolites were separated by high-voltage electrophoresis. Chemical identities were confirmed by three TLC systems. Dopamine was synthesized in the SGG, SGN, superior and inferior œsophageal nerves (SON and ION), inferior ventricular nerve (IVN), and œsophageal and commissural ganglia (OG and CG). This distribution closely matches areas of catecholamine-like fluorescence (Kushner and Maynard) and the location of the smaller of two types of dense core vesicle observed by EM (Friend and Maynard). Octopamine was synthesized in the SGN, SON, CG and in the dorsal ventricular nerve (DVN). Thus octopamine might be a compound stored in the larger type of dense core vesicle, found in non-fluorescent fibers which do not form conventional synapses in the SGG. Another possible source of hormonal modulators is the pericardial organ, which we isolated from the crab Cancer magister. This structure synthesized dopamine and octopamine from ^{3}H-tyrosine, and serotonin from ^{3}H-tryptophan. Each of these amines, when applied to the SGG at 1 to 20μM, modulates ganglion activity in a characteristic fashion. (Supported by USPHS Grant NS-10614.)

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Abstract withdrawn by author

613 EFFECT OF p-CHLOROPHENYLALANINE UPON THE METABOLISM OF SEROTONIN FROM 5-HYDROXYTRYPTOPHAN. P.E. Penn*, T.P. Hyde*, W.J. McBride, J.E. Smith* and J.D. Lane*. The Institute of Psychiatric Research and Depts. of Biochemistry and Psychiatry, Indiana University Med. Ctr., Indianapolis, IN. 46202.

It has been reported (Boggan et al., Psychopharm. 33, 1973) that three consecutive daily injections of p-chlorophenylalanine (PCPA) in rats potentiated the disruptive effects upon responding on an appetative FR 40 schedule produced by an injection of D,L-5-hydroxytryptophan (D,L-5-HTP) 15 days following the initial PCPA injection. Since there is evidence (Aprison et al., Fed. Proc. 34, 1975) to indicate that this type of behavioral disruption may be mediated by serotonin (5-HT), the present studies were undertaken to determine if injections of PCPA could have long-term effects on the metabolism and release of 5-HT. In vivo studies on the uptake and metabolism of [^{3}H]D,L-5-HTP (injected i.v. on day 15) in the telencephalon (TEL) and brain stem (BS) of rats given prior injections of either PCPA or saline demonstrated that the animals pretreated with PCPA had a higher specific activity (S.A. in dpm/nmole) of 5-HIAA (23,000 + 5,220, TEL; 15,200 + 1,010, BS) than the control group (13,900 + 3,060, TEL; 11,400 + 891, BS). In the TEL of the animals pretreated with PCPA, the S.A. of 5-HIAA was 16 times greater than the S.A. of 5-HT while in the control group it was 7 times greater. The data suggest that there is a small compartment of 5-HT that is being metabolized at a greater rate than the total pool of 5-HT and in the animals pretreated with PCPA this small pool appears to be turning over at a higher rate. (Supported in part by Research Grant MH-03225-16 and Postdoctoral Training Grant MH 10695 from NIMH).

614 EFFECT OF 5,7-DIHYDROXYTRYPTAMINE ON SEROTONIN AND TRYPTOPHAN HYDROXYLASE IN DISCRETE REGIONS OF THE RAT BRAIN. Juan M. Saavedra and Julius Axelrod, Laboratory of Clinical Science, NIMH, Bethesda, Md. 20014

5,7-dihydroxytryptamine (50μg) was administered intraventricularly to rats pretreated 30 minutes before with 25 mg/kg of desmethylimipramine (DMI). Fourteen days after the injections, the serotonin content and the tryptophan hydroxylase activity of 13 individual nuclei were examined. A parallel decrease of serotonin and tryptophan hydroxylase was observed in all areas. A 40% decrease was noted in the raphe nuclei, the area postrema and the locus coeruleus. The substantia nigra, medial forebrain bundle, and median eminence showed a reduction of 50%. In the nucleus suprachiasmatis, the subfornical organ and the nucleus arcuatus, the serotonin content and tryptophan hydroxylase activity were reduced to less than 10%. The results show that the local synthesis of serotonin is affected to different degrees by 5,7-dihydroxytryptamine in different brain nuclei, and confirm the presence of serotoninergic terminals in the circumventricular organs, median eminence and nucleus arcuatus.

615 CONTROL OF BRAIN SEROTONIN SYNTHESIS AFTER INHIBITION OF SEROTONIN RE-UPTAKE BY LILLY 110140. Kenneth W. Perry* and Ray W. Fuller. The Lilly Research Laboratories, Indianapolis, Ind. 46206.

Serotonin (5HT) turnover in rat brain appears to be decreased after treatment with Lilly 110140 [3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine hydrochloride] or other inhibitors of 5HT uptake (chlorimipramine, imipramine). Presumably suppression of 5HT synthesis results from enhanced 5HT action on synaptic receptors when inactivation of 5HT by reuptake is blocked. We have the following evidence that 110140 reduces brain 5HT turnover: decrease in 5-hydroxyindoleacetic acid (5HIAA) levels and in rate of 5HIAA accumulation after probenecid, slower decline in 5HT after inhibition of its synthesis, and slower accumulation of 5HTP after decarboxylase inhibition. In addition, Bymaster and Wong (Pharmacologist 16, 244, 1974) reported a decreased rate of ^{3}H-5HT synthesis from ^{3}H-tryptophan in 110140-treated rats. We found, however, that 110140 pretreatment did not alter the rate of brain 5HT accumulation after inhibiting MAO with N-cyclopropyl-2,4-dichlorophenoxyethylamine in rats. As Meek and Fuxe (Biochem. Pharmacol. 20, 693, 1971) suggested, "normal control of 5HT synthesis appears to be lost after MAO inhibition". Brain tryptophan concentration is thought to be a predominant factor influencing 5HT synthesis (R. J. Wurtman and J. D. Fernstrom, in Perspectives in Neuropharmacology, pp. 143-193, 1972). We found that elevating brain tryptophan concentration by the i.p. injection of L-tryptophan or of aminophylline increased 5HT and 5HIAA levels in control and in 110140-pretreated rats. The total 5-hydroxyindole production was less in 110140-pretreated rats than in controls, suggesting that the suppression of synthesis of 5HT after uptake inhibition still occurred even at elevated brain tryptophan concentrations. The exact molecular mechanism for the decrease in serotonin synthesis following inhibition of reuptake remains unknown.

616 EFFECT OF TRANSIENT ISCHEMIA ON MONOAMINE LEVELS IN THE CEREBRAL CORTEX OF GERBILS. R.Gaudet†, Eva Chabi†, K.M.A.Welch and Bessie Wang*. Dept. Neurol., Baylor Coll. Med., Houston, Texas, 77025.

Studies in this laboratory have shown that permanent unilateral occlusion of the common carotid artery (CCA) in the Gerbil causes release and depletion of monoamines in ischemic cerebral cortex. In the present study transient cerebral ischemia (5, 30, 60 min.) was induced by application (occlusion) and removal (reflow) of an aneurysm clip to the right CCA in 219 animals. 41% exhibited neurological signs of cerebral hemispheric ischemia (stroke) upon recovery from light ether anesthesia. Cortical monoamine levels were measured in animals sacrificed under liquid N_2 during ischemia and at intervals after reflow.

5-HT levels decreased in occluded and non-occluded cerebral hemispheres of all animals, the degree of reduction being more marked in ischemic hemispheres of stroked animals. Levels decreased further immediately after reflow and showed slow recovery.

Dopamine (DA) levels initially increased in stroked animals, followed by decrease after prolonged occlusion. Immediate and further decrease followed by a rebound increase to above control values was seen in DA levels after reflow in animals occluded for 5 or 30 minutes. No recovery in DA levels occured in the ischemic hemisphere of stroked animals occluded for 60 minutes.

Norepinepherine levels were unchanged in both stroked and non-stroked animals. In all cases a rebound increase was seen immediately after reflow.

Cortical monoamines, particularly 5-HT, appear exquisitely sensitive to transient ischemia since alterations in levels occured in stroked as well as non-stroked animals after CCA occlusion. In general the degree of reduction , or potential for recovery of monoamine levels after reflow , seems dependant on duration and severity of ischemia.

617 DEVELOPMENTAL PROTEIN MALNUTRITION IN RATS: CHANGES IN BIOGENIC AMINE LEVELS IN REGIONAL BRAIN AREAS AND IN PERIPHERAL TISSUES. Oscar Resnick*, Warren C. Stern, Maravene Miller*, William B. Forbes and Peter J. Morgane. Worcester Fndn. Exp. Biology, Shrewsbury, Mass. 01545.

The development of serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and norepinephrine (NE) from birth to 300 days in regional brain areas and in peripheral tissues was examined in rats fed a normal (laboratory chow or 25% casein) or a low protein (8% casein) diet. The malnourished group, which received the low protein diet starting 5 wks prior to conception, showed significantly elevated brain and peripheral tissue (heart, lung and stomach) levels of the biogenic amines and 5-HIAA at birth (table below). This appears to be one of the earliest ages at which protein malnutrition has been reported to affect a major biochemical measure in the brain. After birth the rats were maintained on their respective diets. In the malnourished groups brain concentration of 5-HT and 5-HIAA remained elevated at older ages, up to 300 days, with the largest effects (200% increases) occurring in subtelencephalic brain regions, especially the midbrain-pons-medulla areas. This suggests that some of the effects of developmental protein malnutrition are localized at the level of indoleamine containing cell bodies since the midbrain-pons-medulla areas contain most of the serotonergic perikarya of the brain. The increase in brain indole concentrations probably represent a general metabolic alteration in indole metabolism since elevated 5-HT and 5-HIAA levels were also observed in the heart, lung and stomach.

At most ages the increase in brain NE concentration in the brains of malnourished rats was less pronounced than for 5-HT. Interestingly, no significant increases in NE levels were observed in peripheral tissues. With respect to NE, however, the brain appears to be more sensitive to the insult of protein malnutrition than do peripheral tissues. The present results demonstrate that maintaining rats on a diet low in protein, but adequate in all other respects, significantly elevates brain biogenic amine content from birth through age 300 days. These results indicate that inadequate availability of dietary protein during the prenatal period significantly influences brain development.

Mean ± S.E. ng/gm on Day of Birth

	Telenceph. n=6	Brainstem n=6	Heart n=12	Lung n=13	Stomach n=12
5-HT					
Lab Chow	294 ± 20	498 ± 39	610 ± 32	540 ± 56	685 ± 119
25%	375 ± 21	294 ± 20	---	---	---
8%	288 ± 25	732 ± 18[a,b]	916 ± 52[b]	762 ± 44[b]	1180 ± 94[b]
5-HIAA					
Lab Chow	383 ± 108	487 ± 73	740 ± 50	846 ± 114	596 ± 80
25%	407 ± 37	605 ± 25	---	---	---
8%	573 ± 33[b]	773 ± 39[b]	1345 ± 138[b]	912 ± 82	1022 ± 102[b]
NE					
Lab Chow	212 ± 34	237 ± 23	343 ± 27	282 ± 29	376 ± 30
25%	118 ± 18	158 ± 20	---	---	---
8%	234 ± 40[a]	761 ± 34[a,b]	421 ± 40	260 ± 33	468 ± 50

[a]$p < 0.05$ compared to 25% casein; [b]$p < 0.05$ compared to Lab Chow.

Supported by grant HD 06364.

618 EXCRETION OF 5-HYDROXYINDOLEACETIC ACID AND HOMOVANILLIC ACID BY HUMANS AND RATS IN RESPONSE TO CHANGES IN DIETARY PROTEIN.

M. Nomura*, J.D. Fernstrom, B. Hammarstrom*, H.N. Munro*, W. Rand*, and R.J. Wurtman. MIT, Cambridge, Mass. 02139.

Fernstrom and Wurtman (*Sci. Am.*, 1974) showed previously that the diet consumed by rats affects brain serotonin synthesis; the formation of this indoleamine varies inversely with the protein content of the meal. However, urinary output of 5-HIAA (the serotonin metabolite 5-hydroxyindoleacetic acid) also reflects serotonin formation in other tissues (e.g., gut) as well as brain. To determine whether the protein content of the diet affects both total urinary output of 5-HIAA from all sources, and output of HVA (homovanillic acid, the major metabolite of dopamine formed in brain and other tissues), 7 normal human subjects received formula diets providing either 0, 75, or 150 g protein (as egg protein) per day with other nutrients in adequate constant amounts. The diets were fed as 3 equal meals for 4 days at each protein level, and urine was collected daily in 8-hr periods (7AM-3PM, 3PM-11PM, 11PM-7AM). With increasing protein intake urinary 5-HIAA output rose from 2.61 ± 0.16 (s.e.m.) through 3.00 ± 0.16 to 3.46 ± 0.18 mg/day, while HVA excretion progressed from 3.18 ± 0.49 through 3.73 ± 0.62 to 4.54 ± 0.59 mg/day. Excretion of 5-HIAA showed no distinct daily rhythm, whereas HVA output was maximal during the period between 7AM and 3PM.

Similar studies were performed on rats (initially weighing 200 g) that received diets providing 0, 18, or 40% protein for 4- to 7-day periods. Urinary output of 5-HIAA again rose significantly with each increase in dietary protein (see table). Administration of the peripheral decarboxylase inhibitor MK-486 (100 mg/kg 3 times daily throughout) eliminated the increase due to dietary protein.

Dietary Protein	5-HIAA Output (µg/kg/day)	
	Controls	MK-486-treated
0%	140 ± 5 (17)	109 ± 6 (9)
18%	166 ± 3 (44)	77 ± 5 (21)
40%	271 ± 16 (20)	95 ± 8 (11)

Numbers of urine collections are given in parentheses.

These observations indicate that (1) the synthesis rates by man of 5-HIAA and HVA (and thus presumably of serotonin and dopamine) increase directly with the protein content of the diet; (2) synthesis of 5-HIAA by the rat shows a similar relationship to dietary protein level; (3) this acceleration of serotonin synthesis induced by protein occurs largely or entirely in tissues other than brain, in agreement with our earlier observation that dietary protein inhibits brain serotonin synthesis; (4) control of dietary protein content is essential in studies relating urinary metabolite output to brain monoamine metabolism.

(Dr. Nomura holds a Fellowship from the Parkinson's Disease Foundation.)

619 CALCIUM-DEPENDENT EFFLUX OF ASPARTATE, GLUTAMATE AND γ-AMINOBUTYRATE FROM RAT HIPPOCAMPAL FORMATION: EFFECTS OF SELECTIVE LESIONS. J. Victor Nadler, Kenneth Vaca*, Carl W. Cotman and Gary S. Lynch. Dept. Psychobiol., University of California, Irvine, Ca. 92664.

Aspartate and glutamate have frequently been proposed as excitatory neurotransmitters in the central nervous system, but they have seldom been associated with particular neurons or fiber tracts. To determine whether these amino acids may be transmitters in the rat hippocampal formation, we have monitored the efflux of endogenous aspartate, glutamate and γ-aminobutyrate (GABA) *in vitro* from slices of hippocampal regions using a continuous perfusion device. When the tissue was depolarized with 56 mM K^+ or 0.1 mM veratridine in Ca^{2+}-free medium, introduction of Ca^{2+} into the medium increased the rate of efflux of all three amino acids by about 500-800 pmol/30s/10 mg tissue. This effect of Ca^{2+} was dependent on depolarization. Ca^{2+} could be replaced by Ba^{2+} and its action was antagonized by Mg^{2+}. Thus the calcium-dependent efflux of aspartate, glutamate and GABA measured *in vitro* possesses some properties of physiological transmitter release.

We have attempted to relate calcium-dependent efflux of aspartate and glutamate to particular excitatory inputs by using animals with selective lesions. Efflux of GABA served as a control in these studies, since GABA is thought to be the transmitter only of intrinsic inhibitory interneurons in the hippocampal formation, and therefore the calcium-dependent efflux of GABA should not be reduced by lesions.

To investigate the possibility that glutamate or aspartate is released by the mossy fibers (granule cell axons), rats were used which had received low-level X-irradiation during the first 15 days after birth, a treatment which reduced the granule cell population by about 75%. In these animals, the tissue content of glutamate was reduced by 15-20% in dentate gyrus and regio inferior, regions in which the mossy fibers terminate, but was unchanged in regio superior, which receives no mossy fiber input. However, X-irradiation did not reduce the calcium-dependent efflux of glutamate from regio inferior, indicating that the high glutamate content of the mossy fibers does not imply a transmitter function. The efflux of both glutamate and aspartate from dentate gyrus was reduced by X-irradiation. This result probably reflected the establishment of fewer synapses which use these amino acids as transmitters because there were fewer granule cells on which to form synapses.

Removal of the commissural afferents reduced the calcium-dependent efflux of aspartate from both dentate gyrus and regio superior by about half. Since efflux of aspartate was reduced in two regions to which commissural fibers project, aspartate may be released by this afferent and could therefore be the commissural transmitter. Commissurotomy also reduced the calcium-dependent efflux of glutamate from regio superior, but not from dentate gyrus. The source of this glutamate efflux is under investigation.

Bilateral removal of the perforant path did not reduce the calcium-dependent efflux of aspartate, glutamate or GABA from dentate gyrus or regio superior. However, efflux of GABA and aspartate from dentate gyrus was increased by 50-100%. This result may be related to previous reports that commissural afferents and GABAnergic interneurons increase their input to the granule cells after lesions of the perforant path.

Our efflux data therefore suggest aspartate as a strong candidate for transmitter of the hippocampal commissural fibers, but do not support a transmitter role for glutamate or aspartate in the mossy fiber or perforant path systems. (Supported by NIH grant NS 08597).

620 NEUROTRANSMITTER RELEASE FROM BRAIN: I. THE INVOLVEMENT OF UPTAKE SYSTEMS. John W. Haycock, William B. Levy and Carl W. Cotman. Dept. Psychobiology, UC Irvine and Dept. Psychology, UC Riverside, California.

These studies investigated the accumulation of ^{3}H-NE and ^{14}C-GABA into synaptosomal fractions from mouse forebrain and the ability of these exogenously loaded pools to support subsequent Ca-dependent release. Synaptosomal fractions accumulated transmitter via high-affinity Na-dependent processes. Release of these molecules was absolutely dependent upon the presence of Na during loading but not during release. Thus although Na-dependent uptake was obligatory for subsequent release of accumulated transmitter, the release process itself was dissociated therefrom.

Temperature, length and substrate concentration of incubation affected the amount of accumulation by the synaptosomes. Fractional release in response to Ca, however, was affected only by the concentration of transmitter during loading. Changes in incubation temperature (22^{o},37^{o}C) or in incubation length (20sec-10min) did not alter release, but loading with concentrations of transmitter above their high-affinity K_ms decreased the percent released. These studies are consistent with a model in which transmitter accumulated by high-affinity Na-dependent processes rapidly equilibrates with intraterminal pools, including those immediately releasable. Attempts to demonstrate preferential release of "newly" vs "previously" accumulated GABA were unsuccessful. Decreasing the interval between loading and stimulation from 10 min to 90 sec (following a 20 sec load) did not affect percent release. Prior to stimulation, then, preferential accumulation of GABA in releasable pools was not obtained.

The rapidity with which accumulated transmitter has access to the release process suggests that, in the physiological situation, a major function of uptake processes may be to maintain the levels of neurotransmitter in releasable pools. (Supported by NIH grant NS 08597)

621 NEUROTRANSMITTER RELEASE FROM BRAIN: II. STIMULATION-DEPENDENT DEPRESSION AND RECOVERY. W.B. Levy, J.W. Haycock and C.W. Cotman. Dept. Psychology, UC Riverside and Dept. Psychobiology, UC Irvine, California.

The present study investigated the release of exogenously loaded transmitters (GABA, NE, DA) from brain synaptosomal preparations in response to twin Ca pulse (1.5mM) stimulation. Percent release of previously accumulated transmitter decreased in response to the 2nd Ca pulse at inter-stimulation intervals (ISI) of 90-120 sec. Release was depressed to approx. 60% of initial release values for these transmitters. Recovery from depression occurred much more slowly than the rate of onset. At an ISI of 10 min., the relative depression of, e.g., GABA release had recovered to 80% of initial release. These data suggest that mobilization of stores remaining following stimulation may be a limiting factor in the recovery from depression when no substrate is present for reuptake.

Although release to the 2nd Ca pulse was depressed, the release process per se was intact. The rapidity with which transmitter can be accumulated and distributed in this system allowed "reloading" of synaptosomes immediately following the 1st Ca pulse. A 2nd incubation (20sec) with the same transmitter having a different label is interposed between stimulations. For ISI's of 120 sec., release from the reloaded pools was comparable to initial release from the previously loaded pools. Thus the availability of transmitter and not the release process itself was depressed by the prior stimulation. Furthermore, prior stimulation allowed demonstration of preferential release of newly vs previously accumulated transmitter.

Previous studies of synaptic depression have not distinguished between decreased transmitter availability and some failure in the release process. The present data suggest that synaptic depression can result specifically from a decrease in available transmitter: In the depressed state, the release process was functional. Further, recovery from depression may result, in the physiological situation, from mobilization and reuptake.

622 DIFFERENTIAL EFFECTS OF ANTICONVULSANTS ON THE ACTIVE UPTAKE OF PUTATIVE NEUROTRANSMITTERS BY RAT BRAIN SYNAPTOSOMES. Jesse Weinberger*, William J. Nicklas* and Soll Berl. (SPON: Roger Duvoisin). Dept. of Neurology, Mount Sinai School of Medicine, New York, New York, 10029.

The effects of pentobarbital and diphenylhydantoin on the kinetics of the high affinity uptake of the putative neurotransmitters GABA, glutamate and norepinephrine were examined using a rat brain synaptosomal preparatio Pentobarbital and diphenylhydantoin significantly inhibited the uptake of norepinephrine (K_I = 1.4 and 0.11mM, respectively). Both drugs appeared to be non-competitive inhibitors of norepinephrine uptake. On the other hand, these drugs enhanced the accumulation of the amino acids. 1mM pentobarbital increased the uptake of GABA two-fold ($p<0.01$) with a concomitant 30-40% increase in the uptake of glutamate. 0.1mM diphenylhydantoin affected the uptake differently from pentobarbital in that glutamate uptake was increased two-fold ($p<0.01$) whereas that of GABA was increased by only 37% ($p<0.01$). These disparate effects of anticonvulsant on the uptake of norepinephrine vs. that of the amino acids suggest that these drugs may play a role in limiting the propagation of seizures throug the balance of excitatory glutamate pathways and inhibitory GABA and norepinephrine pathways. The contrasting effects of these drugs on GABA and glutamate uptake may be related to the hypnotic properties of pentobarbital not possessed by diphenylhydantoin. This work was supported in part by NIH grants MH 25505, NS 11824 and Clinical Center of Research on Parkinson's and Allied Diseases, NS 11631.

623 RELEASE OF ENDOGENOUS SEROTONIN, NOREPINEPHRINE AND DOPAMINE FROM NERVE ENDINGS. J. D. Lane* and M. H. Aprison The Inst. of Psychiatric Research, and Depts. of Biochemistry and Psychiatry, Indiana University Med. Ctr., Indianapolis, IN. 46202

Several investigators have studied the release of transmitter substances from brain slices and subcellular fractions using perfusion and incubation procedures. In most instances, the release was measured as the amount of radioactive isotope which accumulated in the media. Recent developments in methodology have increased assay sensitivities to the picomole level, permitting the measurement of endogenous levels of the biogenic monoamines released into the media. In this study, crude synaptosome fractions (P_2) were prepared from the telencephalon of the rat essentially by the method of Gray and Whittaker (J. Anat., 1962). The fractions were washed with a glucose-bicarbonate-saline (GBS) media (McIlwain et al., J. Neurochem., 1969) containing tranylcypromine, an MAO inhibitor. After centrifugation, the fractions were resuspended in a variety of medias including standard GBS, GBS with elevated potassium (55 mM) and GBS with elevated potassium and reduced calcium (O mM + 3 mM EGTA), and incubated 10 min. at 37°C. Following centrifugation, the pellets and the medias were assayed for serotonin, norepinephrine and dopamine (Smith et al., Anal. Biochem., 1975). Compared to the standard GBS, elevated potassium stimulated release of all three monoamines, with a concomitant decrease in the monoamines remaining in the respective pellets. The release also appeared to be sensitive in part to calcium, suggesting that vesicular release mechanisms may be involved. (Supported by Grant MH-03225-16, NIMH).

624 THE ROLE OF PRESYNAPTIC RECEPTORS IN RELEASE AND SYNTHESIS OF ^{3}H-DOPAMINE BY SLICES OF RAT STRIATUM. Thomas C. Westfall, Marie-Jo Besson*and J. Glowinski; Groupe de Neuropharmacologie Biochimique, College de France, Paris.

Striatal slices were continuously superfused with L,3.5-^{3}H-tyrosine (30-50 uCi/m). ^{3}H-H_2O (index of ^{3}H-DA synthesis) and ^{3}H-DA were estimated in 0.5^{2}ml superfusate fractions. Depolarization with 50 mM potassium (K), for 7.5 min. induced a marked increase in ^{3}H-DA release and a biphasic effect on synthesis (an increase followed by a decrease). Benztropine in a concentration which produces marked inhibition of DA uptake (10^{-6}M) increased the K induced overflow of ^{3}H-DA but failed to alter the inhibition of synthesis. On the other hand, when the powerful neuroleptic, fluphenazine (10^{-6}M), was added to the superfusion medium it potentiated ^{3}H-DA release and prevented the inhibition of synthesis both in the absence or presence of benztropine. A similar effect was seen following the in vivo treatment of rats with fluphenazine (2 mg/kg; 1 1/2 hour before sacrifice). The addition of exogenous DA (6 x 10^{-6}M) or norepinephrine (NE; 10^{-6}M) to the superfusion medium reduced DA synthesis. The DA effect was still observed in the presence of benztropine or $DMPH_4$ (5 mM) while the NE effect was prevented. Isoproterenol did not affect synthesis. The administration of fluphenazine significantly inhibited the decrease in synthesis induced by DA. These studies suggest that the activation of presynaptic DA receptors by the transmitter results in both the inhibition of release and synthesis (supported by INSERM, USPHS NS 10260 and the Macy Foundation).

625 IDENTIFICATION AND CHARACTERIZATION OF β-ADRENERGIC RECEPTORS IN RAT BRAIN BY (-)[^{3}H]ALPRENOLOL BINDING. R.W. Alexander *, J.N. Davis and R.J. Lefkowitz*, Dept. of Med., Duke University Medical Center, Durham, N.C.

Evidence suggests that β-adrenergic receptor (β-AR) mediated responses are important in the physiology of the central nervous system. The study of β-AR has been somewhat limited by the inability to study the receptors directly and by the difficulty of stimulating the β-receptor linked adenylate cyclase in broken cell preparations of brain. New techniques permit the direct characterization of the β-AR by binding with (-)[^{3}H] alprenolol, a potent β-AR antagonist. β-AR were identified in subcellular fractions derived from various areas of the rat CNS. These receptors exhibited a high degree of stereospecificity with the (-)isomers of β-adrenergic agonists and antagonists being more potent than were the (+)isomers in competing with (-)[^{3}H]alprenolol for the binding sites. (-)Propranolol bound to the receptors with high affinity (K_D=30nM). The α-adrenergic antagonist phentolamine and physiologically inactive catechol-containing compounds did not compete for receptor binding. Binding was saturable thus permitting determination of the number of β-AR in 11 CNS areas.

	β-adrenergic receptors pmole/mg protein		β-adrenergic receptor pmole/mg protein
Nucleus accumbens	0.28	Hypothalamus	0.16
Hippocampus	0.26	Pons	0.11
Corpus striatum	0.25	Cerebellum	0.11
Parieto-temporal cortex	0.22	Medulla	0.06
Frontal cortex	0.21	Spinal cord	0.04
Epithalamus-diencephalon	0.20		

There was no close correlation between numbers of β-AR and levels of norepinephrine or dopamine. The present methods offer a unique approach to the study of the central β-AR and should facilitate the study of β-adrenergic mediated mechanisms in the brain.

626 BIOCHEMICAL IDENTIFICATION OF THE β-ADRENERGIC RECEPTOR IN MAMMALIAN BRAIN. David B. Bylund and Solomon H. Snyder. Depts. Pharmacology and Psychiatry, Johns Hopkins Medical School, Baltimore, Md. 21205.

A binding site in mammalian brain, which appears to be the β-adrenergic receptor, has been identified using alprenolol, a potent β-adrenergic antagonist. Alprenolol, labelled with tritium to a specific activity of about 30 Ci/mmole, exhibits rapid, saturable, sterospecific and high affinity binding to a particulate fraction of rat brain prepared by centrifugation of a crude homogenate at 49,000xg for 15 minutes. The inhibition of the binding of the labelled compound by antagonists and agonists is as expected for the β-adrenergic receptor. Thus, the concentration of either (-)-alprenolol or (-)-propranolol needed to reduce the specific binding by 50% is about 5 nM. (Specific binding, defined as the difference in [^{3}H]-alprenolol in the presence and absence of 10^{-6} M (-)-alprenolol, was 70%-80% of the total binding.) The order of potency for the agonists is isoproterenol>norepinephrine>epinephrine. For both agonists and antagonists the (-)-isomers are at least 50 times more potent than the (+)-isomers. In addition, [^{3}H]-alprenolol binding is not inhibited by phenoxybenzamine (an α-adrenergic antagonist), dopamine, pyrocatechol or 2-n-propylphenol. The regional variation and the subcellular distibution of [^{3}H]-alpernolol binding in rat brain will also be discussed. (Supported by NIH grants DA-00266 and MH-18501.)

627 DOPAMINE RECEPTOR BINDING IN MAMMALIAN BRAIN. David R. Burt, S.J. Enna, Ian Creese and Solomon H. Snyder. Dept. Pharmacol., Sch. Med., Johns Hopkins Univ., Baltimore, Maryland 21205

Using appropriate radioactive ligands, we have demonstrated direct binding to a site in bovine corpus striatum which exhibits most of the characteristics expected of the dopamine receptor. In the calf, this site has an apparent affinity for dopamine in the range 5-15nM, with a slightly higher affinity for apomorphine and a 20-fold lower affinity for norepinephrine. The affinity for isoproterenol is about 1000-fold lower. Phenothiazines and other dopamine antagonists appear to bind in a specific fashion to the same or a related site, with relative potencies resembling their published efficacies in inhibiting the dopamine sensitive adenylate cyclase. Certain of these compounds exist in different optical or geometrical isomers which differ markedly in their pharmacological effectiveness. These isomers also show a high degree of specificity in reacting with the binding site. More limited studies with the corpus striatum of the rat have yielded 10-20 fold lower affinities for agonists and somewhat higher affinities for antagonists than appeared to be the case in the calf. This binding method promises to be extremely useful in elucidating the mechanism of pharmacological supersensitivity to dopamine, in clarifying the relationship of the dopamine-sensitive adenylate cyclase to dopamine receptors, and in screening new drugs for dopamine receptor activity. (Supported by NIH grants NS-01654, MH-01598, DA-00266 and MH-18501.)

628 DIFFERENTIATION OF GABAERGIC NEURONS AND THEIR POSTSYNAPTIC RECEPTORS IN THE RAT BRAIN. S.J. Enna and J.T. Coyle. Dept. Pharmacol., Johns Hopkins U., Sch. Med., Baltimore, Md. 21205.

Examination of various biochemical characteristics of the gabaergic nervous system in rat brain was made between 15 days gestation and adult. At birth, the concentration of γ-aminobutyric acid (GABA) in whole brain, midbrain, cerebellum, cortex, striatum and hypothalamus was approximately 50% of adult levels, whereas the medulla-pons had achieved adult levels by birth. Compared to GABA levels, there was a marked lag in the development of glutamate decarboxylase activity (GAD) in all areas studied. In addition, there was no correlation between the development of GABA uptake, GAD activity and GABA levels, with uptake in whole brain and midbrain 100% of adult at birth and only about 40% in the cerebellum and cortex. In all areas, GABA levels and GAD activity increased in a linear fashion from birth to adult, whereas in all areas GABA uptake was significantly higher at 8 days after birth than adult. Studies on the development of the postsynaptic receptor for GABA revealed that in all regions receptor binding was <25% of adult up to 8 days after birth at which time it increased dramatically, approximating adult levels by 4 weeks. The results suggest that presynaptic elements develop prior to the postsynaptic receptor for GABA. (Supported by USPHS Grants MH-01598 and DA-00266)

629 TRANQUILIZER RECEPTORS IN RAT STRIATUM. P. Seeman, M. Wong & J. Tedesco. Pharmacology Department, Univ. of Toronto, Toronto, Canada.

It is known that major tranquilizer drugs interfere with dopaminergic transmission, and Van Rossum in 1966 had suggested that these neuroleptic drugs block dopamine receptors. Our previous work had shown that the specific binding of ^{3}H-dopamine to disrupted synaptosomes (from rat striatum) was blocked by 25 nM haloperidol (Seeman et al., Fed. Proc. 33, 1974, p. 246), thus supporting Van Rossum's hypothesis. In order to confirm this by a different approach, we studied the binding of ^{3}H-haloperidol to disrupted synaptosomes prepared from rat brain striatum. The number of haloperidol binding sites was of the order of 2×10^{-13} moles per mg. of synaptosome protein. The concentrations of drugs which reduced the binding of ^{3}H-haloperidol by 50% are:

drugs:		transmitters:	
haloperidol	2 nM	dopamine	10 μM
(+)-butaclamol	3 nM	(apomorphine	1 μM)
(-)-butaclamol	> 1000 nM	serotonin	70 μM
spiroperidol	10 nM	norepinephrine	200 μM
chlorpromazine	20 nM	phenylephrine	200 μM
clozapine	100 nM	No effect at 10^{-4}M:	
chlordiazepoxide	3000 nM	phenobarbital	
diazepam	> 10^{-4} M	glycine	
diphenylhydantoin	> 10^{-4} M	glutamate	

The data indicate that haloperidol is bound specifically insofar as the dissociation constant is low, and it is displaced stereospecifically by (+)-butaclamol. These haloperidol receptors may be identical to dopamine receptors since dopamine (and apomorphine) was the most effective neurotransmitter to compete for binding. (Supported by Ontario Mental Health Foundation, and the M.R.C. of Canada grant MT-2951).

630 PREPARATION AND CHARACTERIZATION OF HORSERADISH PEROXIDASE CONJUGATED SNAKE NEUROTOXIN. W.A. Lutin*, R.N. Brady*, C.F. Jensen*, P. Skene*, and J.A. Freeman. Vanderbilt Univ., Nashville, TN. 37232. We have prepared and purified a new horseradish peroxidase (HRP) -conjugated snake α-neurotoxin using aldehyde coupling and molecular seive chromatography. The activity of the HRP-toxin is comparable to that of ^{3}H-toxin, ranging from 40 to 60% of the activity of native toxin measured by its ability to inhibit endplate potentials in toad sartorius muscle, to block post-synaptic potentials at the retino-tectal junction, and to compete with ^{3}H-Siamensis toxin of known biological activity for binding of acetyl choline receptor (AChR) protein in a soluble membrane extract from Torpedo electric organ. A quantitative assay for tissue-bound HRP-toxin was developed based on the ability of the peroxidase moiety to oxidize dianisidine dye. This procedure allows the detection of 10^{-14} M of HRP-toxin bound to a brain membrane pellet. The assay is superior to radioassays using ^{125}I-neurotoxin, avoiding the disadvantages of short half life, protein radiation damage, and health hazard. HRP-toxin was applied to toad sartorius muscle or brain and observed to localize in the same sites to which fluorescein isothiocyanate-labeled α-bungarotoxin and ^{3}H-toxin were previously shown to bind, using light microscopy (Freeman and Lutin, Neurochem. Soc. 1975 Abst. #132). Electron microscopy has revealed localization of HRP-toxin binding to the end plate region in muscle and to the sub-synaptic membrane in brain. We conclude that HRP-toxin is a good probe for histochemical localization of AChR in brain, and is a sensitive ligand for assaying AChR in vitro.

631 CORRELATION OF PATTERN OF CATECHOLAMINE DENERVATION AND BEHAVIORAL DEFECT AFTER INTRAHYPOTHALAMIC 6-HYDROXYDOPAMINE. J. Stephen Fink* and Gerard P. Smith, Dept. Psychiat., Cornell U. Med. Coll. and E.W. Bourne Behavioral Research Laboratory, New York Hospital, White Plains, N.Y. 10605.

Anterolateral (AL), but not anteromedial (AM), hypothalamic microinjections of 6-hydroxydopamine (6-OHDA) produce deficits in active avoidance (Brain Res. 88:483, 1975), consummatory behavior (Nature New Biol.235:27, 1972) and visual placing (Exp. Neurol. 41:723, 1973) in rats. To analyze the pattern of catecholamine (CA) damage, forebrain regions from AL (n=10) and AM (n=7) 6-OHDA rats and AL (n=5) and AM (n=5) vehicle rats were sectioned with the Vibratome and processed by the glyoxylic acid histofluorescence method. The loss of CA fluorescence in AL and AM vehicle rats was restricted to the hypothalamic region at the tip of the cannula track. In contrast, loss of CA fluorescence in AL and AM 6-OHDA rats was widespread. Both AL and AM 6-OHDA groups showed severe CA loss in parietal and frontal cortex, hippocampus and the molecular layer of the piriform cortex. Only AL 6-OHDA rats showed evidence of severe CA loss in the AL hypothalamus, the head of the caudate nucleus, nucleus accumbens, the dorsal part of the bed nucleus of stria terminalis, olfactory tubercle, lateral septal nucleus and the submolecular layers of the piriform cortex. Only AM 6-OHDA rats showed severe loss of CA in the AM hypothalamus and medial preoptic area. These data demonstrate a different pattern of CA damage in animals displaying different behavioral effects of AL and AM 6-OHDA microinjections and they suggest that severe loss of CA in AL hypothalamus, nucleus accumbens, head of the caudate, lateral septal nucleus, dorsal bed nucleus stria terminalis, olfactory tubercle, submolecular piriform cortex or some combination of these areas is necessary for the behavioral defects.

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632 PREVENTION OF THE NEURODEGENERATIVE ACTIONS OF 6-HYDROXYDOPAMINE (6-OHDA) AND 6-AMINODOPAMINE (6-ADA). G. Cohen, B. Allis*, B. Winston*, C. Mytilineou and R. Heikkila*. Dept. of Neurology, Mt. Sinai Sch. Med., New York, New York, 10029.

We report that 1-phenyl-3-(2-thiazolyl)-2-thiourea (PTTU) prevents the toxic actions of 6-OHDA and 6-ADA on adrenergic nerve terminals in the mouse atrium and iris. The degree of destruction or damage to nerve terminals was evaluated in two ways: by measurement of the accumulation of ^{3}H-norepinephrine (^{3}H-NE) by nerve terminals in the left atrium, and by direct visualization of the adrenergic nerve plexus in the iris by means of the Falck-Hillarp fluorescence method. PTTU was injected i.p. as a suspension in 0.25% methylcellulose one hour prior to i.v. 6-OHDA or 6-ADA. The following are representative results for accumulation in vitro of ^{3}H-NE (3×10^{-8} M, 10 min., 37°C) by the left atrium. Results are the mean $\pm$ SEM expressed as a % of the accumulation seen in untreated controls. At 72 hours after 6-OHDA·HBr (10 mg/kg), the accumulation of ^{3}H-NE was 17.4% $\pm$ 1.4% of control (N=41) after 6-OHDA alone, and 77.3% $\pm$ 6.9% (N=32) after PTTU (200 mg/kg) plus 6-OHDA. At 24 hours after 6-ADA·2HCl (10 mg/kg), the accumulation of ^{3}H-NE was 31.8% $\pm$ 3.0% (N=22) after 6-ADA alone, and 95.8% $\pm$ 6.9% (N=32) after PTTU plus 6-ADA. Treatment with PTTU had no effect on the accumulation of ^{3}H-NE in vitro. In vivo measurements showed that the accumulation of ^{3}H-NE (i.v., 100 µc/kg) by the left atrium was normal at one hour after PTTU. Inhibition of dopamine beta-hydroxylase and binding of cellular copper by PTTU were both excluded as possible mechanisms of action by the observations that diethyldithiocarbamate (1 gm/kg), a potent copper chelator and dopamine beta-hydroxylase inhibitor, was not protective. An explanation for protection by PTTU may lie in the capacity of thiourea derivatives to scavenge cytoxic hydroxyl radicals that are generated during the autoxidation of 6-OHDA or 6-ADA. (Supp. by Grant NS-05184 from the USPHS).

633 BEHAVIORAL AND BIOCHEMICAL EFFECTS OF NEONATAL 6-HYDROXYDOPA INJECTIONS. Rachel Sweig*, Jack H. McLean*, Richard M. Kostrzewa, and James G. May. Department of Psychology, University of New Orleans, and Veterans Administration Hospital, New Orleans, Louisiana 70122.

Female Sprague-Dawley rats received injections of either 6-hydroxydopa (60 µg/g, i.p.) or saline on days 1 and 3 of life. Animals were tested in adulthood on tasks involving female sexual behavior, pain sensitivity, equilibrium, acquisition and memory for a passive avoidance task, and open-field activity. Day of vaginal opening was recorded and subsequently vaginal smears were taken for a 21-day period. Results indicated that treated animals were less sensitive to thermal pain, fell more often on a rod balancing task, were generally more active in open-field tests, exhibited less of an activity decrease following noxious stimulation, and were lighter in weight in comparison to the control group. Other behavioral measures failed to reach statistical significance.

When compared to saline animals, endogenous norepinephrine of treated rats was significantly reduced in the cortex, hippocampus, amygdala and spinal cord, but was elevated in the cerebellum.

634 COMPETITIVE UPTAKE BETWEEN 6-HYDROXYDOPAMINE AND CATECHOLAMINES BY THE ADRENERGIC TERMINALS. Hideo Uno* and Jack H. Fellman*. (SPON: C. C. Bell). Lab. Path., Oreg. Reg. Primate Res. Ctr., Beaverton, OR 97005 and Dept. Biochem., U. Oreg. Health Sci. Ctr., Portland, OR 97201.

Released noradrenaline is recaptured by the presynaptic terminals of adrenergic neurons. This neuronal uptake by the terminals can occur in the other amines structurally related to noradrenaline (NA) such as adrenaline (A), dopamine, and tyramine (Axelrod, Harvey Lect. 67:175, 1973). Using the following materials and techniques, we examined the competitive uptake of 6-hydroxydopamine (6-OHDA) and other catecholamines by the adrenergic terminals in smooth muscles. Dense plexuses of nerve fibers in the piloarrector muscles of the scalp skin of stump-tailed macaques show the intense fluorescence of catecholamines (Falck & Hillarp method). Ultrastructurally most of the terminal axon profiles in the muscles contain numerous dense-cored vesicles. After an intradermal injection of 6-OHDA (1 mg per 1 cm^2 skin surface area) into the scalp skin, fluorescence completely disappeared from all the muscles examined in the injected area, and the terminal axons showed severe degenerative change. These histochemical and ultrastructural changes in the terminals induced with 6-OHDA provided a morphological index of its uptake. Each of the other amines--5-hydroxydopamine (5-OHDA), NA, A, and dopamine (1 or 5 mg) --was injected simultaneously with 6-OHDA (1 mg) into the same area of the skin and specimens were taken 24 hrs after the injection. The results are shown in the following Table:

	Terminals in piloarrector muscles		
Intradermal Injection (mg)	Fluores*	Ultrastr. change in axon	No.
6-OHDA(1)	-	degeneration of axon	3
6-OHDA(1) + 5-OHDA(1)	-	degeneration of axon	4
6-OHDA(1) + NA(1)	+	degranulation of vesicles	2
6-OHDA(1) + A(1)	+	degranulation of vesicles	2
6-OHDA(1) + 5-OHDA(5)	+	increased granularity in vesicles	4
6-OHDA(1) + NA(5)	++	no change	4
6-OHDA(1) + A(5)	++	no change	2
6-OHDA(1) + Dopamine(5)	++	no change	2

*Fluorescence: - negative, + weak, ++ strong

These results demonstrate that the uptake of 6-OHDA by the adrenergic terminals is inhibited by all of the amines examined in this study. The degree of inhibition depended largely on their concentration in the local environment. When 5-OHDA was administered in the same concentration as 6-OHDA, it did not interfere with the uptake of 6-OHDA since among the isomers, 6-OHDA apparently has a stronger affinity for uptake by the terminals than 5-OHDA. When concentrations of A and NA equal to 6-OHDA were injected, the uptake of 6-OHDA was inhibited and the changes effected in the terminals by 6-OHDA were greatly reduced. When the dosage of the amines was five times that of 6-OHDA, the effect of 6-OHDA on the terminals was completely blocked. Our previous histochemical and ultrastructural observations showed that when each of these catecholamines was injected alone, the adrenergic neurons did not undergo detectable changes. (Supported by NIH Grants RR00163 and RR05694 and AM08445 from the National Institute of Arthritis and Metabolic Diseases.)

635 EFFECT OF 6-HYDROXYDOPAMINE PRETREATMENT ON LOCOMOTION IN THE MESENCEPHALIC CAT. Larry M. Jordan and John D. Steeves*. Dept. of Physiology Faculty of Medicine, University of Manitoba, Winnipeg, Canada R3E 0W3.

Stimulation of the "mesencephalic locomotor region"(MLR) described by Shik, et al. (Biofizyka 11: 659-666,1966) induces locomotion in mesencephalic cats, and Grillner and Shik (Acta physiol. Scand.,87: 320-333,1973) have suggested that MLR stimulation activates a descending noradrenergic system arising from the brainstem which initiates activity in an intrinsic spinal mechanism responsible for locomotion. Previous evidence from this laboratory indicates that stimulation in the MLR excites catecholaminergic neurones in the locus coeruleus. In order to determine whether noradrenaline(NA)is essential for the initiation of locomotion by stimulation of the MLR, cats were injected with 6-hydroxydopamine (6-OHDA) at the twelfth thoracic level. In each cat, 2µl of 6-OHDA (2µg/µl)in Ringer containing 0.1% ascorbic acid was injected into the gray matter bilaterally (total dose,8µg). The animals were allowed to recover for 7 to 11 days;they were then anaesthetized, decerebrated at the precollicular level, and placed on a treadmill. Following recovery from anaesthesia, the MLR was stimulated and the evoked locomotion was observed. The 6-OHDA treatment did not alter the capability of the mesencephalic cats to walk on a treadmill in response to stimulation of the MLR;coordinated locomotion could be observed in all 4 limbs, and the hindlimbs frequently began locomotor movements prior to commencement of locomotion in the forelimbs. Spectrophotofluorometric analysis revealed that NA levels in the lumbar enlargement were reduced to less than 20% of control values, while 5-HT levels were unaffected. NA levels in the cervical enlargement were reduced by approximately 50%. These results indicate that NA levels in the spinal cord can be substantially reduced without altering the locomotion produced by MLR stimulation in the mesencephalic cat.(Supported by Medical Research Council of Canada).

636 INVESTIGATION OF THE DEVELOPMENT OF CEREBELLAR NORADRENERGIC NEURONS AFTER 6-HYDROXYDOPA. Richard M. Kostrzewa and Richard E. Garey. Veterans Administration Hospital and Depts. of Pharmacology and Psychiatry, Tulane University Medical Center, New Orleans, Louisiana 70146 (U.S.A.).

In these studies the development of noradrenergic neurons was followed with measurement of cerebellar norepinephrine (NE) levels and in vitro uptake studies of ^{3}H-NE by cerebellar synaptosomes. It was found that NE levels at 5 weeks were increased by 100% over controls, when rats received a single injection of 6-hydroxydopa (6-OHDOPA) (60 µg/g i.p.) on the day of birth. Synaptosomes from the cerebellum of treated rats were incubated with various concentrations of ^{3}H-NE, and data were analyzed by Michaelis-Menten type kinetics. Lineweaver-Burke plots or best fit lines determined by linear regression analysis showed that the K_m for uptake was not altered, but that the V_{max} was elevated. Therefore, the data suggest that there is an increase in the number of synaptosomes or an increase in the number of functional nerve terminals in the cerebellum of rats treated with 6-OHDOPA on the day of birth. In support of such findings it was also observed that 6-hydroxydopamine (6-OHDA) (60 µg/g i.p.) brought about a similar rise in cerebellar NE levels when rats were treated on the day of birth and sacrificed at 5 weeks. The agent L-α-hydrazine methyldopa (carbidopa), a peripherally-acting dopa decarboxylase inhibitor, prevented alteration of NE levels by 6-OHDOPA treatment at birth, further indicating incomplete formation of a blood-brain barrier at birth. In contrast to these findings a 50% reduction in cerebellar NE levels was brought about when 6-OHDOPA was administered to rats at any time between 3 and 14 days of age. In vitro uptake of ^{3}H-NE by cerebellar synaptosomes indicated that the V_{max} was reduced, while the K_m was still unchanged, in rats treated at day 6 with 6-OHDOPA. Carbidopa protected from the NE alterations by 6-OHDOPA during this period, while 6-OHDA brought about a reduction in NE up to 11 days of age. When multiple doses of 6-OHDOPA (60 µg/g i.p. X 3, 48 hr intervals from birth) were given, light microscopic evidence of degeneration was apparent and levels of NE were again initially reduced, but elevated above control at 5 weeks. Correlative study of the neocortex and hippocampus, regions innervated by other projections of the locus coeruleus, shows that NE levels and ^{3}H-NE uptake were reduced after all treatments with 6-OHDOPA. These studies indicate an apparent regenerative sprouting of noradrenergic projections of one group of fibers from the locus coeruleus, after 6-OHDOPA. The phenomenon appears to involve an "initiating mechanism", which is present on the day of birth and which is not readily abolished by subsequent treatment with the neurotoxic agent 6-OHDOPA. This system appears to represent a good model for study of the growth/development, and regenerative/sprouting of noradrenergic neurons.

637 EFFECT OF 6-HYDROXYDOPAMINE ON THE ACQUISITION OF A CONDITIONED EMOTIONAL RESPONSE. John J. Clancy, Donald F. Caldwell* and Charles E. Frohman*. Psychobiology Department, Lafayette Clinic, Detroit, Michigan 48207.

Cooper, Breese, Howard and Grant (PHYSIOL. BEHAV. 9:727, 1972) evaluated the effect of centrally administered 6-hydroxydopamine (6-OHDA) on the acquisition of a two-way conditioned avoidance response (CAR) in rats. Six-OHDA produced central adrenergic lesions, inhibited CAR acquisition in four rats and significantly reduced rate of acquisition in the eight remaining animals. The present investigation will attempt to extend the Cooper, et al. findings by evaluating the effect of 6-OHDA lesions on acquisition of a one-way CAR. Both central and peripheral catecholamine levels will be simultaneously depleted by 6-OHDA. This procedure will determine if CAR inhibition in only some of the animals, as found by Cooper, et al., is due to peripheral adrenergic functioning.

Thirty male Sprague-Dawley rats, 14 weeks old during behavioral testing, were randomly assigned to one of three treatment conditions: control, sham drug and 6-OHDA. Chronic cannulas were surgically implanted in the right lateral ventricles of the animals in the latter two groups. The 6-OHDA animals received intraventricular and IP injections of the drug while the sham control rats were given intraventricular and IP injections of artificial cerebral spinal fluid (CSF). The control subjects were neither cannulated nor injected. All 6-OHDA subjects exhibited "6-OHDA sickness" which ran its course in seven days. Behavioral testing commenced 10 days after the last 6-OHDA injection at which time all 6-OHDA rats appeared and behaved normally. Acquisition of a one-way, active avoidance response was evaluated in a totally automated jump-box.

The results of spectrophotofluometric assays on the brain and spleen of 6-OHDA treated rats were expressed as percent of NE concentrations for the pooled CSF control animal tissues. A mean reduction of 84% in brain tissue and 79% in spleen tissue was observed for the 6-OHDA treatment group. In comparison to controls, rate of acquisition for the one-way CAR was statistically inferior for 6-OHDA treated animals on both the latency and percent avoidance criteria. Half of the 6-OHDA subjects failed to show any avoidance behavior and the remaining animals manifested significantly fewer and slower CARS than the CSF and control rats. On the latency criterion the inhibited 6-OHDA rats never made an average avoidance response over the entire 150 trials. On the final trial block they were avoiding only 2% of the time in comparison to a 85% avoidance rate for the CSF animals.

The present results substantiate the recent findings of Cooper, et al. and extend the detrimental effect of reduced adrenergic functioning from a two-way CAR to a one-way CAR learning situation. The simultaneous central and peripheral adrenergic lesions did not account for the lack of avoidance inhibition in all 6-OHDA rats, as reported by Cooper, et al., since the present study found similar results. A review of the NE assays and 6-OHDA concentrations involved, suggests that the level of NE reduction in both studies is at the animals' threshold for total elimination of avoidance behavior. This is a possible explanation for lack of total avoidance inhibition in all animals.

The DiGiusto and King (J. COMP. PHYSIOL. 81:491, 1972) "difficulty of task" hypothesis is rejected in favor of a "drug dosage level" hypothesis to account for discrepancies in the 6-OHDA literature. The finding that adrenergic lesions of equal magnitude have similar effects on both one-way and two-way CAR learning argues against a "difficulty of task" explanation. The present results support two basic tenets of Mowrer's (PSYCHOL. REV. 63:114, 1956) two factor learning theory: first, adrenergic functioning is necessary for emotional response learning and, secondly, the acquisition of emotional behavior is a two factor process. The finding that adrenergic lesions have a differential effect on escape and avoidance acquisition supports the latter tenet.

638 MONOAMINE NEUROTOXINS: SELECTIVE AND DELAYED EFFECTS ON BEHAVIOR IN A RAT COLONY. Gaylord D. Ellison* (SPON: R. Gorski). Dept. Psychology, UCLA, Los Angeles, 90024.

Male rats were raised in an enriched colony environment and then administered intraventricular injections of 6-OHDA, or of 5,6 DHT, or of saline. They were then returned to the colony enclosure and free behavior during the next 50 days was observed. Shortly after lesioning the 6-OHDA animals spent more time in the burrows than Controls and when out were inactive, whereas the 5,6 DHT rats conversely spent more time in the open than Controls, running in activity wheels and approaching humans. Some of these effects reached a peak at 14 days after lesioning and then recovered, but other selective effects of the monoamine neurotoxins were more delayed. Progressive changes in social behaviors developed in the colony, with fighting, mounting, and hoarding gradually increasing and reaching a peak 25 days after lesioning. During this time the 6-OHDA rats were progressively falling in dominance whereas the 5,6 DHT animals were progressively becoming more vicious and were increasing in dominance measures. Social behaviors returned to more normal levels after 50 days. Several successive stages develop following monoamine neurotoxins, and recovery from neurotoxins is different in caged and colony animals. Supported by NS 11846.

639 ALTERATIONS IN DOPAMINERGIC SENSITIVITY IN CHRONICALLY PRETREATED RATS. P. Muller, P. Seeman. (SPON: Y. Israel) Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada, M5S 1A8.

Dopaminergic supersensitivity following pharmacological blockade of the dopaminergic pathway has been demonstrated using stereotypy and turning behaviour as a model. (Tarsy and Baldessarini, Neuropharmacol. 13: 927, 1974). In our experiments we tried to demonstrate supersensitivity and also subsensitivity using catalepsy as a model. The effect of chronic apomorphine and haloperidol on haloperidol-induced catalepsy was studied on two different tests. Chronic apomorphine treatment potentiated the cataleptic potency of haloperidol. Chronic haloperidol treatment induced tolerance to haloperidol. The difference in cataleptic potency of haloperidol before and after chronic haloperidol was potentiated if the test was done in the presence of apomorphine. Our findings suggest that chronic apomorphine treatment causes subsensitivity of the dopamine transmission system due to chronic stimulation. Chronic haloperidol induces supersensitivity by chronic blockade of dopamine transmission.

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640 HUMAN PLASMA NOREPINEPHRINE: I. VARIATIONS IN NORMAL SUBJECTS. C.R. Lake,* M.G. Ziegler* and I.J. Kopin. LCS, NIMH, Bethesda, Md. 20014.

Norepinephrine (NE) is released from sympathetic nerve endings and appears in plasma, where it is rapidly metabolized; plasma half life of NE is less than 2 min. Significant changes in plasma levels can occur rapidly, particularly under conditions of excitment, pain, anxiety, etc. Recently methods have been developed which are sufficiently sensitive and specific to assay levels of this amine in human plasma. The radioenzymatic method of Henry et al. (1975) was modified and yielded 8CPM/pg NE over a range of 10-1000 pg NE with an average blank of 110 CPM, so that levels as low as 12 pg/ml could be reliably determined.

NE levels in blood withdrawn from resting, supine, normal volunteers immediately after venipuncture were higher by a mean of 86 pg/ml ($p < 0.05$) than levels drawn 20 min post venipuncture with an indwelling butterfly needle. The latter value was defined as the basal level and for 42 control subjects was 285 pg/ml. The levels were not significantly different ($p > 0.1$) in blood drawn one week apart from the same 15 patients. No diurnal rhythm was found. When subjects were asked to stand, NE levels were double basal values at 5 min ($p < 0.0005$) and increased by an additional 10% at 10 min. A standing isometric hand grip at 30% of maximal force for 5 min increased plasma NE to 158% above basal. Sitting for 10 min increased NE 70% above base; a 60° tilt, 82%; and a 4 min cold pressor test increased NE 137%.

In 42 healthy normotensive volunteers there was a significant positive correlation ($cc = 0.498$, $p < 0.001$) between age and basal levels of plasma NE. Thus the levels of plasma NE in man varies between individuals depending upon the technique of blood drawing, age, and various stresses which alter NE secretion.

641 CENTRAL NORADRENERGIC REGULATION OF BRAIN MICROCIRCULATION. M. Raichle, B. Hartman, J. Eichling*, and L. Sharpe. Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Missouri 63110.

Using the technique of immunofluorescence localization of dopamine-ß-hydroxylase (DBHase; E.C.1.14.2.1) it has been possible to clearly demonstrate a close association of DBHase-containing central noradrenergic terminal fibers with small parenchymal blood vessels including capillaries deep within brain substance (1).

From these anatomical findings evolved the working hypothesis that the central noradrenergic system is analogous to the peripheral sympathetic system except that it is specialized for performing specific functions related to the brain microvasculature. This hypothesis was tested in 4 adult rhesus monkeys (3 with chronic bilateral superior cervical ganglionectomies) with stereotaxically placed cannulae permanently located in the lateral ventricles and the locus coeruleus for the injection of drugs. Cerebral blood flow (CBF) and the blood-brain-barrier (BBB) permeability for water ($H_2{}^{15}O$) were simultaneously measured in a manner previously described (2). Our data demonstrate that stimulation of the locus coeruleus with carbachol (8 µg) produces a prompt reduction in CBF and an increase in BBB permeability. The intraventricular administration of the rapid acting α-adrenergic blocker phentolamine (25-100 µg) has the opposite effect. These suggest that the central noradrenergic system in brain may have the unique function of regulating capillary permeability as well as blood flow.

1. Hartman, B.K., et al (1972): Proc. Nat. Acad. Sci. 69:2722.
2. Eichling, J.O., et al (1974): Circ. Res. 35:358.

642 EFFECTS OF INTRACISTERNALLY ADMINISTERED NGF OR ANTI-NGF ON THE POST-NATAL DEVELOPMENT OF CATECHOLAMINE NEURONS. R.J. Konkol*, G.R. Breese, and B.R. Cooper* The Neurobiology Program, Depts. of Psychiatry and Pharmacology, Univ. North Carolina, Sch. of Med., Chapel Hill, N.C. 27514

Nerve growth factor (NGF) extracted from mouse submaxillary gland has been found to effectively augment growth of peripheral catecholamine neurons, and in some cases, regrowth of injured central adrenergic fibers into peripheral tissues transplanted within the central nervous system. Antiserum to NGF (anti-NGF) has been reported to inhibit development and growth of peripheral adrenergic neurons while also retarding regrowth of transected central monoamine containing fibers into transplants. In this study, we wished to determine the effect of intracisternal administration of NGF or anti-NGF on developing central adrenergic neurons during the first several days after birth. This action of NGF or anti-NGF was analyzed both at an early (1-2 weeks) and a later stage (4 weeks) after birth. Following such treatment, areas containing predominately cell bodies and regions consisting largely of adrenergic terminals showed no consistent change in monoamine levels with either NGF or anti-NGF treatment. However, examination of tissues innervated by the peripheral sympathetic system revealed a significant reduction of norepinephrine content in the heart. This reduction was attributed to a disruption of peripheral adrenergic fibers. In contrast, introduction of NGF into the cisterna magna did not alter levels of catecholamines in peripheral tissues. These observations are consistent with the belief that neither exogenous NGF nor anti-NGF exert a major direct action on the development of central adrenergic neurons. (Supported by The Sloan Foundation and USPHS Grants MH-16522, MH-00013, and HD-03110.)

643 EXISTENCE OF INTERNEURONS VERIFIED IN THE SYMPATHETIC GANGLIA OF THE GUINEA PIG AND RHESUS MONKEY. Tanemichi Chiba, Asa C. Black, Jr., and Terence H. Williams. Dept. of Anatomy, Univ. of Iowa, Iowa City, Ia.52242

At least some of the small, intensely fluorescent (SIF) cells in the superior cervical ganglion of the rat have been proven to be interneurons (Williams, 1967, Nature, 214:309). In other mammals, however, the primary morphological criterion for the identification of the SIF cell as an interneuron - the presence of an efferent synapse on the principal ganglionic neuron, as identified by ultrastructural analysis - has not been demonstrated. For this reason we examined the SIF cells in the superior cervical ganglion of the rhesus monkey (Macaca mulatta) and the guinea pig by fluorescence and electronmicroscopy.

The fine structural details of the ganglionic interneurons in both species were similar to those in other species (Williams, et al., Nature in press). SIF cells received afferent cholinergic synapses, and some exhibited efferent synapses on presumed principal ganglionic neurons. Thus, at least some SIF cells in the monkey and guinea pig sympathetic ganglia are interneurons.

Quantitative results were as follows: the total number of SIF cells in the superior cervical ganglion of the monkey was 68.8 ± 19 (mean ± standard error of the mean), 372 ± 103 in the guinea pig. These numbers are equivalent to 2.3 ± 0.7 per milligram wet tissue for the monkey and 76 ± 20 per mg. wet tissue for the guinea pig.

The ability of dopamine to stimulate cyclic AMP production was also studied. The presence of such a dopamine-sensitive adenylate cyclase is regarded as diagnostic for the efferent synapse. In the monkey, we found such a dopamine-sensitive adenylate cyclase. For example, 100 micromolar dopamine elevates cyclic AMP levels to 345% of control values. Similar evidence is being sought in the guinea pig ganglion.

644 RELATIONSHIP BETWEEN GLUCOCORTICOIDS AND ADRENAL PHENETHANOLAMINE-N-METHYL TRANSFERASE ACTIVITY DURING DEVELOPMENT IN THE RAT. Richard L. Rotundo* and Victor H. Denenberg, Dept.Biobehavioral Sciences, Univ.of Connecticut, Storrs, Conn. 06268

The enzyme Phenethanolamine-N-Methyl Transferase (PNMT) which catalyses the conversion of norepinephrine to epinephrine in the adrenal medulla has been shown to be under the regulatory influences of both glucocorticoids (Wurtman and Axelrod, J Biol Chem 241:2301,1966) and neuronal stimulation (Kvetnansky,Gewirtz,Weise,and Kopin, Endo 87:1323,1970). As part of a study involving the influence of glucocorticoids upon the developing adrenal medulla we measured the levels of both plasma corticosterone and adrenal PNMT from birth to 91 days of age. Plasma corticosteroids, after an initial decrease, showed a sharp elevation from days 5 to 18 followed by an abrupt decline to adult levels on day 21. Parallel with this early increase in corticosterone, adrenal PNMT activity increased 40-fold from days 1 to 18 reaching a transient plateau coincident with the fall in hormone levels. Thereafter the enzyme activity continued to rise exhibiting a 5-fold increase over the next 70 days while plasma corticoids remained essentially unchanged. Heat stress in newborn pups produced a rapid increase (65% within 8 hours) in adrenal PNMT; these effects could be mimicked by dexamethasone. This increase is apparent on day 7 as well, but is no longer detectable at 15 days. These results suggest that a positive correlation may exist between circulating levels of plasma corticosteroids and the activity of the adrenal enzyme during the period from birth to 21 days. Thereafter the increase in PNMT activity appears not to be related to changes in the plasma level of this hormone. The possibility of a direct influence of glucocorticoids on PNMT activity is supported by experiments showing an increased sensitivity of PNMT to these hormones during early development. (Supported by NIH grant # HD 08195 to VHD).

645 THE EFFECT OF PUTATIVE NEUROTRANSMITTERS ON SYNAPTOSOMAL ATPase. Vincent P. Calabrese. Department of Neurology, Medical College of Virginia and McGuire VAH, Richmond, Virginia 23298

The reuptake of neurotransmitters by presynaptic nerve terminals has been shown to be an energy dependent system which also requires Na. There is some question whether K is also required. This study was undertaken to see if neurotransmitters had a direct effect on the Na-ATPase or Na-K ATPase of synaptosomes.

Synaptosomes were prepared from beef brain cortex. There were then freeze-thawed prior to assay to disrupt the membranes. The assay medium consisted of 1.25 mM/l ATP, 2.5 mM/lMg, 1×10^{-5} M/l neurotransmitters and Na/K conc of 150 mM/O, 140 mM/10mM and 100 mM/50mM 5HT, dopamine, norepinepherine, glutamic acid and GABA had no effect on the Na ATPase or on the Na-K ATPase with a Na/K conc of 150 mM/O or 140 mM/10mM, but at 100 mM/50mM there appeared to be a slight stimulation. This was statistically significant for 5HT ($p < 0.05$) and approached it for dopamine, norepinepherine and GABA. The increase in activity is only 5-10% which would not seem to indicate functional significance and would be consistent with the theory that the function of the ATPase is to restore ionic separation after Na is transported into the cell with the neurotransmitter, and that this enzyme is not an integral part of the neurotransmitter transport system.

646 REFLEXIVE THERMOREGULATORY RESPONSES AFTER INTRA-HYPOTHALAMIC INJECTION OF NOREPINEPHRINE IN RATS. Evelyn Satinoff and E. R. Hackett*. Depts. of Psychol. and Physiol., University of Illinois, Champaign, 61820.

Low doses of norepinephrine (NE) injected into the preoptic/anterior hypothalamic area (PO/AH) raise body temperature in rats. If the rise is to be interpreted as an upward shift in set-point, all of the animal's reflexive thermoregulatory responses should act in an integrated fashion to increase heat production and decrease heat loss. In unrestrained rats, we have measured brain temperature, as well as oxygen consumption, EMG activity and tail temperature to give us indices of metabolic rate, shivering and vasomotor tone, respectively, the main avenues of heat production and heat loss in rats. The experiments were done at ambient temperatures of 4° C, 24° C, and 30-32° C. After bilateral injection of NE (0.15-1.35ug/0.5ul) into the PO/AH, brain temperature and oxygen consumption increased and tail temperature decreased. Shivering was enhanced in the cold and some was seen even in the 24° C environment. These effects were not seen after NE injected into extra-PO/AH hypothalamic areas. These results imply that the increased body temperature seen after low doses of NE injected into the PO/AH are caused by an upward resetting of the temperature around which the animal regulates. (This work was supported by Grant #NS 12033 from NINDS and the Research Board of the University of Illinois.)

647 AN EXTENSIVE NEUROANATOMICAL MAPPING OF THE RAT BRAIN FOR SITES WHICH MEDIATE PROSTAGLANDIN-INDUCED HYPERTHERMIA.

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Prostaglandin E_1 (PGE_1) evokes a short-latency, short-lasting hyperthermic response when injected intracerebroventricularly in any of several species. It has been suggested that this effect could be mediated by an action on the anterior hypothalamic/preoptic region (AH/PO), an area known to be both pyrogen-sensitive and thermosensitive. This suggestion receives support from experiments demonstrating that similar hyperthermic responses can be evoked in several species by PGE_1 injected directly into the AH/PO. However, there has been no systematic, extensive examination of the rest of the brain for sites at which an injection of PGE_1 evokes hyperthermia. Only the posterior hypothalamus and a few scattered lower brainstem sites have been examined. In view of this sampling bias, it would seem premature to conclude that the AH/PO represents the site of action of PGE_1. The present study was undertaken in order to determine whether PGE_1 can evoke hyperthermia in the rat through an action at sites other than AH/PO.

Each of 17 male albino rats (Sprague-Dawley), weighing 200-400 g, was implanted with 2-4 23G intracerebral injection guides. So that injections could be made at a number of depths below the tip, the guides penetrated only a few mm into the brain. The placement of the guides in the 17 rats was designed so that, when the study was completed, the injection sites would form a uniform matrix covering about 75% of the subcortical tissue rostral to the medulla. During experimental sessions, each rat was restrained in a wire mesh cage at 20°C. To monitor core temperature, a thermistor probe was inserted into the rectum and taped to the base of the tail. After core temperature had stabilized (1-2 hours), an intracerebral injection of 0.5 or 1.0 µL of 0.9% saline or of PGE_1 (100 ng/µL) was performed. An increase in core temperature (ΔT_c) of 0.5°C or more was accepted as a positive hyperthermic response. Sites which proved active at a dose of 100 ng were also tested with the 50 ng dose and with vehicle. Positive responses to either dose were replicated at least once.

The brain region explored included all tissue bounded by frontal planes AP(10)-AP(-2), lateral planes L(0)-L(4) and horizontal planes H(+2)-H(-6) (Pellegrino atlas). The enclosed volume encompasses most of the subcortical tissue anterior to the medulla. Thus far, 210 different injection loci have been evaluated. An injection of 100 ng PGE_1 in 175 of these sites had no effect on body temperature. Twenty-five sites responded to 100 ng, but not to 50 ng ($\Delta T_c = 1.22 \pm 0.10$°C; latency = 2.9 ± 0.4 min.). Ten additional sites were sensitive to the 50 ng dose ($\Delta T_c = 1.15 \pm 0.14$°C; latency = 4.1 ± 0.8 min.). Of the 35 active sites, 31 were located between AP planes 6 and 8, within 2 mm of midline and below horizontal plane H(+1). This tissue block encompasses the preoptic area, the anterior hypothalamus and parts of the septum and anterior thalamus. All of the sites which were sensitive to the low dose of PGE_1 lay within 1 mm of midline between AP planes 7 and 8. Nine of the 10 sites were in the anterior hypothalamic-preoptic junctional tissue between the anterior commissure and the optic chiasm. It can be concluded that, in the rat brain, the AH/PO region is the only subcortical, supra-medullary site at which PGE_1 can act to evoke hyperthermia.

648 CRITERIA FOR IDENTIFICATION OF BRAIN STEM SYMPATHETIC RELATED NEURONS. P.M. Gootman, M.I. Cohen and A.P. Rudell. Departments of Physiology, Downstate Medical Center, State University of New York, Brooklyn, N.Y. 11203 and Albert Einstein College of Medicine, Bronx, N.Y. 10461

In order to determine if brain stem neurons can be identified as involved with regulation of sympathetic (Symp) discharge, we are examining the temporal relations between unit and Symp activity, using crosscorrelation and signal-averaging methods. Since Symp nerves have specific activity patterns (combinations of 10/sec oscillation, cardiac and respiratory periodicity), brain stem neurons whose activity has such periodicities, reliably locked to those in Symp nerves, could be considered functionally related to the Symp-driving system. Experiments were carried out on decerebrate or urethane-anesthetized cats with neuromuscular blockade and artificial ventilation on 100% oxygen. Efferent discharges of preganglionic Symp nerves (splanchnic and cervical Symp) and the phrenic nerve (indicating central respiratory activity) were recorded monophasically together with medullary neurons' spike activity. Neurons were found in the classically defined "pressor region" of the medullary dorsolateral reticular formation which had mixtures of cardiac and respiratory-related periodicity locked to such periodicity in Symp discharge; the phase relations differed between neurons. These results are sufficient to tentatively identify some medullary neurons as related to Symp-driving networks. (Supported by USPHS Grants NS-12031, NS-03970 and NS-10987.)

649 LOCALIZATION OF THE MEDULLARY RESPIRATORY NEURONS IN RATS BY MICROELECTRODE RECORDING. M.Tabatabai* and B.R. Howard* (SPON: Robert B. Livingston). Depts. Physiol. and Animal Biol., Pahlavi Univ., Shiraz,Iran.

The medulla oblongatas of sodium pentobarbital-anesthetized rats were explored by extracellular microelectrode recording. Units whose discharge activity was synchronous with some phase of respiration were located bilaterally between 1.5 and 2 mm lateral to the midline, extending from 1.5 mm rostral to 1 mm caudal to the obex in the ventral two thirds of the medulla. The expiratory units predominated (63%) and tended to lie dorsocaudally to the inspiratory units (37%), although there was much intermingling of the cell types. Ten different patterns of discharge were distinguished, varying from a short burst at the beginning of expiration to a resting discharge which increased in frequency during either inspiration or expiration. Evidence was also obtained that respiratory fibers cross the midline just caudal to the obex.

650 PNEUMOTAXIC CENTER AND PULMONARY AFFERENTS INTERACTION IN RESPIRATORY PATTERN CONTROL. Jack L. Feldman and Henry Gautier*. Lab. de Physiol., Univ. Paris VI, Paris 75571 France.

The interaction between pulmonary afferents (PA) and pneumotaxic center (PC) in control of respiratory pattern was studied in lightly anesthetized paralyzed cats. Phrenic nerve discharges served as an indicator of respiratory center output. Introduction of a delay between inspiratory onset and the commencement of an inflation at constant flow resulted in increases of the durations of inspiration (T_I) and expiration (T_E) and amplitude of the integrated phrenic nerve discharge. The lung volume at inspiratory cutoff, i.e. the volume threshold, decreased markedly as T_I increased. There was a linear relationship between T_I and T_E. The effects of introducing a delay in inflation onset were flow dependent. Bilateral vagotomy abolished the effects of delay and flow. PC lesions produced the following changes in cats with their vagi intact: a) the volume threshold for zero delay doubled and its rate of decrease with increased T_I was significantly smaller; b) the change in T_E for a given change in T_I was reduced markedly. Cycle triggered electrical stimulation of the afferent vagi resulted in effects similar to those seen for delays in cycle triggered inflations.

These results suggest that: a) the inspiratory cutoff mechanism is responsive to the rate of lung inflation; b) all of the lung volume information affecting inspiratory cutoff in paralyzed cats is carried via the vagi; c) an intact PC is necessary for the generation of a normal time dependence of the volume threshold for inspiratory cutoff; d) the PC plays an important role in matching T_E to T_I when the latter changes. (Supported by Centre National de la Recherche Scientifique grant LA 204.)

651 CHANGES IN THE BREUER-HERING REFLEXES FOLLOWING LESIONS IN THE PARABRACHIAL NUCLEUS OF THE CAT. C. K. Knox and G. W. King*. Lab. of Neurophysiol., 315 Millard Hall, University of Minnesota, Minneapolis, 55455.

The Breuer-Hering lung inflation reflex was studied in decerebrate cats before and after bilateral electrolytic lesions of the medial parabrachial nucleus (NPBM) of the rostral pons (L 3.5, P 3.0, HC-3mm). A servo-respirator was used to impose known changes in lung volume at various times during the respiratory cycle while recording the associated changes in tidal volume (V_T), inspiratory duration (T_I) and expiratory duration (T_E). Following the lesions it was found that: (1) V_T, T_I and T_E increased, as expected, with no significant changes in the animal's CO_2 sensitivity. (2) During inspiration the hyperbolic V_T-T_I characteristic describing the volume threshold for inspiratory inhibition by lung inflation was shifted toward larger asymptotic V_T and T_I values. (3) During expiration the reflex prolongation of T_E by step or pulse inflations was increased. (4) Subsequent bilateral vagotomy resulted in apneusis. The results are in agreement with the well known inspiratory inhibitory function of the pontine pneumotaxic mechanism. In addition, they show that the time-dependent V_T-T_I threshold characteristic is modified by but does not depend on NPBM. Furthermore, the central mechanisms which mediate the expiratory phase inflation reflex become more excitable following NPBM destruction. Supported by USPHS Grants HL16430 and GM0572.

652 NEURAL AND RESPIRATORY CORRELATES OF UPPER RESPIRATORY TRACT STIMULATION IN CATS AND KITTENS. L.F. Greenwood, J.P. Lund, B.J. Sessle and A.T. Storey. Fac. Dentistry, Univ. Toronto, Toronto, Canada M5G 1G6.

Stimulation of the upper respiratory tract causes (especially in young animals) an apnea which has been implicated in the etiology of the Sudden Infant Death Syndrome (SIDS). We have noted the effects of a variety of natural and artificial upper respiratory tract stimuli on respiration and on respiratory-related neurones in 25 cats and kittens.

Low-intensity (< 1 mA, 0.1 msec, 10/sec) stimulation of the superior laryngeal nerve (SLN) that supplies the larynx produced prolonged apnea i all kittens and most cats tested: some natural stimuli (water, histamine serotonin, ethanol, synthetic gastric juice) applied to the larynx gave less apnea, water being the most effective, while others (ammonium chloride, halothane, ether) increased ventilation and others (sulphur dioxide, saline) had no apparent effect. The electrical SLN stimulus was insufficient to cause swallowing which is itself associated with apnea.

Some of these stimuli were applied while recording in the region of the solitary tract nucleus from single cells which discharged in phase with the animal's natural ventilatory rhythm even when the animal was paralyzed. We could find no excitatory input to 20% of these cells in adult cats, despite the use of many electrical and natural stimuli (bilateral SLN, vagus, glossopharyngeal, infraorbital, recurrent laryngeal nerves, forepaw and nasal mucosa) and in half of these (10% of total) the rhythmic discharge could be suppressed only by SLN stimulation at 10/sec. The proportion of similar cells tested to date in kittens is much greater (50%), which suggests that the suppressive effect of upper respiratory tract feedback on ventilation may be greater in young animals and may be significant in the etiology of SIDS.

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653 POWERFUL INFLUENCE OF PULMONARY AFFERENT ACTIVITY ON ROSTRAL PONTINE (PNEUMOTAXIC CENTER) RESPIRATORY NEURONS. M.I. Cohen, J.L. Feldman and P. Wolotsky*. Dept. Physiol., Albert Einstein Col. Med., New York, N.Y.

The effects of pulmonary afferent (PA) activity produced by cycle-triggered lung inflations on medullary and pontine respiratory neuron discharge were studied in decerebrate, paralyzed cats. Phrenic nerve discharge served as an indicator of respiratory center output. Lung inflation at constant flow was applied coincidentally with phrenic discharges during control cycles by means of a phrenic-triggered pressure system; and during test cycles (every 10th cycle), no inflation was applied. With no inflation, the classical Breuer-Hering reflex was seen: prolongation of the inspiratory phase with no change in the slope of the directly integrated phrenic discharge. Some medullary inspiratory units responded in a manner similar to phrenic discharge, whereas others showed a decrease in frequency when there was no inflation, implying that they were excited by inflation.

The most striking effects of inflation were exhibited by neurons in the dorsolateral rostral pons (pneumotaxic center, PC); we observed many units with tonic activity, not respiratory-modulated, which then became strongly inspiratory-modulated when inflation was absent. This effect indicates the existence of a strong ascending inhibitory pathway from the PA to the PC inspiratory-modulated units and also suggests that the role of the PA in the generation of inspiratory cutoff may be mediated through pontine neuronal circuits as well as through the dorsal infrasolitary nucleus of the medulla. (Supported by NS 03970.)

654 ANATOMICAL AND PHYSIOLOGICAL ANALYSIS OF AFFERENTS AND EFFERENTS IN THE SYMPATHETIC CARDIAC NERVE OF THE PIGEON. John B. Cabot and David H. Cohen. Dept. of Physiology, Univ. of Virginia Medical School, Charlottesville, Virginia 22901

For several years we have been developing visually conditioned heart rate change in the pigeon as a vertebrate model system for cellular investigations of long-term, associative learning. Of particular concern has been delineating the neural pathways mediating conditioned response development (cardioacceleration), and substantial effort has been directed to characterizing its final common path. It has been shown that the response is mediated exclusively by the extrinsic cardiac nerves and that both vagal and sympathetic cardiac innervations participate, the sympathetic contribution being the most prominent. Regarding this sympathetic component, it has been established that: (a) chronotropic influences are exerted almost entirely through the right cardiac nerve; (b) the cells of origin of this nerve are located in sympathetic ganglia 12-14, being most concentrated in ganglion 14; and (c) these cells are preferentially localized within their ganglia.

The present study describes the right cardiac sympathetic innervation in more detail. Compound action potential experiments indicate the fastest component conducts at 2.5-5.6 m/sec and is not associated with cardioacceleration. Another component conducts at 0.5-1.9 m/sec, and its activation is accompanied by tachycardia. Electron microscopy of entire cross-sections of the nerve (placed on slotted, formvar-coated grids) was then used to determine its fiber composition. Myelinated fibers constitute 34% of the total population and range in diameter from 1.3-3.2 μ; unmyelinated fibers range in diameter from 0.3-1.3 μ. Since there is a close correspondence between the fiber distribution and the conduction velocities measured from the nerve compound action potential, this suggests the myelinated fibers have no chronotropic effect and that the unmyelinated fibers (though not necessarily all) constitute the cardiac postganglionics. Further supporting this were data from single unit studies of sympathetic ganglion 14 (4M NaCl, 2-10 MΩ micropipettes). Extracellular recordings from 73 neurons antidromically identified as cells of origin of cardiac nerve fibers indicate their axons conduct at 0.4-1.9 m/sec, consistent with their being the unmyelinated fiber contingent of the nerve.

The following evidence suggests that the myelinated fibers are visceral afferents. First, the cut ends of left and right cardiac nerves were soaked in 10% horseradish peroxidase for 15-30 min; the animals were perfused 2 days later; and frozen sections of dorsal root and sympathetic ganglia were incubated in 3,3'-diaminobenzidine and H_2O_2. Horseradish peroxidase-positive cells were clearly evident in dorsal root, as well as sympathetic, ganglia; control exposures of the uncut nerve to horseradish peroxidase gave negative results. Second, cells in sympathetic ganglion 14 were synaptically driven by cardiac nerve stimulation, and their response latencies definitely implicated the faster conducting, myelinated fibers of the cardiac nerve. Since these neurons were not antidromically activated, it appears cardiac nerve afferents can reflexly affect postganglionic neurons not contributing to the cardiac nerve. Finally, stimulation of the central cut end of the right cardiac nerve elicited a marked decrease in systemic arterial blood pressure at a latency of approximately 1 sec and at a threshold coinciding with that of the early component of the nerve compound action potential. This reflex effect survived bilateral vagotomy but was blocked by atropine, suggesting cholinergic vasodilatation.

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655 NEURAL PATHWAYS MEDIATING CARDIOVASCULAR RESPONSES ELICITED BY STIMULATION OF THE SEPTUM IN THE RAT. J. Ciriello*, F. R. Calaresu and G. J. Mogenson. Dept. of Physiology, University of Western Ontario, London, Ontario, Canada N6A 5C1.

The effects of electrical stimulation of the septum on arterial pressure and heart rate were investigated in 18 rats anaesthetized with sodium pentobarbital. Stimulation of 23 histologically verified sites in the lateral septum (LS) elicited a decrease in arterial pressure (32 ± 3.0 mm Hg) and a decrease in heart rate (15 ± 4.5 beats per min). Stimulation of 19 sites in the medial septum (MS) elicited an increase in arterial pressure (21 ± 2.1 mm Hg) and a decrease in heart rate (41 ± 8.8 beats per min). To identify the pathways mediating the cardiovascular changes, lesions were made to disrupt fibre tracts, which had previously been shown (Fink-Heimer II technique) to contain marked degeneration following lesions of the cardiovascular responsive sites in the septum. Only lesions (n = 12) in the stria medullaris (SM) significantly reduced the LS depressor response, while lesions (n = 10) in the medial forebrain bundle (MFB) in the region of the lateral hypothalamus abolished the MS pressor response. It is concluded that the hypotensive response elicited from the LS is mediated via the SM to lower brain stem structures previously implicated in vasomotor control whereas the hypertensive response elicited by stimulation of the MS is mediated via the MFB to the hypothalamus and lower brain stem structures.

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656 RELATIONSHIP BETWEEN CENTRAL ADRENERGIC AND CHOLINERGIC MECHANISMS MEDIATING SYSTEMIC BLOOD PRESSURE. J. Buccafusco* and H. E. Brezenoff. Dept. Pharmacol., CMDNJ, N. J. Medical School, Newark, N. J., 07103.

In continuing studies of the central adrenergic and cholinergic control of cardiovascular function, experiments were conducted to determine whether these two systems in brain interact with each other to exert their effects. In the ketamine anesthetized rat, injection of physostigmine, either i.v. (100 μg/Kg) or intracerebroventricularly (icv) (35 μg), evoked a centrally mediated increase in systolic blood pressure (SBP) of 35 ± 4 (mean ± S.E.M.) and 45 ± 8 mm Hg, respectively. A small but consistent fall in heart rate (HR) was also observed. Icv injection of norepinephrine (N), 1-20 μg, caused a dramatic fall in HR (147 ± 11 beats/min after 2 μg) and a bimodal change in SBP; a fall after low doses (1-4 μg) and a rise after higher doses (10-20 μg). Phentolamine, 50 μg, icv, abolished the bradycardia and hypotensive response to 2 μg of N (icv) but had no effect on the response to physostigmine. Conversely, atropine, either icv (10 μg) or i.v. (1 mg/Kg) failed to modify the cardiovascular responses to icv injected N although it abolished the pressor effects of physostigmine. These data suggest that central adrenergic and cholinergic mechanisms modulate systemic cardiovascular function via separate and independent central pathways.

657 PROBABILISTIC BEHAVIOR OF CENTRAL 'VASOMOTOR' NEURONS. Gerard L. Gebber. Dept. of Pharmacol. Michigan State Univ., East Lansing, Michigan, 48824.

Bursts of activity recorded from pre- and postganglionic sympathetic nerve bundles usually are coupled in a 1:1 relation to the cardiac cycle. Yet, with the exception of neurons in the solitary nucleus which receive direct input from the baroreceptor nerves, attempts to locate medullary units whose spontaneous discharge is locked to the cardiac cycle have failed. This observation can be interpreted in at least two ways. First, the failure to locate neurons within the pressor region of the medulla which exhibit a cardiac rhythm in their discharge might be related to a microelectrode sampling problem. Second, absence of a cardiac rhythm in the spontaneous discharges of medullary units might signify that only a small percentage of the total population of brain stem vasomotor neurons participates in each cardiac related burst of activity recorded from peripheral sympathetic nerve bundles; and that the active subpopulation changes from heart beat to heart beat. In this case, it might be possible to identify single vasomotor neurons in the brain stem by determining the probability of their discharge during the phases of the cardiac cycle. This possibility was studied by constructing post-R wave time interval histograms of spontaneously occurring unitary discharge in the cat medulla. Six of the 38 units sampled in the lateral portions of nucleus reticularis gigantocellularis and nucleus reticularis ventralis yielded positive results. The probability of discharge of these neurons was considerably higher during early and mid-diastole than during the rest of the cardiac cycle. Interspike interval histograms showed that the discharge patterns of these units were quite irregular. The interspike interval of one unit ranged from 0.4 to 9.6 sec. This neuron remained quiescent for as long as 18 heart beats. Spontaneous unitary discharge was inhibited by baroreceptor reflex activation, further suggesting that the 6 neurons were contained within a vasopressor circuit. These results support the contention that only a small and continuously changing segment of the total population of brain stem vasomotor neurons participates in each cardiac related burst of activity recorded from peripheral sympathetic nerve bundles. It is probabilistic behavior at the unit level, therefore, which has alluded us and consequently made the identification of brain stem vasomotor neurons so difficult in the past. In addition, the data raise the possibility that individual preganglionic sympathetic neurons (PSN) serve as the final common pathway for a large number of brain stem neurons. This possibility was tested on the following assumption. Redundancy, in combination with a continually changing population of brain stem neurons receptive to excitation should lead to a degree of unpredictability regarding the latency of PSN discharge to successive stimuli applied to the same medullary pressor site; providing that impulses can be transmitted to the spinal cord over a number of different routes, each having a different conduction time. This assumption seems reasonable in view of the extensive interconnections known to exist between large numbers of reticulospinal neurons at medullary and pontine levels. Ten PSN in the seventh thoracic spinal segment were antidromically identified by stimulation of the splanchnic nerve. Post-stimulus histograms of PSN discharge to single shock stimulation of a medullary pressor site were constructed. Although PSN responded no more than once to each successive shock applied to the same medullary site, the onset latency of the spike was quite variable ranging from 35 to 100 msec. These results suggest that redundancy of brain stem excitatory input functions to enhance the probability of PSN discharge. (Supported by USPHS Grant HL-13187.)

658 ACTIVATION OF SOLITARY NUCLEUS NEURONS FROM THE LOCUS COERULEUS AND VICINITY. David G. Ward*, Alex J. Baertschi* and Donald S. Gann. Dept. Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Preliminary evoked potential studies have shown short latency evoked responses in the locus coeruleus and vicinity to electrical stimulation of the carotid sinus nerve and solitary tract nucleus (Ward and Gunn, unpublished). To define further ascending system(s) originating from the solitary tract nucleus and vicinity (NTS) which convey cardiovascular information, single shock stimuli were applied to the rostral pons and midbrain while single cell responses were recorded in NTS. A region extending 2 mm around the obex was explored for antidromic and orthodromic responses in cats lightly anesthetized with chloralose and urethane. Four stimulation regions were defined: locus coeruleus-subcoeruleus (LC-SLC), lateral locus coeruleus-brachium conjunctivum (LLC-BC), dorsal tegmental nucleus-central gray (Gudden-CG) and medial longitudinal fasciculus-decussation of brachium conjunctivum (MLF-BCX). Twenty-seven cells were tested for responses to stimulation of LC-SLC, 29 to stimulation of LLC-BC, 25 to stimulation of Gudden-CG and 10 to stimulation of MLF-BCX. Orthodromic (ortho), antidromic (anti) and non-responses(NR) were observed as summarized in the following table.

	Stimulation Loci											
	LC-SLC			LLC-BC			Gudden-CG			MLF-BCX		
	ortho	anti	NR	ortho	anti	NR	ortho	anti	NR	ortho	anti	NR
	15	6	6	15	5	8	5	6	14	1	1	8
%	55	27	22	53	18	29	20	24	56	10	10	80
cv tests	10	5	3	6	2	2	5	2	7	1	0	6
response	8	3	1	4	2	1	5	0	4	1	0	6
%	80	60	33	66	100	50	100	0	57	100	0	100

Responses considered to be antidromic displayed unvarying latencies, would follow 2 msec delayed twin pulses or could be abolished with a collision test. For all stimulus response tests the antidromic latencies ranged from 0.8 to 1.2 msec thereby requiring conduction velocities of 10 to 15 m/sec. Many of the cells were also tested for responsiveness to electrical stimulation of the carotid sinus nerve, aortic depressor nerve or to withdrawal of blood (CV tests). The majority of these cells which were activated by the above (CV test) could also be driven antidromically or orthodromically (response) as summarized in the above table. Cells responsive to CV tests were predominantly activated from LC-SLC, LLC-BC and Gudden-CG. However many cells responsive to CV tests could not be activated from Gudden-CG and nearly all cells tested could not be activated from MLF-BCX. Nearly all cells which were activated from LC-SLC were located in the ventromedial solitary nucleus or the underlying reticular formation immediately beneath the caudal extent of the dorsal motor nucleus of the vagus. Cells responsive to LLC-BC and Gudden-CG were found scattered around the parahypoglossal nucleus, dorsal motor nucleus of the vagus and solitary tract. It is suggested that neural systems which are traveling through or are a component of the locus coeruleus and vicinity are reciprocally connected with NTS. This system(s) may be responsible for 1) mediating "arousal" inhibiting effects arising from NTS and the cardiovascular system or 2) conveying cardiovascular information to regions of the hypothalamus subserving cardiovascular and endocrine function. (Supported in part by grant AM14952 from NIH)

659 SUDDEN DEATH FOLLOWING BILATERAL LESIONS OF NUCLEUS LOCUS COERULEUS.

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In rats bilateral electrolytic lesions of the nucleus locus coeruleus (LC) are often associated with unexplained and sudden death (SD) (Ross et al, Fed. Proc. 32: 708, 1973). We sought to establish the mechanism leading to SD. Rats were anesthetized with halothane and a catheter was inserted in the carotid artery to record arterial pressure (AP) and heart rate (HR). In 41 animals small bilateral lesions (<1mm diameter) were placed stereotaxically through stainless steel electrodes in the region of LC. Controls had electrodes inserted through LC without placement of lesion. Following recovery from anesthesia, AP and HR were continuously monitored for up to 24 hours. SD occurred in 16 (39%) of the lesioned animals within 9 hours. Within 1 hour all lesioned animals developed an elevated AP (134 $\pm$ 3 mmHg; $P<0.01$) but not HR (422 $\pm$ 10 bpm) when compared to sham operated controls (AP = 106 $\pm$ 4 mmHg; HR = 421 $\pm$ 14 bpm). In the 25 lesioned survivors, the hypertension was maintained for 5 hours before gradually returning to control. In the animals developing SD the AP began to return to control after 2 hours coincident with the development of a progressive tachycardia (498 $\pm$ 17 bpm, $P<0.01$). Blood gases were not changed. SD occurred between 29 hours (mean = 5.5 hours) in two ways: (a) following a fall in AP over 30 minutes to shock levels (<50 mmHg mean pressure); or (b) from cardiac arrest without any antecedent hypotension.

Histological examination indicated that in 12 of the SD group the lesions destroyed over 90% of LC bilaterally. In the other 4 animals the lesions were preponderantly destroying the ventral LC including the nucleus subcoeruleus. In the lesioned survivors the lesions were outside of one or both LC, sparing the subcoeruleus area and destroying less than 60% of the nucleus bilaterally. Electrical stimulation in anesthetized rats via electrodes stereotaxically inserted through the LC region resulted in elevations in AP and HR with the largest responses elicited in the LC.

We conclude that bilateral lesions which destroy all of LC or possibly the ventral portion alone, may result in SD due to development of a progressive tachycardia leading to a fall of cardiac output and consequent collapse of AP or to the development of lethal arrhythmias. Tachycardia is due to excitation of pathways from or immediately adjacent to the LC. The SD syndrome may be a consequence of degeneration activation of projections from this noradrenergic system which serves to accelerate the heart.

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660 POSTERIOR ORBITAL CORTEX INVOLVEMENT IN CARDIAC DYSFUNCTION.

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Previous studies have shown that stimulation of the posterior orbital cortex can induce autonomic and somatic responses. Recently orbital cortex stimulation (OCS) capable of eliciting hemodynamic changes were correlated with cardiopathies which resemble lesions seen in animals treated with catecholamines. Since the limbic orbital cortex appears to modulate autonomic activities during emotional stress, its role in the pathogensis of these cardiopathies may include the adrenal steroids as well as catecholamine and hemodynamic influences. Nine conscious, rhesus monkeys (Macaca mulatta) received chronically implanted pressure catheters and stereotaxically implanted stimulating electrodes into the orbital cortex. All animals were acclimated to primate restraining chairs prior to the surgical implantation. The monkeys received 3 separate daily stimulation sessions. Each session consisted of biphasic pulses of 2.0 to 5.0 msec. duration, 20 to 40 Hz and intensities of 0.6 to 2.5 ma that induced OCS for 15 sec. every 5 minutes for one hour and 15 minutes on each day of stimulation. The monkeys were killed with nembutal 2 days after the final session of OCS. Electrode location was verified histologically and hearts were removed for gross and histological examination. OCS induced a significant depressor response (21% @ $P<.001$) in 4 monkeys and pressor response (22% @ $P<.001$) in 3 monkeys. An immediate bradycardia occurred during OCS in all animals and 5 of 7 animals demonstrated plasma cortisol elevations subsequent to OCS. Ectopic beats were frequently recorded immediately after OCS in all animals. These hemodynamic and plasma cortisol responses along with the gross myocardial examinations strongly suggest cardiac dysfunction in these monkeys. Histological confirmation of ischemic necrosis is presently being conducted. Since these responses were induced by stimulation of the orbital cortex which is a part of the limbic system, their presence suggest a pathophysiologic role by which emotional stress might cause shifts in cardiovascular activity and glucocorticoid release by which emotion might contribute to permanent myocardial damage.

661 LOCALIZATION OF BRAINSTEM REGIONS MEDIATING THE CEREBRAL ISCHEMIC REFLEX. R.A.L. Dampney*, M. Kumada and D.J. Reis. Dept. Neurol., Cornell Univ. Med. Coll., New York, 10021.

We sought to identify regions of the brain mediating the pressor response elicited by cerebral ischemia, the cerebral ischemic reflex (CIR). Rabbits were anesthetized (Urethane 1.25 g/kg), paralyzed, and artificially ventilated and both vertebral and one carotid artery ligated. The CIR was elicited by occluding the remaining carotid artery for up to 1 min. Under these conditions the CIR is stable and reproducible for hours. The CIR consists of a large elevation (to 176% of control) in arterial pressure (AP) and bradycardia. The increase in AP, which is blocked by phentolamine, is a consequence of increased peripheral vascular resistance due to vasoconstriction in the renal (mean vascular resistance >1000% of control), mesenteric (238%) and femoral (208%) arterial beds. After sympathetic denervation of each of these beds, the CIR elicited a vasodilatation in the femoral bed and a vasoconstriction in the mesenteric and renal beds. Both effects were abolished by subsequent adrenalectomy, indicating that adrenomedullary catecholamines are released as part of the response. Cardiac output is reduced (to 42% of control). The bradycardia is of vagal origin and is due to both direct excitation of cardiovagal neurons and indirect baroreceptor reflex activation. In spontaneously breathing animals, the CIR was accompanied by a delayed apnea, whereas restoration of the cerebral blood flow was immediately followed by hyperpnea.

The CIR persisted after section of cranial nerves VII-XI combined with brainstem transection at the ponto-medullary junction, but was abolished by spinal cord transection at C1, indicating that the CIR is mediated by regions within the medulla. A powerful pressor response similar in magnitude to and with changes in regional blood flow indistinguishable from the CIR was elicited by punctate electrical stimulation from a highly restricted region of the dorsal reticular formation of medulla and lower pons. The active region extends from the level of the rostral part of the inferior olive to the level of entry of the facial nerve and includes the nucleus parvocellularis. Bilateral electrical lesions placed at the caudal limit of this region greatly reduced or abolished the CIR but did not alter the pressor response to stimulation of points in the medulla caudal to the lesions. The lesions also resulted in a fall in AP to a level comparable to that in spinal animals.

The CIR therefore is a consequence of excitation of neurons in a highly restricted region of the dorsal reticular formation of medulla. The region mediating the CIR corresponds anatomically to the distortion-sensitive region in the cat mediating the Cushing response (Doba & Reis, Brain Res. 47: 487-491 (1972)). It also appears to be critical in maintenance of tonic vasomotor tone, and thereby may correspond to the so-called tonic vasomotor center of the brainstem.

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662 PREGANGLIONIC INNERVATION OF THE ADRENAL GLAND OF THE RAT. A STUDY USING HORSERADISH PEROXIDASE. Lawrence P. Schramm, J. Randle Adair, Judith M. Stribling* and Louis P. Gray*. Dept. of Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Adrenal medullae of 15-day-old and adult rats were injected with 20 to 40% solutions of horseradish peroxidase (Sigma, Type VI) dissolved in dimethyl sulphoxide. Rats were permitted to survive for between 2 and 97 hours after injection. Preganglionic neurons in the 15-day-old rats were readily identified in the intermediate zone of the spinal cord following staining with diaminobenzidine tetrahydrochloride. Peroxidase uptake could not be demonstrated in adult rats. Adrenal preganglionic neurons were distributed between spinal segments T1 and L1. Approximately 60% of the neurons were located between segments T7 and T10. Only slight differences were observed between the distributions of neurons projecting to the right and left adrenal. An estimation was made of the number of axons present in nerve segments close to the adrenal, and it was calculated that approximately 15% of the neurons projecting to the adrenal demonstrated uptake of horseradish peroxidase. The velocity of transport was calculated to be approximately 4 mm/hour. Horseradish peroxidase filled neurons so that it was possible to observe the major orientation of perikarya and dendrites. The perikarya and dendrites exhibited a predominately transverse orientation. Extremely close apposition of dendrites from different neurons was regularly observed.

663 THE ELECTRODERMAL RESPONSE AS A MODEL FOR CENTRAL SYMPATHETIC REACTIVITY: THE EFFECTS OF CLONIDINE. M. C. Koss and M. A. Davison. Dept. Pharmacology, Univ. of Okla. Col. Med., Oklahoma City, Okla. 73190.

Electrodermal responses (EDR) were evoked centrally by stimulation of reactive loci in the posterior hypothalamus and peripherally by stimulation of the distal portion of the sectioned median or ulnar nerve in pentobarbital anesthetized cats. Moderate doses of clonidine (3-30 µg/kg, i.v.) reduced the amplitude of the centrally evoked EDR while having no effect on the peripherally evoked responses. This central action of clonidine occurred concomitantly with the clonidine-induced bradycardia and hypotension. We have found that both the centrally and peripherally evoked EDR are frequency dependent when a maximal current is used. Administration of clonidine shifted the centrally evoked EDR frequency-response curve to the right in a dose related manner at 3, 10, and 30 µg/kg i.v. One µg/kg was without effect on these responses. Doses as high as 100 µg/kg i.v. were without effect on the peripherally evoked frequency-response curves. In addition, the central depressant action of clonidine was partially reversed following administration of yohimbine (0.25 - 1.0 mg/kg, i.v.). These results suggest that clonidine inhibits central reactivity in this sympathetic-cholinergic system in a manner analogous to its action on other sympathetic systems, and that a central adrenergic inhibitory mechanism may be involved. (Supported by MH 24083-01 and grants from the Oklahoma Heart Association)

664 THE ELECTRODERMAL RESPONSE AS A MODEL FOR CENTRAL SYMPATHETIC REACTIVITY: BASIC CHARACTERISTICS AND PERIPHERAL INNERVATION. M. A. Davison and M. C. Koss. Dept. Pharmacology, Univ. of Okla. Col. Med., Oklahoma City, Okla. 73190.

The electrodermal response (EDR) of the cholinergic-sympathetic sudomotor system was investigated in pentobarbital anesthetized cats. Responses were evoked either from brainstem loci or by stimulation of post-ganglionic peripheral nerves. This response was found to be dependent upon innervation of both the median and ulnar nerves (approximately 40 and 60 per cent respectively). The evoked potentials were large in amplitude when elicited by central (mean = 14.7 mV) or peripheral stimulation (mean = 9.25 mV). Both the centrally and the peripherally evoked responses were found to be frequency dependent when a maximal current was used. The peripherally evoked EDR reached its maximal amplitude at 16 Hz whereas the EDR evoked from central loci did not reach maximal amplitude until 32-64 Hz. With optimal stimulation parameters EDR were stable over time with only 5% depression from control amplitude after three hours of continuous, once per minute stimulation. Centrally evoked responses were abolished following ganglionic blockade (C_6) and all responsivity was suppressed by small doses of atropine (100 µg/kg, i.v.). The EDR was also found to be independent of blood flow in that occlusion of the peripheral blood supply for 10 minutes did not greatly alter the amplitude of either the centrally or peripherally evoked responses. Since the peripheral neurotransmitter is acetylcholine, it appears that the EDR is a sympathetic effector which may serve as an effective model system with which to investigate the actions of various adrenergic agents on central sympathetic control. (Supported by MH 24083-01 and grants from the Oklahoma Heart Association)

665 CEREBELLAR PRESSOR RESPONSE IN THE DOG. K.J. Dormer* & H.L. Stone. Marine Biomedical Institute, Univ. Texas Med. Br., Galveston, Texas 77550.

The fastigial pressor response has been previously described for the cat and monkey. This study concerns the pressor-tachycardia response elicited by stimulation in the fastigial nucleus of mongrel dogs. An angled-electrode, subtentorial approach to the nucleus gave consistent access to the target areas. Rostral portions of this cerebellar nucleus and its nearby white matter and also its subjacent vermis when stimulated caused cardiovascular changes. Mean arterial pressure increased up to 150 mmHg above control. A concomitant tachycardia was proportional to the pressure rise and reached heart rates as high as 190 beats/min above control. These responses are the result of widespread sympathetic activation. The peripheral pressor response was reduced by injection of alpha-adrenergic blocking agents and the tachycardia was obliterated by bilateral stellate ganglionectomy. Only the cardiac portion of the response, however, was subject to buffering through the baroreceptor reflex. The evoked tachycardia peaked after four seconds of stimulation and immediately began to subside, often into a bradycardia while the stimulation continued. By contrast, the mean arterial pressure remained elevated throughout stimulations as long as 30 seconds. Disruption of the baroreceptor feedback loop by bilateral carotid sinus isolation caused the bradycardia to disappear from the response. The rate of rise in heart rate increased and the peak tachycardia was enhanced. Bilateral vagotomy showed similar effects with a sustained tachycardia during stimulation. Thresholds for the response were near 0.25 mA at 80pps. Stimulus intensity curves are linear for currents approaching 2 mA whereupon the curves plateau and begin to fall off. The response remains viable under submaximal anesthetic doses of alpha chloralose and is not obliterated by high levels of barbiturates (30-40 mg/kg). (Supported by NASA Grant NGR 44 088 002.)

666 TEMPORAL RELATIONSHIPS DURING BAROSENSORY MODULATION IN THE CONSCIOUS RABBIT. Michael P. Gimpl*,Andrew L. Brickman*, Mark P. Kaufman* and Neil S. Schneiderman. Dept. of Psychology, Univ. of Miami, Coral Gables, Florida, 33124.

Electrical stimulation (10 sec. train) of the anterior (lateral or medial) or posterior (lateral or medial) hypothalamus in conjunction with stimulation (5 sec. train) of the aortic depressor nerve (ADN) was used to assess the temporal relationships involved during barosensory modulation in the unanesthetized rabbit. Stimulation of each hypothalamic location (8 anterior; 8 posterior) elicited blood pressure and heart rate decreases although the highest thresholds and smallest responses occurred to stimulation of the medial posterior hypothalamus. Response magnitudes to hypothalamic stimulation peaked approximately 15 sec. after stimulation onset. Presentation of anterior hypothalamic stimulation onset coincident with or 5, 10 or 15 sec. prior to stimulation of the ADN augmented the depressor - decelerator response to ADN stimulation. The augmenting effect was more pronounced for heart rate than for blood pressure. Simultaneous onset of ADN and posterior hypothalamic stimulation did not influence ADN responses. In contrast, when ADN stimulation was delayed until 5, 10 or 15 sec. after onset of posterior hypothalamic stimulation, small, moderate, and full attenuation of ADN responses occurred, respectively. Since ADN and posterior hypothalamic stimulation each ordinarily led to depressor - decelerator responses, the "gating" of the ADN responses cannot be accounted for by a simple summation of the cardiovascular responses elicited by the brain and ADN stimulation. Similarly, augmenting of the ADN responses by anterior hypothalamic stimulation cannot be accounted for by the simple summation of cardiovascular responses. Thus, when ADN and anterior hypothalamic stimulation onset together, augmented ADN responses occurred prior to the normal onset latency of the cardiovascular changes to brain stimulation.

667 REFLEX ACTIVATION OF THE SYMPATHETIC INHIBITORY PATHWAY TO THE URINARY BLADDER BY ELECTRICAL STIMULATION OF VESICAL AFFERENTS. W. C. de Groat and R. J. Theobald*. Dept. Pharmacology, Sch. Med. Univ. of Pittsburgh, Pittsburgh, Pa. 15261

Experiments were conducted on chloralose anesthetized cats in which the sacral parasympathetic outflow to the urinary bladder was interrupted by bilateral pelvic nerve transection. Electrical stimulation (3-20 cps) of vesical afferents central to the point of transection elicited a depression of transmission in vesical parasympathetic ganglia and a depression of bladder contractions produced by intermittent stimulation (1-5 cps) of vesical efferent fibers. The former depression was antagonized by the administration of dihydroergotamine (30-75 ug, i.a.), an alpha adrenergic blocking agent, and the latter by propranolol (200-400 ug, i.a.) a beta adrenergic blocking agent. Both depressions were blocked by bilateral transection of the sympathetic innervation (i.e. hypogastric nerves) to the bladder. Similar inhibitory responses were elicited by stimulation of the hypogastric nerves. The inhibitory responses were observed in acute spinal preparations and in cats with an intact spinal cord. Stimulation of vesical afferents also elicited reflex firing on nerve filaments on the surface of the urinary bladder and on hypogastric nerves at latencies of 125-150 msec and 50-80 msec, respectively. The firing on bladder nerve filaments was abolished by transecting the ipsilateral hypogastric nerves. The afferents eliciting the reflex firing and bladder inhibition were A$\gamma\delta$ fibers. It is concluded that the urinary bladder is subject to an inhibitory feedback control through a spinal sympathetic reflex pathway. The inhibitory mechanism is activated by vesical afferents entering the sacral cord and is mediated by depression of bladder ganglia as well as bladder smooth muscle.

668 THE HIPPOCAMPUS AS A POSSIBLE SITE OF PROPRANOLOL'S ANTIHYPERTENSIVE ACTION. Willa B. Phyall* and H. Lloyd Garvey. Department of Pharmacology, Howard University College of Medicine, Washington, DC 20059, U.S.A.

The precise mechanism of the antihypertensive action of beta adrenergic blocking drugs is not known. Both peripheral and central sites of action have been proposed. In previous studies, it was demonstrated that the antihypertensive action of dl-propranolol was associated with significant concentrations of the drug as well as active metabolites at specific brain sites. The present study was designed to localize specific brain sites involved in this antihypertensive action. Using chloralose anesthetized cats and Wistar rats, it was observed that discrete stereotaxic administration of dl-propranolol at dorsal hippocampal sites produced decreases in both blood pressure and heart rate. Ablation of these sites prevented these responses. Similar drug induced changes were not observed when dl-propranolol was administered at ventral hippocampal sites. Chronic studies in rats indicated that daily oral or subcutaneous drug administration significantly decreased blood pressure and heart rate within 6 days. Chronic drug therapy in rats with dorsal hippocampal lesions produced no alteration in either blood pressure or heart rate. Further studies using dextro, levo as well as 4-OH propranolol are being performed. In addition, degeneration studies (Fink-Heimer technique) are being conducted in order to trace fibre pathways involved in these responses. The results indicate that central mechanisms in the dorsal hippocampus may be involved in the centrally mediated antihypertensive action of propranolol. (Supported by the Sinsheimer Trust Fund, Washington Heart Association and N.I.H. Grant #RR-08016.)

669 INHIBITION AND FACILITATION OF CHEMORECEPTOR-EVOKED SYMPATHETIC REFLEXES IN THE CAT. P.M.Lalley and W.C.DeGroat. Dept. Pharmacol. and Therap., Coll. Med., U.Florida, Gainesville,32610 and Dept. Pharmacol., Sch. Med., U.of Pittsburgh, Pittsburgh 15261.

Electrical stimulation of myelinated chemoreceptor afferents in the carotid sinus nerve (CSN) of the cat evoked reflex firing on thoracic and lumbar sympathetic nerves (DeGroat and Lalley,Brain Res.80:17-40,1974). Activation of low threshold afferents in the aortic depressor nerve (ADN) or vagus nerve (VN) with single shocks or trains of stimuli (100 to 300 Hz) inhibited CSN-evoked sympathetic firing. The inhibition occurred with a short latency, being detectable when conditioning ADN or VN volleys were applied at the same time as CSN test stimuli.Maximum inhibition occurred with C-T intervals of 20 to 30 msec.The duration of inhibition ranged from 200 to 300 msec following short trains of stimuli. ADN afferents mediating inhibition had conduction velocities ranging between 20 and 40 M/sec. Stimulation of slowly conducting myelinated afferents (8 to 16 M/sec.) in the ADN evoked sympathetic discharges which resembled those evoked by CSN stimulation. Activation of low-threshold myelinated afferents in the CSN did not inhibit sympathetic reflexes. However, stimulation of nonmyelinated afferents in the CSN with trains of shocks (100 to 300 Hz, 50 to 150 msec train duration) produced prolonged inhibition (500 to 1000 msec) of chemoreceptor-evoked sympathetic reflexes. Repetitive stimulation (5 to 30 Hz for 5 to 30 sec) of CSN at intensities below threshold for unmyelinated afferents produced a long lasting posttetanic potentiation (PTP) of CSN-sympathetic reflexes (mean duration, 6.0 min) but did not potentiate sympathetic reflexes mediated by other visceral or somatic afferents. These results support the following conclusions: (1) the chemoreceptor-evoked sympathetic reflex can be inhibited by myelinated afferents in the ADN and unmyelinated afferents in the CSN which are probably baroreceptor in function.(2) The inhibition probably occurred within the medulla, as suggested by its early onset.(3) PTP may have occurred at the first synapse on the reflex pathway, or at least at some point central to the input of other visceral and somatic afferents.

670 EFFECT OF INTRAVENOUS INFUSIONS OF AN ANGIOTENSIN II ANTAGONIST ON THE DRINKING AND PRESSOR RESPONSES TO INTRACEREBROVENTRICULAR INJECTIONS OF ANGIOTENSIN II. William E. Hoffman* and M.I. Phillips. Dept. Physiology, University of Iowa, Iowa City, Iowa 52240

When injected into the cerebral ventricles of unanesthetized rats angiotensin II(AII) produces a short latency pressor and drinking response. Both are reported to be mediated by the central nervous system. We have used intravenous(IV) infusions of an AII antagonist, 1-Sar-8-ala-angiotensin II (P113), to examine the pressor and dipsogenic responses to intraventricular(IVT) injections of AII in unanesthetized rats. These experiments were designed to determine whether P113, infused in concentrations capable of blocking the pressor response to a larger IV dose of AII, is capable of blocking the pressor response of IVT injections of AII.

Experiment 1. Twelve male Sprague-Dawley rats were implanted with a lateral ventricular cannula three days before the start of the test. On the day of the test a femoral artery catheter, intended for measuring blood pressure,was inserted under ether anesthesia. A femoral vein, to be used for infusions, was catheterized at the same time. Upon recovery the femoral artery catheter was connected to a blood pressure transducer and testing was started. Testing procedure was as follows: test 0- a control IVT infection of 5ul artificial cerebrospinal fluid(CSF), test 1- 50ng AII IVT injected in a 5ul CSF vehicle, test2- 50ng AII IVT with a simultaneous IV infusion of saline(18ul/min), test 3, 50ng AII IVT with a simultaneous IV infusion of P113. Two doses of IV P113 were used. In group 1 (n=5) 500ng/min P113 was infused IV during the IVT AII test. In group 2 (n=7) 1800ng/min P113 was used. IV infusions were started seven minutes before and continued seven minutes after the IVT test injections. All of the above tests were separated by 1 hour. Control experiments showed that drinking and pressor responses to IVT AII injections at 1 hour intervals were reliably reproduced. For group 1 the drinking and pressor responses were: test 1- 6.00±2.02 ml (mean±SD), 24.20±6.01 mmHg; test 2- 3.68±2.44 ml, 14.40±7.86 mmHg; test 3- 7.18±1.79 ml, 21.80± 2.71 mmHg. For group 2 the responses were: test 1- 5.27±3.71 ml, 16.00± 6.63 mmHg; test 2- 4.01±2.79 ml, 18.14±5.89 mmHg; test 3- 5.30±2.85 ml, 17.28±3.61 mmHg. None of the differences were significantly changed from test 1. These data indicate IV infusion of these 2 doses of P113 has no significant effect on drinking and pressor responses to IVT AII injections.

Experiment 2. In 12 animals the effect of IV P113 on the pressor response to IV AII (180ng/min) was examined. Rats were catheterized with 1 femoral artery and 2 femoral vein catheters under ether anesthesia. Two hours later the pressor response to AII (180ng/min, IV) was recorded. One hour later the AII pressor response with a simultaneous IV infusion of 500ng/min (n=5) or 1800ng/min (n=7) P113 was tested. P113 infusions were started seven minutes before and continued with the IV AII infusions (five minutes). In contrast to its lack of effect on central AII responses 500ng/min IV P113 attenuated the IV AII pressor response 53% (53.00±8.12 mmHg→ 25.00 mmHg, $p<.01$) while 1800ng/min IV P113 attenuated the AII pressor response 79% (46.57±14.06 mmHg→10.14±7.09 mmHg, $p<.01$). IV P113 ifusions were thus shown capable of blocking, in a dose-response relationship, the pressor response of a peripheral infusion of AII.

The results of these experiments show the IVT AII has its pressor and dipsogenic action on a site or sites inside the blood brain barrier. Peripheral infusions of P113 in the dosages and time parameters measured apparently do not cross the blood brain barrier in sufficient concentrations to effectively compete with these sites.

671 CENTRAL SYMPATHETIC STIMULATION BY TRIFLUOROETHYL VINYL ETHER (FLUROXENE)

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In a study of the cardiovascular effects of 2,2,2-trifluoroethyl vinyl ether (fluroxene) in human volunteers, Cullen et al. (Anesthesiology 32: 218, 1970) reported that a 12% alveolar concentration of this inhalational anesthetic produced increases in both heart rate and cardiac output of over 50% above control values and a 20% increase in mean arterial pressure. In contrast, alveolar concentrations of 5% and 9% fluroxene produced no significant changes (acutely) in any of these parameters. To investigate the mechanism of this unique dose-dependent hyperdynamic circulatory state, we continuously recorded eight channels of bipolar EEG data, arterial pressure, heart rate, P_ACO_2 and alveolar fluroxene concentration (infrared analysis of end-tidal expired gas) in three healthy, informed, unpremedicated volunteer patients undergoing fluroxene anesthesia. A parallel study was done in cats with electrodes chronically implanted in selected areas of the limbic, reticular activating and diffusely projecting systems and eight parasagittal and temporal ECoG leads. No other EEG-active agents were used.

Continuous generalized electrographic seizure activity consisting of high-frequency, high-amplitude spikes interrupted by brief periods of inter-ictal silence occurred in each subject at alveolar fluroxene concentrations of 9.8% ± 0.4% (standard error), independently of P_ACO_2. Subjects were paralyzed with pancuronium bromide at the time and thus no motor activity was observed. This EEG phenomenon, previously unreported in humans anesthetized with fluroxene, was associated with sustained increases in heart rate, arterial pulse pressure, mean arterial pressure and pupillary diameter. The inspired fluroxene was discontinued immediately with appearance of the seizure pattern in the EEG, but this pattern and its circulatory correlates persisted for a number of minutes despite falling alveolar anesthestic concentration. The relative constancy of the above concentration threshold for electrographic seizure activity with fluroxene and the prolonged duration of the activity once initiated both suggest that the subjects equilibrated at 12% fluroxene in the study of Cullen et al. were in electrical "status epilepticus", although no motor activity was observed.

Depth recordings in the cat revealed that fluroxene-induced spike activity originated in the amygdala and hippocampus and increased in incidence and amplitude in association with a progressive dose-related decline in multiple unit activity (MUA) recorded from the midbrain reticular formation; when the latter decreased to approximately one-fourth of the pre-anesthetic REM sleep control value, high-frequency limbic spikes developed and rapidly involved all cortical regions. Pre-tectal (supracollicular) transection of the brainstem was performed in one animal equilibrated at an alveolar fluroxene concentration below the seizure threshold and this resulted in the immediate appearance of limbic and neocortical seizure activity without autonomic correlates. This was the only fluroxene seizure in the cat which was not associated with peripheral sympathetic responses.

In summary, the findings of this study suggest that the marked circulatory stimulation which has been reported to develop in humans at alveolar fluroxene concentrations somewhere between 9 and 12% may be the result of electrical seizure activity involving cerebral centers governing autonomic outflow, particularly limbic structures and orbito-frontal cortex. The absence of seizure-associated sympathomimetic effects of fluroxene after high midbrain transection is also consistent with a cerebral origin of the sympathetic excitation, which appears to be predominantly β-adrenergic in nature although with distinct α components. Suppression of brainstem inhibitory influences over more rostral structures (either pharmacologically by increased anesthetic concentration or surgically by transection) appears to precipitate generalized electrocerebral seizures at high concentrations of fluroxene by unmasking the direct irritative effect of the agent. A similar mechanism for ether-induced seizures has been proposed by Mori et al.

672 HISTOCHEMICAL EVIDENCE FOR THE PERMANENT DESTRUCTION OF ARCUATE DOPAMINE NEURONS BY NEONATAL MONOSODIUM L-GLUTAMATE IN THE RAT. Charles B. Nemeroff, Lester D. Grant, Lindy E. Harrell*, Garth Bissette*, Gregory N. Ervin*, and Arthur J. Prange, Jr.*. The Neurobiology Program, Depts. Psychiatry and Anatomy, Univ. North Carolina, Chapel Hill, North Carolina 27514.

It is well established that high doses of the sodium salt of l-glutamic acid (MSG) administered neonatally, induce a hypothalamic lesion in rodents and primates. As adults MSG-treated animals show growth, endocrinological and behavioral deficits. The hormonal changes observed in these animals suggest an impairment of the hypothalamic-pituitary-gonadal axis. Because of the growing number of reports implicating the tuberulo-infundibular dopamine system in the control of gonadotrophin-releasing hormone secretion, we assessed the effects of high doses of MSG, administered neonatally, on the arcuate dopamine neurons. Timed-pregnant Sprague-Dawley rats were maintained on laboratory chow and water ad libitum. Their offspring were injected with either 5 injections of MSG(4 g/kg) or 0.9% saline intraperitoneally at 2 day intervals in the first 10 days of life. As adults MSG-treated animals were extremely stunted and obese although food and water intake appeared normal. When sacrificed, MSG-treated females had significantly smaller ovaries, uteri, and pituitaries, while MSG-treated males had significantly smaller testes and pituitaries when compared with controls. Conventional light microscopic methods (hematoxylin and eosin) did not reveal any cytopathology in MSG-treated animals. The fluorescent histochemical method for visualization of biogenic amines (Falk-Hillarp technique) revealed a complete loss of dopaminergic perikarya in the arcuate nucleus. Other catecholamine systems appeared normal. These results suggest that neonatally administered MSG may be useful as a tool to selectively destroy a single monoaminergic tract: the tuberulo-infundibular dopamine system.(Supported by NIMH Grant 11107, NICHHD Grant HD-03110 and the Alfred P. Sloan Foundation).

673 THE ROLE OF THE SUPRACHIASMATIC NUCLEI IN REPRODUCTIVE CYCLICITY. E. Barry Smalstig* and James A. Clemens, Dept. of Physiol. Research, The Lilly Research Labs., Indianapolis, IN 46206.

To clarify the role of the hypothalamic suprachiasmatic nuclei (SCH N) in maintenance of reproductive cyclicity the following procedures were attempted: 1) improvement of lesioning technique limiting damage to SCH N, 2) following subsequent estrous cycles (or acyclicity), 3) endocrinological characterization of the resulting reproductive states, 4) physiological manipulation of lesioned rats to identify any aberrations or idiosyncrasies in responses to endocrine stimuli, and 5) correlation of SCH N damage with reproductive states and responses to these stimuli.

Using platinum electrodes and an anodic current of 1mA for 12 sec. bilateral lesions were placed (Burés Atlas) in the SCH N of normally cycling female rats. Daily vaginal cytology was followed for 2-5 mo. revealing a variety of reproductive patterns. Rats were either sacrificed to obtain organ weights and serum hormone levels or exposed to the following stimuli: 1) ether stress, 2) cervical stimulation, 3) mating, 4) hormone treatments. 80% of the rats with SCH N damage exhibited constant estrus (CE) and failed to ovulate and/or become pseudopregnant (PSP) in response to stress or cervical stimulation. Pregnancy could not be maintained in these animals although most did mate. CE rats exhibited smaller ovaries, but larger pituitaries and uteri than estrous controls. LH levels were slightly higher but prolactin (PRL) tended to be lower. Dorsal and medial damage by cut or lesion produced the same CE effect as lesion of the nucleus itself. Repeated PSP resulted from preoptic lesions. The dramatic effects of these small SCH N lesions indicate that these nuclei are a dominant part of neural paths controlling pituitary LH, and possibly PRL, release. Further studies are in progress.

674 HYPOPHYSECTOMY FACILITATES LORDOSIS RESPONDING IN FEMALE RATS. J.F. Rodriguez-Sierra*, W.R. Crowley*, and B.R. Komisaruk. Institute of Animal Behavior, Rutgers University, Newark, New Jersey, 07102.

Lordosis was elicited in 49% of 87 hypophysectomized (H)-ovariectomized (O)-hormonally-untreated (U) rats in response to palpation of the flanks and perineum. In contrast, only 12% of 113 O-U rats showed lordosis to this stimulation ($p=8x10^{-9}$). Heightened lordosis responsiveness of H-O, as compared to O rats was further substantiated in that low daily dosages of estradiol benzoate (EB) (1ug/Kg/day subcu) induced sexual receptivity in the H-O rats earlier than the O rats. For example, after 6 days of EB, 8/9 H-O rats mated whereas only 2/10 O rats mated ($p=0.004$); furthermore, the group mean lordosis quotients were 73 and 8 respectively ($p<0.01$). Recent studies (Moss and McCann, Science, 181: 177, 1973; Pfaff, Science, 182: 1148, 1973) have demonstrated that luteinizing hormone -releasing hormone (LRH) induces sexual receptivity in estrogen-treated rats when injected systemically. We hypothesize that hypophysectomy disinhibits the synthesis and/or release of LRH which may then act locally in the hypothalamus to increase lordosis responsiveness even in the absence of estrogen. Preliminary support for this hypothesis is that dihydrotestosterone propionate, which depresses plasma LH (Naftolin and Feder, J. Endocr. 56: 155, 1973) perhaps by depressing synthesis and/or release of LRH, (500 ug/rat/day, subcu) inhibited lordosis responsiveness in 9/14 H-O rats.

675 ENHANCEMENT OF LORDOTIC BEHAVIOR BY INTRAHYPOTHALAMIC INFUSION OF LUTEINIZING HORMONE-RELEASING HORMONE. Mark M. Foreman* and Robert L. Moss. Dept. Physiol., Univ. Tx. Hlth. Sci. Ctr., Southwestern Med. Sch., Dallas, Texas, 75235.

The effects of intrahypothalamic infusion of luteinizing hormone-releasing hormone (LRH), thyrotropin-releasing hormone (TRH) and saline on the initiation of lordosis behavior were investigated. Seventy-five ovariectomized female rats, known to be sexually active, had stainless steel (23 gauge) cannulae surgically implanted into either the medial preoptic area (MPOA; N=30), arcuate-ventromedial complex (ARC-VM; N=30), lateral hypothalamic area (LHA; N=6) or the cerebral cortex (CC; N=9). Following a post surgical recovery period, each animal was pretreated with estrone, in doses too low to initiate consistent or substantial lordotic behavior. Forty-eight hours later, these animals were infused over a 60 sec period with a total volume of 0.5ul. Fifty nanograms (ng) of LRH injected systemically or infused into the LHA or CC did not enhance lordosis behavior. Similar findings were observed with sham, saline or TRH infusion into the MPOA or ARC-VM complex. However, 50ng of LRH infused into either the MPOA (L/M=0.58, 0.05; M±SEM) or ARC-VM complex (L/M=0.53; 0.03; M±SEM) significantly increased ($p < .001$) the lordosis-to-mount ratio (L/M) as compared to pre-infusion behavior (MPOA: L/M=0.13, 0.03; M±SEM; ARC-VM: L/M=0.03, 0.08; M±SEM). This effect was most evident one hour and 45 minutes post-infusion. These results support the hypothesis that LRH acts on neural tissue to initiate lordosis behavior and suggest a possible role of LRH in synchronizing the release of luteinizing hormone and sexual behavior in the female rat. (Supported by NSF Grant #GB- 43494).

676 INTRACEREBRAL INFUSIONS OF LUTEINIZING HORMONE - RELEASING FACTOR INDUCE LORDOSIS IN RATS. Lorraine R. Herrenkohl and Ingrid M. Verhulst. Dept. Psychol., Temple Univ., Philadelphia, 19122.

Luteinizing hormone-releasing factor (LH-RF) recently isolated from the hypothalamus and synthesized, causes the release of luteinizing hormone from the adenohypophysis, thereby inducing ovulation. More recently this neurohormone has been found to induce sexual behavior. Systemic injections of LH-RF were reported to facilitate lordosis not only in estrogen-primed ovariectomized rats (Moss and McCann, Science 181: 177, 1973) but in similarly treated hypophypectomized rats as well (Pfaff, Science 182: 1148, 1974). We induced lordosis by bilateral infusions of 100 ng LH-RF/rl saline into the medial preoptic-anterior hypothalamus of estrogen-primed ovariectomized rats. Doses of 20 ng LH-RF /rl saline were less effective; saline alone produced virtually no effect. We have evidence suggesting that sensitivity to intracerebral infusions of LH-RF wanes during the dark phase of the light cycle and that priming doses of .002 mg estradiol benzoate/ml sesame oil were optimal for LH-RF induction of lordosis. The results demonstrate therefore that LH-RF acts centrally to induce mating behavior.

677 THE LH-RH SYSTEM IN THE POSTNATAL RAT. J.C. King*, A.A.Gerall K.E. Elkind* and J.B. Fishback*. Dept. Psychology, Tulane University, New Orleans, La. 70118.

The development of fibers immunoreactive with LH-RH was traced using the technique of immunocytochemistry in the hypothalamus of male and female rats during their critical postnatal period of sexual differentiation.

Positive fibers are present in both sexes on the day of birth in the arcuate-median eminence region and in a zone at the beginning of the third ventricle at the plane of the preoptic area. Control adjacent sections treated with antisera absorbed with LH-RH did not show staining in either of these regions. In both sexes staining diminishes from day 1 (day of birth) to a minimum on day 5. Subsequent to this time, in the female the number of LH-RH immunoreactive fibers increases in both the arcuate-median eminence and preoptic regions until day 9, when it is equivalent to the density observed in adult animals. In the male, the number of stained fibers increases only slightly from day 5 to day 9. Thus, the difference between female and male animals in stained fibers is greatest on day 9. In addition, the male has more positive elements in the region of the infundibular recess and less in the region of the tuberoinfundibular sulcus than the female.

These results parallel LH levels in rats reported by Brown Grant (INSERM 32: 357, 1974). In the female both LH-RH reactive fibers and LH plasma levels reach a peak on day 9. Comparable changes are not recorded in male rats in either LH-RH fibers or LH plasma levels.

678 TESTICULAR RESPONSES TO PHOTOPERIOD ARE BLOCKED BY LESIONS OF THE SUPRACHIASMATIC NUCLEI IN GOLDEN HAMSTERS. Benjamin Rusak* and Lawrence P. Morin* (SPON: I. Zucker). Dept. Psychol., Univ. Calif., Berkeley, CA 94720.

Lesions of the suprachiasmatic nuclei (SCN) of golden hamsters (Mesocricetus auratus) prevented the testicular regression ordinarily observed in short photoperiods or after peripheral enucleation. These lesions also induced testicular regrowth in hamsters whose testes had previously been regressed by the absence of adequate photostimulation. Interruption of the primary optic tracts just caudal to the chiasm did not prevent testicular regression by short photoperiods, nor prevent recrudescence of regressed testes by long photoperiods. We hypothesize that testicular responses to photoperiod are mediated by the retino-hypothalamic tract, which projects to the SCN, and that the SCN in turn control the secretion of a pineal antigonadal substance. The mechanism for this control may involve entrainment by light of hormonal rhythms in the pineal that are regulated by the SCN (Klein, Neurosciences III: 509, 1974; Rusak, unpublished dissertation, 1975). Alternatives to this hypothesis will be discussed.

679 FORMATION OF LAMELLAR WHORLS IN ARCUATE NEURONS OF THE HYPOTHALAMUS OF CASTRATED AND MORPHINE-TREATED MALE RATS. M.T. Price, J.W. Olney and T.J. Cicero.* Wash. Univ. Sch. Med., Dept. Psychiat., St. Louis, Mo. 63110.

Brawer (J. Comp. Neur. 143:411,1971) reported that whorls of endoplasmic reticulum develop in neurons of the arcuate nucleus of the hypothalamus of rats following castration. Testosterone (TS.) replacement prevented whorl formation and no whorls were detected in controls. Ford et al. (Neurobiol. 4:1,1974) found abundant whorls in arcuate neurons of male rats following morphine (MO.) treatment (60 mg/kg or more I.P.) while a few whorls were detected in only 1 of 5 controls. MO.-induced whorl formation may have resulted from TS. depletion in that Cicero et al. (J. Pharm. Exp. Therap. 192:542,1974) have reported 85% reduction in plasma TS. following MO. treatment of male rats. In the present study we attempted to reproduce the findings of Brawer and Ford et al. but we employed the method of MO. treatment of Cicero et al. (75 mg MO. pellet implant S.C.)

Our findings differ from theirs in that an average of 7 whorl-containing neurons (WCN)/section of arcuate nuclei was found in control male rats, those treated with MO. for up to 3 d. or MO. + TS. for up to 14 d. Castration or 14 d. MO. treatment without TS. replacement caused an increased incidence of WCN (20 & 39/section respectively). These findings and those of Brawer and Ford et al. suggest a correlation between whorl formation and TS. deficiency.

Mapping the pattern of whorl formation in the arcuate nuclei of all animals in this study, 58% WCN were clustered near the infundibular recess and 24% were located in the dorsal periventricular quadrant of the nucleus. Cells containing luteinizing hormone releasing factor (LH-RF) have also been localized in these regions (Scott et al. Cell Tiss. Res. 149:371,1974; Zimmerman et al., Endocrin. 5:1,1974). Thus, WCN may produce LH-RF perhaps under TS. negative feedback regulation.

680 ANDROGEN ACCUMULATION BY SYRINGEAL MOTONEURONS AND NEURONS IN OTHER SONG CONTROL AREAS IN THE BRAIN OF THE ZEBRA FINCH (Poephila guttata). Arthur P. Arnold* (SPON:C. Pfaffmann). The Rockefeller University, New York, N.Y. 10021.

Using the autoradiographic method in the zebra finch, areas of the brain were identified which contain cells which accumulate testosterone or its metabolites after intramuscular injection of tritiated testosterone. Among these areas are the caudal nucleus of the hyperstriatum ventrale (HVc), nucleus intercollicularis of the midbrain (ICo), and the tracheosyringeal portion of the hypoglossal nucleus in the medulla (nXIIts). These three have been shown by other investigators to control or influence song and other vocalizations in passeriform birds: lesions of HVc specifically disrupt song, stimulation of ICo elicits vocalizations, and histological studies demonstrate that nXIIts contains the motoneurons supplying the syringeal (vocal) muscles. Since song in zebra finches is under the influence of androgens, this suggests that these three areas are sites of action of androgens in modulating singing behavior. The accumulation by motoneurons is of especial interest, since it suggests that androgens modify the function of the final common path, through which all neuronal influences act to control the syringeal muscles.

Other areas of the zebra finch brain which contain hormone-concentrating cells include the magnocellular nucleus of the anterior neostriatum, lateral septum, medial preoptic area, periventricular magnocellular nucleus of the anterior hypothalamus, dorsal infundibular areas, dorsomedial thalamus, periventricular medial neostriatum, nucleus taeniae of the archistriatum, and ventral paleostriatum. Some of these areas may be involved in the control of androgen-dependent events such as sexual and agonistic behavior, and feedback regulation of the pituitary.

681 LOCATIONS OF STEROID HORMONE-CONCENTRATING CELLS IN THE CENTRAL NERVOUS SYSTEM OF RANA PIPIENS. Darcy B. Kelley and Donald W. Pfaff. The Rockefeller University, New York, NY 10021

The distribution of steroid hormone-concentrating cells in the central nervous system of leopard frogs was determined autoradiographically. Five adult, female Rana pipiens were ovariectomized. One week later, two frogs were injected intraperitoneally with tritiated estradiol and two frogs received tritiated testosterone. The frogs were sacrificed and the brains quickly removed and frozen. Unfixed, unembedded frozen sections, 4 μ thick, were mounted directly on emulsion coated slides. Brain sections from the fifth, uninjected frog served as controls for negative and positive chemography. Slides were exposed for 5 to 10 months and scanned systematically for labelled cells (grain reduction of at least 5 times background).

Estrogen-concentrating cells in the telencephalon were found in the ventral striatum, ventral septum, nucleus accumbens and rostral amygdala. The anterior preoptic area (APOA) and ventral infundibular nucleus (VIN) contained large numbers of labelled cells. Labelled cells were also found in the ventral thalamus. In the mesencephalon, estradiol-concentrating cells were found in the laminar nucleus of the torus semicircularis. The intensity of labelling of testosterone-concentrating cells was less than that of estradiol. So far, testosterone-labelled cells have been found in the APOA.

Steroid hormone-concentrating cells have also been reported in the brain of another anuran amphibian, Xenopus laevis (Kelley et al., Morrell et al., J. Comp. Neurol., 1975). The distribution of labelled cells in the brains of these two frogs appears very similar. Some of the regions which concentrate sex hormones in Rana and Xenopus (notably the APOA and VIN) have been implicated, using other techniques, in neuroendocrine and behavioral regulation.

682 POSTNATAL ONTOGENY OF CYTOPLASMIC AND NUCLEAR ESTROGEN-SPECIFIC BINDING IN THE BRAIN AND PITUITARY OF THE RAT. N. MacLusky, C. Chaptal, and B. McEwen. The Rockefeller University, New York, N.Y. 10021.

Cell nuclear isolation, following in vivo administration of ^{3}H-17β-estradiol (^{3}HE) was used to examine the postnatal development of nuclear estrogen binding in pituitary (P), hypothalamus (H), preoptic area (PO), amygdala (A), cerebral cortex (C), mid-brain (MB) and cerebellum (CB) of female Wistar rats. Saturable estrogen specific ^{3}HE binding was observed in nuclei from all 7 tissues in the youngest animals studied (day 3): pre-treatment with unlabeled E, 17α-estradiol, or the anti-estrogen CI628, but not with progesterone, 5α-dihydrotestosterone (DHT), 19-OH DHT, androstan-3α-17β-diol, or androstan-3β-17β-diol, decreased ^{3}HE retention in all tissue nuclear fractions. However, the tissue distribution of nuclear binding at this age differed markedly from that observed in adult females, with relatively high levels of nuclear uptake in C, and low levels in P and PO, in the neonates. From days 3-10 nuclear ^{3}HE binding capacity increased in all tissues, but most strikingly in P and H. Thereafter C uptake decreased to very low levels, P,H,A,MB and CB uptake declined slightly, while PO binding increased - resulting, by day 25, in an uptake pattern similar to that of adults. The distribution of cytosol high-affinity estrogen specific binding, determined using Sephadex LH-20 gel filtration, resembled that of nuclear ^{3}HE retention at all ages studied. However, only in PO was the rise in nuclear binding at day 10 paralleled by increased binding at the cytosol level. These results confirm previous reports indicating that the neonatal rat possesses brain and pituitary estrogen binding mechanisms similar to those of the adult rat, but suggest that the distribution, capacities and relationship between these mechanisms change during early postnatal life. (Supported by grants from USPHS and The Rockefeller Foundation.)

683 NEUROCHEMICAL CORRELATES OF ESTROGEN RECEPTOR FUNCTION. V. Luine, G. Wallach*, and B. McEwen. The Rockefeller University, New York, N.Y. 10021.

The activities of a number of enzymes are altered by estradiol (E) in ovariectomized (OVX) rats in specific brain regions and pituitary, which contain E receptors. Experiments were designed to determine if enzymatic changes occur with physiological levels of E and what mechanisms are responsible for these changes. Female rats were sham OVX or OVX. At 8 and 36 days after surgery, activities of glucose-6-phosphate dehydrogenase and lactic dehydrogenase were decreased 30% in the pituitary, monamine oxidase (MAO) was increased 36% in the amygdala (A), and levels of MAO and isocitric dehydrogenase were decreased 20-30% in the hypothalamus (H). OVX females were implanted with silastic capsules containing cholesterol or E. Enzyme changes were observed with blood levels approximating those found in proestrus. Administration of CI628, known to occupy E receptors in pituitary, brain and uterus and to be a weak estrogen, resulted in small enzyme changes. CI628 also antagonized the larger enzyme effects of subsequent E injections. Pargyline, administered I.V. (1mg/100g B.W.), results in irreversible inhibition (90%) of MAO in cortex, H, and A within 1 hr. In contrast, short term I.V. doses of E as high as 100μg/rat do not affect MAO activity, while daily S.C. doses inhibit MAO in the H and A. To study the turnover of MAO, OVX female rats were injected I.V. with saline or pargyline, and then half of the pargyline group received E for 1-3 wks. Resynthesis of 50% of the control MAO activity required, respectively, 4 and 8 days longer in the A and H of pargyline-E treated rats than in pargyline treated rats. Resynthesis of MAO in the cortex was not altered by E. Experiments completed suggest that enzyme changes can occur at physiological levels of estrogen possibly via estrogen action on the cell nucleus resulting in altered synthesis of enzyme protein. (Supported by grants from USPHS and The Rockefeller Foundation.)

684 CYCLIC AMP-DEPENDENT PROTEIN KINASE: MECHANISM OF REGULATORY SUBUNIT INHIBITION OF THE CATALYTIC SUBUNIT. Robert Roskoski, Jr. and Jonathan J. Witt*, Department of Biochemistry, The University of Iowa, Iowa City, Iowa 52242

Protein kinase (EC 2.7.1.37) catalyzes the phosphorylation of polypeptidic serine and threonine hydroxyl groups according to the following chemical equation: ATP + protein → phosphoprotein + ADP. Cyclic 3',5'-adenosinemonophosphate (cAMP)-dependent protein kinase from bovine brain is composed of two dissimilar subunits. In the absence of cAMP, the regulatory subunit combines with the catalytic subunit to form a catalytically inactive holoenzyme. Added cAMP combines with the regulatory subunit and dissociates it from the catalytic subunit. The catalytic subunit is thereby activated. We find that the holoenzyme is resistant to N-acetylimidazole (NAI) inhibition. After addition of cAMP, however, the enzyme becomes susceptible to NAI inactivation. Thus, NAI (10 mM) inactivates 50% in the presence of 10^{-5} M cAMP and less than 1% in its absence. Pre-incubation with the substrate histone fails to protect the enzyme from inhibitory chemical modification. However, the other substrate, Mg^{2+}-ATP, fully protects the enzyme from NAI inhibition. This substrate protection suggests that the modification of the residue associated with inhibition is in the active site. NAI treatment of the catalytic subunit, chromatographically separated from the regulatory subunit, inactivates the enzyme. Addition of the regulatory subunit or Mg^{2+}-ATP protects against inhibition. Protection by the regulatory subunit and Mg^{2+}-ATP suggests that these molecules interact with the same region of the catalytic subunit. We propose that the regulatory subunit shields the active site of the catalytic subunit thereby rendering it inactive. Addition of cAMP dissociates the regulatory subunit from the catalytic subunit, and frees the active site. This form of the enzyme, which is inhibited by NAI, is catalytically active. (Supported by N.I.H. grant NS-11310).

685 ELECTROPHYSIOLOGICAL EVIDENCE TO SUGGEST THAT HYPOTHALAMIC RELEASING (INHIBITING) PEPTIDES MAY BE LIBERATED FROM NERVE TERMINALS IN THE CNS. L.P. Renaud. Division of Neurology, Montreal General Hospital, McGill University, Montreal, Canada.

Hypothalamic regulation of adenohypophyseal hormones is mediated by peptide messengers which are apparently produced by parvicellular 'tuberoinfundibular' hypothalamic neurons, and released into the pituitary portal plexus from their median eminence nerve terminals. This report presents evidence that these peptides may also be released in the CNS from hypothalamic and extrahypothalamic terminals of axon collaterals of tuberoinfundibular neurons.

Electrophysiological techniques were utilized to study the activity pattern of 134 tuberoinfundibular neurons located within the arcuate and ventromedial nuclei and the periventricular region in male pentobarbital anaesthetized rats. Only 38% of these tuberoinfundibular neurons (n=51) were spontaneously active : many of these exhibited irregular activity punctuated by bursts of spikes; discharges from other tuberoinfundibular neurons were apparently randomly distributed.

Thirty-nine tuberoinfundibular neurons specifically localized in the ventromedial nucleus (HVM) demonstrated evidence of afferent connections from amygdala. Stimulation of stria terminalis evoked orthodromic excitation (latency range 8-12 msec) from 13 tuberoinfundibular neurons. Amygdala stimulation evoked excitation (latency range 11-30 msec) followed by a silent period of 50-95 msec from 21 cells; synaptic inhibition without initial excitation characterized the responses of 5 other cells.

In addition to antidromic invasion, median eminence stimulation evoked two patterns of activity which support the presence of axon collaterals in the tuberoinfundibular system: recurrent inhibition, at latencies coincident with but not necessarily dependent upon the antidromic spike and with durations of 45-120 msec, was evident in 28 of 33 tuberoinfundibular cells tested; orthodromic activation (latency range 1.5 - 9.0 msec) suggestive of recurrent excitation was observed from 32 medial hypothalamic non-tuberoinfundibular neurons.

Seven tuberoinfundibular neurons also presented evidence of antidromic invasion from either the anterior hypothalamic area, or from extrahypothalamic sites (preoptic area, medialis dorsalis of the thalamus), indicative of axon bifurcation. Coupled with data to indicate local recurrent influences from axon collaterals of tuberoinfundibular neurons, these observations suggest that both peripheral release (into portal blood) and central release (at synaptic terminals) of peptides is associated with activity in the tuberoinfundibular system. The evidence that these peptides are found in the synaptosomal fraction of specific areas of brain, and have a potent but selective depressant action on central neurons, implies a neurobiological role different from their actions in the adenohypophysis. (Supported by MRC)

686 ACQUIRED SUPPRESSION OF PLASMA CORTICOSTERONE CONCENTRATION BY STIMULI PAIRED WITH FEEDING OF DEPRIVED RATS. Gary D. Coover, Betty R. Sutton* and John P. Heybach*. Dept. Psych., Northern Illinois Univ., DeKalb, IL 60115.

There exists a broad neural substrate for inhibition of the secretory activity of the hypothalamo-pituitary-adrenocortical system that need not be solely involved in the negative feedback action of glucocorticoids. Though the forms of stimulation that activate the pituitary-adrenal system are many, there are few reports of forms of stimulation that diminish secretory activity. The presentation of water to deprived rats has been shown to cause a rapid decline in plasma corticosterone concentration (Coover, Goldman and Levine, 1972). The present data demonstrate the ability of previously neutral stimuli to suppress corticosterone concentration by virtue of pairing with daily feeding periods (consisting of a fixed amount of wet food mash and 1 hr access to water). The conditioned stimulus consisted of lifting the rats' cages off the shelf and into soundproof chambers, where they remained for 30 min on each daily trial. The rats were run in large groups between 9 am and 12:30 pm (lights on 7 am, off 7 pm), but each rat was run at a different time each day in order to decrease the influences of circadian variation, light onset and experimenter presence on corticosterone levels. Feeding occurred within ½ to 6 min of placement in the chamber. The exact time was varied, according to a schedule, in order to minimize any potential influence of perceived delay of reinforcement. The conditioned stimulus complex is not actually neutral, but presented alone should cause pituitary-adrenal activation. To demonstrate this, additional animals were run under a pseudoconditioning procedure, wherein they were placed in the chamber but fed daily 1½ - 2½ hrs either before or after the stimulus. Blood samples were obtained from all animals by decapitation after 1,6,14 or 24 trials, and after 0, 10 or 20 min from onset of the conditioned stimulus. Fluorometrically assayed plasma indicated that "basal" (time 0) corticosterone concentrations were fairly constant across the morning testing hours, averaging 21 µg/100 ml plasma (these elevated concentrations are expected due to the deprivation condition). Rats that were placed in the chamber and fed immediately on the sampling day exhibited a decline in corticosterone level to 14 µg/100 ml plasma by 10 min and to 8 µg/100 ml by 20 min. The time course of this suppression was well established and unchanging after 6 trials, depending only upon rapid consumption of the available food and water. The pseudoconditioned rats exhibited a rise in corticosterone levels upon placement in the chamber, with the magnitude of the rise diminishing with trials toward a flat response by 24 trials as expected (habituation). The conditioned groups exhibited only slight responses to chamber placement (not fed on blood sampling day) after 1 or 6 trials, but after 14 trials showed a decline to 14 µg by 10 min and equivalently low values by 20 min. This acquired, rapid decline in corticosterone concentration seems most easily interpretable as due to activity in neural circuits that inhibit release of hypothalamic corticotrophin releasing factor, and thereby decreased ACTH and corticosterone secretion. Regardless of the mechanism producing the decline, it occurs in response to manipulations similar to those used in many behavioral experiments, and represents a measurable correlate of a conditionable central state that is interpretable as pleasurable.

687 EXCITABILITY CHANGES INDUCED BY STEROIDS IN THE CENTRAL NERVOUS SYSTEM.
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Earlier studies have shown that in intact male Sprague-Dawley rats physiological doses of intravenously (iv) administered DOC lead to a decrease in the amplitude (with no accompanying latency changes) in sciatic evoked potentials in the pontine reticular formation (RF) and that 5 min - 1 hr. following the iv injection of 1,2-^{3}H-DOC the highest concentrations of neural tissue label are recovered from the same area (Kraulis, et al, Brain Res. 88:1, '75). Chromatographic analysis has pointed to a relatively uniform intracerebral distribution of 1,2-^{3}H-DOC and has implied that the high concentration of brainstem radioactivity may be due to allo-TH-DOC (5α-Pregnane-3α,21-diol-20-one), a ring A reduced metabolite of DOC and an anaesthetic-type steroid. It may therefore be that the depressant effects of DOC are mediated via allo-TH-DOC, or the intermediary metabolite, dihydro-DOC (5α-Pregnan-21-ol-3,20-dione). To investigate this possibility we have, in the present study, compared the effects of intraperitoneally (ip) administered DOC, 5α-dihydro-DOC and allo-TH-DOC on sciatic evoked potentials in the pontine RF. To evaluate the specificity of action of these hormones, we have also studied the effects of hydroxydione (5β-Pregnan-21-ol-3,20-dione), a known steroid anaesthetic, and of corticosterone, the major glucocorticoid in the rat and a steroid not expected to posses central depressant properties. The rats were anaesthetized with 25% urethane (4 cc/kg/iv). A bone defect was made over the left occipital regions and the dura was retracted. The sciatic nerve of the right leg was prepared for stimulation and was kept moist in a pool of warm parrafin oil. Tungsten microelectrodes were placed stereotaxically in the RF and their position was verified histologically. In some experiments a second electrode was placed in the thalamus (VPL), an area not found to concentrate radioactivity. Sciatic evoked potentials were recorded and averaged from the RF before and after hormone administration. Steroids were dissolved in sesame oil, and administered ip (0.75mg/cc/300g. A decrease in the RF potential with means of 22-40% of the original amplitude, with no concurrent changes in the thalamic potential, was observed following the injection of DOC, dihydro-DOC, allo-TH-DOC, and hydroxydione. Corticosterone showed a mean increase of 22% in the amplitude of the RF potential. Sesame oil had no effect. The later components of the potentials were generally affected earlier, and showed greater amplitude changes than the first components, indicating a higher susceptibility for polysynaptic pathways. Both metabolites of DOC showed a faster acting time than DOC, with a mean of 9.4 min for DOC, as compared to 2.7 and 3.5 for dihydro-DOC and allo-TH-DOC, respectively. This suggests that the depressant effects of DOC may be mediated via its less polar metabolites, a situation analogous to that described for progesterone (Gyermek et al, Int. J. Neuropharmacol. 6:91, '67). The steroid effects were transient and the potentials recovered to preinjection levels within 50 min. We believe that the decrease in the amplitude of the sciatic evoked potentials with DOC and its metabolites is related to reduced brain excitability in the pontine RF. The fact that there was no effect on the amplitude of the potentials in the thalamic relay nuclei indicates that peripheral mechanisms were not affected. Moreover, increasing the intensity of stimulation from 1.5 threshold (T) used during testing to 3T reinstated the amplitude of the RF potential to preinjection levels.

688 EFFECT OF HYPOPHYSECTOMY, ACTH AND DEXAMETHASONE ON BRAIN DOPAMINE BETA HYDROXYLASE ACTIVITY IN RATS. Glen R. Van Loon* and R. N. Mascardo* (Spon: O. Hornykiewicz). Departments of Medicine and Physiology, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

The central nervous system plays a significant role in the regulation of ACTH secretion. Previous studies have demonstrated that the neuroanatomical substrate for this regulation involves pathways from brain regions that converge in the hypothalamus, probably on neurons secreting corticotropin releasing factor. Regulation appears to involve stimulation and inhibition of ACTH secretion. The catecholamines, dopamine and norepinephrine, are putative transmitters of the neuroanatomical substrate for the regulation of ACTH secretion. Several studies suggest a role for these amines in the regulation of ACTH secretion, although no unifying concept in this regard is currently established. Furthermore, manipulations of the pituitary-adrenal axis through adrenalectomy or hypophysectomy are associated with alterations in brain catecholamine metabolism. To gain greater insight into this relationship, we studied the effects of altering plasma ACTH and corticosteroids on the activity in brain of dopamine-β-hydroxylase (DBH), the terminal enzyme in the biosynthesis of norepinephrine. Adult male Sprague-Dawley rats were housed in pairs in an environmentally-regulated room with constant temperature (23°C) and humidity (44%), and regular light-dark cycles (0700-1900 hr, light). The hypophysectomized (hypox) rats (152 ± 3 g body wt at sacrifice; mean ± SE) were provided with normal saline drinking solution and fed ad libitum. A group of control rats (227 ± 2 g) was underfed to compensate partially for the marked differences in body weight (bw) between hypox and control rats fed ad libitum (308 ± 5 g). ACTH (Cosyntropin), 0.5 or 0.1 μg/100 gm bw, dexamethasone (Dex), 2 μg/100 gm, or saline, 0.25 ml/100 gm, were injected sc qd at 1830 hr from Days 3-15 post-hypophysectomy. Control animals received comparable injections. Rats were sacrificed 2 and 3 hr after the final injections of ACTH and Dex, respectively. Brain was removed and the hypothalamus, brain stem, cerebellum, and cerebral cortex dissected for assay of DBH activity (ηmol/min/gm wet tissue; mean ± SE) by the method of Kato et al. ACTH (0.5 μg/100 gm) produced a transient increase in plasma corticosterone of control rats 15 min after ACTH administration, but plasma corticosterone had returned to normal at 120 min at the time of assay for DBH. Stress of underfeeding did not alter hypothalamic and cerebellar DBH significantly. ACTH and Dexamethasone increased hypothalamic DBH ($p<0.05$) and decreased cortical DBH ($p<0.05$) in control rats. Hypophysectomy decreased DBH in the four brain areas. ACTH (both 0.5 and 0.1 μg/100 gm) reversed the effect of hypophysectomy in the hypothalamus and brain stem which are innervated primarily by the ventral noradrenergic pathway. The effect of hypophysectomy on the cerebellum and cerebral cortex, areas mainly supplied by the dorsal noradrenergic bundle, was not altered by either dose of ACTH. Dexamethasone failed to alter the hypophysectomy-induced decrease in DBH in the four areas. These data provide further evidence for a relationship between alterations in plasma ACTH and brain catecholamine metabolism. They also suggest that the ventral noradrenergic pathway plays an important role in the regulation of ACTH secretion, and that noradrenergic neurons appear to be responsive to very small doses of ACTH. It is possible that some of the previously reported behavioral effects of ACTH-like peptides may be mediated via the ventral noradrenergic pathway. (Supported by MRC Grant MA-5183 and Urquhart Fund).

689 HYPOTHALAMIC-PITUITARY-ADRENAL AXIS AND NOREPINEPHRINE METABOLISM IN DEPRESSIVE ILLNESS. C.T. Hengeveld,* S. Pandey, M. Dysken,* G. Bakas,* and J.M. Davis (SPON: H. Dekirmenjian). Univ. of Chicago, and Ill. State Psychiatric Institute, Chicago, Illinois 60612.

Recent endocrine studies in depressive illness have reported changes in the hypothalamic-pituitary-adrenal (HPA) axis: a subpopulation of depressed patients shows elevated serum cortisol levels and suppression-resistance to dexamethasone. While previous investigators have considered these changes secondary to the stress of the illness, current theoretians postulate that the neuroendocrine changes may be a manifestation of central neurotransmitter changes in depression, namely, that there is a functional hypoactivity of the central noradrenergic system which releases the HPA from inhibitory NE control. One would expect, therefore, to find a hyperfunctioning HPA system in depression because of the disinhibition. Elevated serum cortisols and suppression-resistance to dexamethasone may be related to the postulated deficit of NE in depressive illness. To test these hypotheses we have compared both baseline serum cortisol levels including diurnal variation and suppressibility of diurnal cortisol variation by dexamethasone with a commonly used index of central NE turnover, 24 hour urinary MHPG. Our findings will be discussed both in light of known mechanisms of action of NE upon the HPA axis and in the light of diagnostic subtypes of depressive diseases by the use of Spitzer and Endicott's Research Diagnostic Criteria in each patient group. Implication of the work for the continuing development of neuroendocrine strategies in the study of major psychiatric syndromes will be brought into focus.

690 ON THE RELATIONSHIP OF CATECHOLAMINES TO ACTH SECRETION AND THE FEEDBACK MECHANISM. Kevin L. Keim and Ernest B. Sigg*. Dept. of Pharmacology, Hoffmann-La Roche Inc., Nutley, New Jersey 07110.

The central catecholamines have been claimed to exert an inhibitory and/or facilitatory control over the secretion of ACTH. This was investigated under various conditions of restraint stress (RS) in 70 day old male rats when applied for 30 minutes. RS produced reliable and significant biochemical changes, e.g. a reduction in hypothalamic norepinephrine (hNE) and forebrain norepinephrine (fNE) concomitant with a rise in hypothalamic dopamine (hDA) and plasma 11-hydroxycorticosterone (CS). When RS was applied daily for 17 consecutive days, the 24 hr post-stress hNE was progressively reduced from 2.4 µg/gm to 0.8 µg/gm, while resting CS remained at values < 10 µg%. Moreover, the elevation in CS induced by repeated RS was also diminished simultaneously with an attenuated RS-evoked hNE depletion. The possibility that the aminergic substrates may be involved in corticosteroid feedback was tested by giving dexamethasone (DEXA, 0.5 mg/kg sc 1 hr prior to RS of 30 min duration). The RS-induced reduction of hNE, but not that of fNE, could be prevented by the DEXA pretreatment. In contrast, the RS-induced increase in hDA was slightly enhanced; the CS response was markedly reduced. This specificity by DEXA on hNE was not shared by agents known to decrease the turnover of central norepinephrine. Thus, diazepam (5 mg/kg sc 1 hr prior to RS) reduced the CS increase and prevented the loss of hNE as well as of fNE. These data indicate that those pharmacological manipulations which prevent a stress-induced depletion of central catecholamines also diminish the CS response; the effect of DEXA suggests that the hypothalamic catecholamines may be specifically responsive to steroid feedback. The marked lowering of endogenous hNE by repeated RS is associated with a diminished CS response. Hence, this study does not support an inhibitory action of hNE on the secretion of ACTH.

691 BASOLATERAL AMYGDALOID CONTROL OF PITUITARY-ADRENAL FUNCTION. I. Lourie*, M. M. Krieger* and J. S. Burgess* (SPON: Max L. Fogel). Res. Dept., Norristown State Hospital, Norristown, Pa. 19401.

Small radiofrequency lesions (mean diameter 0.8mm) were placed bilaterally in the lateral aspect of the basolateral amygdaloid nucleus in 90 day old male rats (BL). When tested two weeks postsurgery these animals exhibit a characteristic behavior pattern consisting of rapid habituation of exploratory activity and deficits in social interaction. Food and water uptake and weight gain remain normal. BL animals meeting these behavioral criteria (n=4) and controls (C) (n=4) were subjected to restraint induced stress for a period of 3 hrs. Tail vein blood samples were obtained at 0, 1-1/2 and 3 hrs and corticosterone (OHCS) levels were determined by a competitive protein binding method. The mean OHCS values in temporal order for BL were 2.0, 39.1 and 33.5; and for C, 2.4, 18.4 and 27.6μg/dl.

These data are consistent with the view that BL lesions do not affect basal pituitary-adrenal function but cause a disinhibition of a modulating effect in the presence of neurogenic stress stimuli possibly by affecting a rapid release of available ACTH stores. The temporal course of the hyper OHCS response suggests that circulating OHCS in response to arousal stimuli could be a factor in explaining the behavioral effects of the lesion.

692 THE SUPRACHIASMATIC NUCLEI AND CIRCADIAN RHYTHMS. Kiyomi Koizumi, Hitoo Nishino* and David Colman*. Dept. Physiology, SUNY, Downstate Medical Center, Brooklyn, New York 11203.

The circadian rhythm in rat pineal gland secretory activity is known to be controlled by the optic system acting through the cervical sympathetic nerves. We investigated the suggestion that the suprachiasmatic nuclei of the hypothalamus might be involved and found that anterograde migration of horseradish peroxidase injected into the vitreous body revealed a distinct bilateral projection from the retina to these nuclei. Furthermore, recordings from neurons in the suprachiasmatic nuclei of urethane or chloralose-urethane anesthetized rats, by glass capillary microelectrodes, showed that stimulation of the optic nerve at a rate of 5 to 10/sec produces an increase in firing frequency in one third of neurons tested while inhibiting some others. Stimulation by light gave similar results. Since earlier studies (Brain Res. 87:181, 1975) showed that optic nerve stimulation inhibits sympathetic nerves which evoke electrical activity in the pineal, the suprachiasmatic nuclei were stimulated and effects on the sympathetic nerve observed. It was found that stimuli at a rate of 10-20/sec similarly inhibited tonic activity in the cervical sympathetic neurons. Our results support the view that the suprachiasmatic nuclei possess neurons which mediate circadian rhythmicity in pineal activity. (Supported by USPHS Grant #NS00814 and NSF Grant #OIP74-19337.)

693 ALTERATIONS IN BEHAVIORAL ONTOGENY FOLLOWING NEONATAL RADIOTHYROIDECTOMY IN THE RAT. Sonya K. Sobrian*, Nina Edson*, Radhey L. Singhal* and Bruce A. Pappas. Dept. Psych., Carleton Univ. and Dept. Pharmacol., Univ. Ottawa, Ottawa, Ontario K1S-5B6.

Hypothyroidism, induced in the rat by a single injection of 200 µCi of ^{131}I on the day of birth, altered the ontogeny of several behaviors and impaired somatic and reflex development. Hypothyroid animals did not show the characteristic increase in locomotor activity exhibited by controls between 10 and 15 days of age or the subsequent decline. Activity levels in the cretinous rats increased between 5 and 10 days of age at the same rate as controls and then remained unchanged. The delayed appearance of thyroidectomy-induced alterations in behavioral ontogeny is coincidental with previously reported changes in biochemical development following neonatal thyroidectomy. The maturation of spontaneous alternation (SA) behavior was also retarded. While controls showed the normal age-related increase in SA, with reliable levels first apparent in 27 day old pups, hypothyroid rats did not alternate above chance levels between 17 and 37 days of age. However, the percentage of SA behavior exhibited by 90 day old hypothyroid animals was not significantly different from that of age-matched controls. This eventual development of SA might reflect the delayed but not suppressed maturation of various brain structures which has been shown to occur in the cretinous rats. Although no persistent effects of neonatal thyroidectomy were observed on adult 2-way shuttle avoidance, hypothyroid animals were more active in the open field and did not habituate to repeated testing in a T-maze. The results of this study provide the first experimental evidence for the role of the thyroid gland in the development of behavior above the reflex level and are consistent with reports of retarded anatomical and biochemical development of the CNS in hypothyroid rats.

694 EFFECTS OF THYROTROPIN RELEASING HORMONE ON CHOLINERGIC SYNAPTIC TRANSMISSSION IN APLYSIA. Rafiq Waziri, Dept. of Psychiat., Sch. Med., Univ. of Iowa, Iowa City, Iowa 52242

Thyrotropin Releasing Harmone (TRH) is a ubiquitious substance in the brain (Winokur and Utiger, Science, 185, 265, 1974) and in addition to its effects on the pituitary, it has been implicated in having a role in synaptic transmission. Some studies have indicated that TRH increases the release and turnover of catecholeamines (Dale Horst and Spirt, Life Sci., 15, 1073, 1974) and this may be important in it's reported antidepressant properties in affectively ill patients. In the abdominal ganglion of Aplysia, where cholinergic synaptic transmission between identified pre- and postsynaptic neurons can be accurately studied (Frazier et al, J. Neurophys., 32, 1288, 1967) the effects of TRH in concentrations of 10-20 ugm/ml in artificial sea water bathing the ganglion were studied. The initial response of cholinergic PSPs between the presynaptic neuron L_{10} and its follower neurons, was enhancement of both the IPSPs and the EPSPs. With prolonged exposure, these PSPs gradually decremented to about 25% of their control, but were never totally abolished. No changes in the membrane properties of the pre- or postsynaptic elements were observed. Preliminary analysis of the data indicate that TRH in these experiments may have an eserine - like anticholinesterase activity.

695 NEUROENDOCRINE CONTROL OF ROTATIONAL BEHAVIOR IN THE NONLESIONED RAT. M. L. Cohn, M. Cohn* and B. Petro*, Dept. Anes., Magee-Womens Hospital, Univ. of Pitt. Sch. Med., Pittsburgh, Pa. 15213.

Neuroleptic drugs e.g. (+) amphetamine and apomorphine induce stereotype behavior and hyperactivity in rats. Reserpine causes sedation and blepharoptosis in hunch-backed rats. Behavioral effects of neuroleptic drugs have been attributed to dopamine-mediated activity in CNS. In nonlesioned rats, following IP injection of apomorphine 6 mg kg^{-1}, (+) amphetamine (150 ug), centrally injected, induces tight head to tail rotations on a flat surface (Cohn, M. L.: In Molecular Mechanisms of Anesthesia, 1975). That haloperidol 4 mg kg^{-1} and pimozide 0.5 mg kg^{-1} inhibit such rotations supports our contention that rotation in nonlesioned rats is mediated through central dopaminergic neurons. Thyrotropin-releasing factor (TRF) induces rotations similar to those induced by (+) amphetamine. In contrast, somatostatin produces "barrel" rotation (through an axis at right angles to that of TRF-induced rotation) which is inhibited by atropine (0.2 mg). Four other brain polypeptides were examined in our system: melanocyte-stimulating hormone-release inhibiting factor (MIF), leuteinizing hormone releasing hormone (LRH), substance P, and glutathione. While substance P induces head to tail rotation, LRH produces both head to tail and barrel rotation. Haloperidol inhibits the head to tail and atropine the barrel rotation induced by LRH. Our findings and the fact that the brain polypeptides applied by microiontophoresis alter populations of CNS neurons suggest that these compounds act as neurotransmitters or modulators in well defined pathways of the brain. (Supported by NIMH DA00605)

696 Parallelism between behavioral and adrenal hyperreactivity in the septal rat. Jo A. Seggie, I. Uhlir* and G.M. Brown, Neuroendocrine Research Section, Clarke Institute of Psychiatry, Toronto.

Rats lesioned in the septal area are behaviorally hyperreactive to environmental stimuli (septal syndrome), however this disturbance attenuates with time. The adrenal response to stress in septal rats is also hyperreactive as indicated by a decreased latency and increased magnitude of corticosterone response, (Neuroendo.,1974, 16: 225-236). In order to determine if the behavioral and adrenal changes co-vary, septal rats were maintained until their behavioral syndrome had attenuated to a level comparable to non-lesioned animals. Resting and stressed levels of corticosterone were measured in normal, sham-operated and septally lesioned rats 3, 8, 13 and 61 days after surgery. Resting corticosterone levels of all groups were comparable. Septal rats that exhibited the septal syndrome 2 and 7 days postoperatively also exhibited exaggerated stress responses. By 61 days after surgery, when the septal hyperreactivity had attenuated and all groups were behaviorally equivalent, the exaggerated plasma corticosterone response to stress in septal rats was also found to have disappeared. Thus, it appears that adrenal changes in the septal rat occur in parallel with the altered behavioral state. Modification of plasma corticosterone or ACTH levels (J.Comp.Physiol.Psy. 1971, 74: 11-19; 1973, 83: 60-65) had no influence on the septal syndrome indicating that the behavior changes are not secondary to changes in the pituitary-adrenal axis. The present study suggests that adrenal changes following septal ablation may be secondary to the behavioral state, or alternatively that both adrenal and behavior changes in septal rats are secondary to some common factor such as an altered ability to cope with environmental demand. Dr. Jo Seggie is an Ontario Mental Health Foundation (O.M.H.F.) Research Scholar and Dr. Brown is an O.M.H.F. Research Associate.

697 NEUROPHYSIN, VASOPRESSIN AND THE HYPOPHYSIAL PORTAL SYSTEM. EVIDENCE THAT ADRENALECTOMY SELECTIVELY INCREASES THE NEUROPHYSIN ASSOCIATED WITH VASOPRESSIN. Margaret A. Stillman*, Larry D. Recht*, Hilda W. Sokol*, Said M. Seif* and Earl A. Zimmerman. Dept. Neurol., College of P&S, Columbia Univ., N.Y., N.Y. 10032, Dept. Physiol. Dartmouth Med. Sch., Hanover, N.H. 03755, and Dept. Med. Univ. Pittsburgh, Pa., 15261.

It has recently been shown by immunocytochemical methods that fibers containing neurophysin (NP) and vasopressin (VP) project to the portal system in the zona externa (ZE) of the median eminence. Although there are separate reports that the immunostaining of both NP and VP increases in ZE after adrenalectomy (ADX), it is uncertain whether the enhanced NP is solely the NP associated with VP (VP-NP) or includes in addition the NP related to oxytocin (OT), and possibly another NP formed with corticotropin releasing factor (CRF). Since specific antisera to rat VP-NP and OT-NP are not available, in the present studies we have compared the effects of adrenalectomy on the immunoperoxidase staining of NP in normal rat with the homozygous Brattleboro diabetes insipidus rat (DI rat) which lacks VP and VP-NP. Hypothalamic tissues were reacted with antisera to VP, OT and rat and human NP. In non-ADX normal rat some NP and VP positive processes were seen in ZE, and only a few NP and OT projections to ZE were found in non-ADX DI rat. One to 3 weeks after ADX a marked increase in NP and VP and no change in OT was found in normal and no increase in NP and OT in DI rat. These studies suggest that ADX selectively stimulates VP and VP-NP in neurosecretory pathways to the hypophysial portal system and has no effect on OT and OT-NP. Since DI rat is known to have CRF, the lack of any change in NP in DI rat after ADX is against the existence of a CRF-NP. These data lend further support to the concept that VP has a real role in the function of the hypothalamic-pituitary-adrenal system.

698 CHARACTERIZATION OF THE INHIBITORY ACTION OF THE SEPTUM ON PITUITARY-ADRENAL ACTIVITY. Anna N. Taylor, Berrilyn J. Branch* and Barbara B. Turner*. Dept. Anat. & Brain Res. Inst , UCLA, Los Angeles, CA 90024.

We have reported that stimulation of the lateral septum through chronically implanted electrodes in freely behaving rats exerts inhibitory actions on basal and stress-induced pituitary-adrenal activity. The response has now been further characterized with respect to latency of onset, anatomical localization and diurnal relationships. Male rats were exposed to 10-min restraint with a wire-mesh screen at 5 or 35 min after the termination of septal stimulation (50 cps, 1 ms/pulse, 15 sec/min for 30 min, 22.5-50 μA). Plasma corticosterone was measured fluorometrically in 0.3 ml blood samples withdrawn at half-hourly intervals from an indwelling jugular vein catheter. When restraint was applied in the afternoon, commencing 5 min after the end of stimulation of the lateral septum, the normal steroid response to stress was completely blocked. Stimulation of sites in the medial septum or around the septum, as in bed nucleus of the stria terminalis and fornix, did not affect the stress response. When the stress was applied in the morning, 5 min after the end of septal stimulation, the stress response was not affected by stimulation of the lateral septum and stimulation of the sites outside of the lateral septum significantly enhanced the stress response. When the stress was applied in the morning, commencing 35 min after the end of stimulation, lateral septal stimulation significantly reduced the magnitude of the stress response. Thus, the generally accepted modulatory action of the septum on pituitary-adrenal function involves inhibition by the lateral, but not the medial septum of basal and stress-induced activities of this system. The latency of onset and extent of inhibition of the stress response vary relative to the phase of the diurnal pituitary-adrenal rhythm.
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699 SUBCELLULAR DISTRIBUTION OF SOMATOSTATIN IN EXTRAHYPOTHALAMIC BRAIN TISSUE. D. Tsang*, A.T. Tan*, P. Brazeau*, S. Lal*, L.P. Renaud, and J.B. Martin. Depts. Neurology, Psychiatry and Anesthesia Research, McGill University, Montreal, Quebec, Canada.

Recent studies using radioimmunoassay and bioassay have indicated that significant concentrations of somatostatin (SRIF) are present in extrahypothalamic regions of the CNS, particularily cerebral cortex and amygdala. The present investigations were undertaken to determine the subcellular distribution of SRIF, as measured by bioassay, in cerebral cortex and amygdala. In addition, in vitro studies of synaptosome preparations of cortex were used to investigate the mechanism of action of SRIF.

Homogenates of male rat hypothalamus, cerebral cortex and amygdala prepared in 0.32 M sucrose were fractionated by differential centrifugation and sucrose gradients. SRIF activity was measured by a bioassay using monolayer cultures of anterior pituitary (sensitivity; 10^{-12} to 10^{-9} M). The cytoplasmic marker, lactic dehydrogenase (LDH), was used to monitor the purification.

The ratio of SRIF activity /mg protein in the total homogenate of cortex, amygdala and hypothalamus was approximately 1:3:10. Hypothalamic SRIF content was 5.0 ±.3 ng/hypothalamic fragment. The majority of SRIF activity was found in the P_2 (17,000 g) fraction in each brain region. After separation of the P_2 on a discontinuous sucrose density gradient, over 50% of the activity was present in the synaptosome fraction. This zone also contained the bulk of LDH activity. Synaptosome preparations of guinea pig cerebral cortex were studied to assess possible mechanisms of SRIF activity in brain tissue. Synaptosomes were incubated in Krebs-Ringer phosphate medium with radioactive calcium (^{45}Ca) in the presence of glutamate. The glutamate-enhanced ^{45}Ca uptake was increased 2 fold in the presence of SRIF. This effect was not observed with TRH.

These results indicate that synaptosome fractions of rat cerebral cortex and amygdala contain significant SRIF activity suggesting that SRIF is localized in nerve terminals in these regions of brain. Evidence that SRIF enhances calcium uptake in synaptic membrane may indicate one mechanism for the observed depressant action of SRIF on the electrical activity of single CNS neurons (Renaud, Martin and Brazeau, Nature 255: 233-235, 1975). These findings suggest that SRIF, in addition to its role in the regulation of adenohypophyseal secretion, may have a neurobiological role in the modulation of central neuronal excitability.

700 SOMATOSTATIN (SRIF) EFFECTS IN VIVO AND IN VITRO ON CYCLIC AMP CONCENTRATIONS IN RAT BRAIN. D. Enock* and M. L. Cohn (SPON: S. K. Wolfson, Jr.), Dept. Anes., Magee-Womens Hospital, Univ. of Pitt. Sch. Med., Pittsburgh, Pa., 15213.

It has become increasingly evident that SRIF, a growth hormone-release inhibiting factor, may regulate many behavioral functions unrelated to its endocrine-metabolic role. We have reported that SRIF, injected centrally, prolongs barbiturate narcosis, reduces body temperature and locomotor activity and induces tranquilization of prolonged duration (Cohn, M. L.: In Molecular Mechanisms of Anesthesia, 1975). SRIF also induces "barrel" rotational behavior on a flat surface. Oka et al. (1974) reported that in in vitro incubation studies with whole rat anterior pituitary glands, SRIF decreases the level of cyclic AMP. In the present study we describe in vivo and in vitro effects of SRIF on cyclic AMP concentrations in rat brain. Sprague-Dawley rats (200-250 g) were sacrificed in a high-intensity microwave oven ten minutes after injection of SRIF (5-50 ug) into the right lateral ventricle. The brain was sectioned and extracted with $HClO_4$. In another series of experiments rats were decapitated; the brains were removed and the striata isolated and homogenized. The homogenate was incubated in tris buffer containing sucrose, Mg++, ATP, phosphodiesterase inhibitor and SRIF (10^{-3}-10^{-9}M). Analysis of cyclic AMP in in vivo extracts and in supernatants from in vitro incubation was performed by purification on ion-exchange columns and subsequent RIA. SRIF significantly increases cyclic AMP levels both in vivo and in vitro. This contrasts with data we obtained previously with thyrotropin releasing factor. Our results suggest that somatostatin may act in the CNS by increasing levels of cyclic AMP. (Supported by NIMH DA00605).

701 PLASMA VOLUME MEDIATED GROWTH HORMONE (GH) RESPONSES IN THE RHESUS MONKEY. J.W. Chambers* and G.M. Brown. Clarke Institute of Psychiatry, Toronto.

While examining the effects of several agents used routinely for the clinical assessment of GH release, it was discovered in conscious adult male rhesus monkeys that the intraatrial infusion of 0.9% saline in a volume of 8.3 ml/kg (about 30 ml total) over 30 min caused a very significant elevation of plasma GH ($p<0.01$ vs 15 ml 0.9% saline) beginning immediately following completion of the infusion and peaking 30 min (115.9 ± 32.6 ng/ml) after the end of the infusion. This volume was chosen as equivalent to that used in humans by many clinical investigators. Infusion of the same volume of sterile water containing L-Arginine 0.5 g/kg or of Dextran 75 (Abbott - 6% dextran MW 75,000 in 0.9% saline) produced GH responses with comparable amplitudes. However, responses were significantly delayed ($p<0.05$) peaking at 45 min (34.5 ± 10.4 ng/ml) and 75 min (68.1 ± 51.5 ng/ml) after infusion respectively. A GH response was not demonstrated following a 30 min infusion of 15 ml of 0.9% saline. Thus, the GH response to 0.9% saline would seem to be volume dependent. The significant delay in the GH response following Dextran 75, a plasma expander, which is maintained in the intravascular compartment longer than 0.9% saline further suggests that the GH response is related to an intravascular volume control mechanism. It was noted that the rebound elevation of GH following completion of a 30 min infusion of somatostatin (30 µg/kg) which peaks 30 min after infusion (72.1 ± 22.8 ng/ml) is statistically equivalent ($p<0.01$) in both amplitude and timing to that of 0.9% saline. It is suggested that the volume stimulus may induce somatostatin secretion and that the observed GH responses may be rebound elevations following the termination of somatostatin secretion. (Supported by OMHF and MRC.)

702 CATECHOLAMINE REGULATION OF GROWTH HORMONE IN MAN. David L. Garver, James C. Garbutt*, Stephen E. Ericksen*, Haroutine Dekermenjian and John M. Davis. Illinois State Psychiatric Institute, Chicago 60612

Since neuroendocrine systems are modulated by many of the same central neurotransmitters which have become suspect in major psychiatric syndromes (serotonin, dopamine and norepinephrine(NE)), study of specific neuroendocrine systems may provide additional lines of evidence for or against particular hypotheses of neurotransmitter abnormalities in such states.

Because abnormalities of Growth Hormone(GH) secretion have been reported in some unipolar, depressed patients, we examined GH response following hypoglycemia induced by insulin in such patients. We related that response to an independent measure of whole brain NE tunrover, urinary 3-methoxy-4-hydroxyphenethylene glycol(MHPG).

Our findings in eight unipolar, depressed patients indicates that GH peaks following hypoglycemia are significantly correlated quantatively with urinary MHPG ($p<0.01$).

Such correlation provides evidence that abnormalities of NE function in some affective disease states are not limited to isolated brain areas such as those centers responsible for modulating affective states, but such abnormalities equally effect other brain sites such as those responsible for regulation of the release of hypothalamic-pituitary hormones. Abnormalities of NE function in affective disease therefore appear to be generalized, effecting rather wide-spread areas of the central nervous system. The detailed study of neuroendocrine systems may be able to detect functional states of neurotransmitter systems throughout the brain in many psychiatric syndromes.

703 ABSENCE OF NOCTURNAL ELEVATION OF PLASMA PROLACTIN CONCENTRATIONS IN CUSHING'S DISEASE. Dorothy T. Krieger, Peter J. Howanitz* and Andrew G. Frantz*. Dept. of Medicine, The Mount Sinai School of Medicine and the Dept. of Medicine, Columbia College of Physicians and Surgeons, N.Y.C., N.Y.

The percent change (increase) in the nocturnal elevation of plasma prolactin concentrations was significantly reduced ($p < 0.01$) in 6 untreated patients with clinically active Cushing's Disease, when compared to that seen in normal subjects. Patients with hypercorticism secondary to adrenal adenoma (n=2) or receiving long term, high dose corticosteroid therapy, (n=6) did not differ significantly from normal subjects in the percentage change seen in the nocturnal elevation of their plasma prolactin concentrations. The difference between the percentage change observed in the patients with Cushing's Disease versus that seen in these two categories of hypercorticism was significant at $p < .02$. Four untreated patients with localized hypothalamic tumors also showed a significant reduction in the percentage change in the nocturnal elevation of plasma prolactin concentrations ($p < .05$).

In contrast to these findings, the prolactin responses to thyrotropin-releasing hormone (indicative of pituitary responsiveness) in patients with Cushing's Disease, adrenal adenoma, or receiving chronic corticosteroid therapy, were all within the normal range seen in sex matched control subjects.

These findings provide additional evidence for the suggestion that there is altered hypothalamic function in patients with Cushing's Disease.

704 **CHLORPROMAZINE-ACTIVATED ADENYLATE CYCLASE FROM A PROLACTIN PRODUCING TUMOR CELL LINE (GH_3).** *Yvonne Clement-Cormier*, Jerrold J. Heindel* and G. Alan Robison* **Dept. of Pharmacology, The University of Texas Medical School, Houston, Texas 77025.**

An adenylate cyclase present in homogenates of GH_3 cells, a clonal pituitary tumor cell line which releases prolactin and growth hormone under appropriate conditions, was observed to be selectively stimulated by low concentrations of chlorpromazine. A half-maximal increase in activity of the GH_3 adenylate cyclase occurred in the presence of 0.7 - 1.0 x 10^{-6}M and a significant increase in activity was observed with concentrations of chlorpromazine as low as 10^{-7}M. The chlorpromazine derivatives, 7-methoxy-chlorpromazine, 7-hydroxychlorpromazine, and 8-hydroxychlorpromazine were found to mimic the stimulatory action of chlorpromazine on adenylate cyclase whereas chlorpromazine-5,N-dioxide was virtually ineffective. In addition, NaF (10^{-4}M) caused a four-fold increase in adenylate cyclase activity. Under the assay conditions utilized, dopamine was ineffective in stimulating adenylate cyclase at concentrations up to 3 x 10^{-4}M. The three-fold stimulation of adenylate cyclase activity observed in the presence of chlorpromazine (10^{-5}M) was blocked by the ergot alkaloids, ergotamine and ergocryptine. These results considered with other data suggest that hyperprolactinemia resulting as a side effect of phenothiazine treatment may be attributable to a direct action of these drugs to increase adenylate cyclase activity in prolactin containing cells of the anterior pituitary and that increased cyclic AMP levels in these cells enhance prolactin release. The chlorpromazine derivatives used in this study were given to us by Dr. Albert A. Manian. This research was supported by grants from the NSF (GB 41337) and the NIH (1F22-AM 01482).

705 LIGHT AND ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL LOCALIZATION OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) IN THE HYPOTHALAMUS OF THE GUINEA PIG. A. J. Silverman* and P. A. Desnoyers* (SPON: R. W. Goy). Regional Primate Research Center, University of Wisconsin, Madison, WI. 53706

The distribution of LHRH was investigated in the adult male guinea pig hypothalamus by light microscopic immunocytochemistry. Tissue was immersion-fixed in Bouin's solution, embedded in paraffin, and 6 μ sections were cut and stained by sequential application of antiserum to LHRH (supplied by S. Sorrentino), sheep anti-rabbit globulin serum, the peroxidase-anti-peroxidase (PAP) complex (supplied by L. Sternberger) and diaminobenzidine-H_2O_2. LHRH was localized as a brown granular reaction product. LHRH-positive axons were observed as far anterior as the preoptic area and appeared to run in a ventromedial direction through the suprachiasmatic nucleus to the median eminence (ME), but it was not possible to trace fibers throughout their entire length. Fibers were also observed to branch frequently. Tracing of axonal pathways was made more difficult by our inability to observe LHRH-positive cell bodies even in castrate, colchicine-treated animals. In the external layer of the median eminence immunoreactive deposits were most heavily concentrated in the dorsal medial portion of the stalk region. Addition of 50 mg of LHRH to the specific antiserum prior to its application to tissue sections eliminated all staining while the addition of vasopressin (1 μg) or oxytocin (5.7 μg) had no effect. For electron microscopy median eminence was fixed either by freeze-substitution or by perfusion with various mixtures of glutaraldehyde and paraformaldehyde and embedded in Araldite. Thin sections were stained in the same manner as were light microscopic sections. PAP molecules indicating the presence of LHRH were present over granules of 900-1100 Å; these granules were located within axon profiles and nerve terminals. LHRH-positive nerve terminals were found primarily on the capillary loops which penetrate into the external layer of the median eminence rather than on the larger capillaries of the Mantelplexus between the pars tuberalis and the median eminence. Cells of the pars tuberalis were negative. (Supported by USPHS grants RR-00167 and HD-09636).

706 RELATIONSHIP OF AROMATIZATION TO ANDROGEN STIMULATION OF GERBIL SCENT MARKING. Pauline Yahr* (SPON: P. W. Landfield). Dept. Psychobiology, Univ. California, Irvine, CA 92664.

Male Mongolian gerbils (Meriones unguiculatus) scent mark using a ventral sebaceous gland. Scent gland size and marking frequency are both androgen dependent. Estrogen, as well as testosterone, reinstates scent marking in castrated male gerbils, but dihydrotestosterone (DHT) does not. DHT and estrogen both promote scent gland growth. Similar observations on the androgen dependent mating behavior of male rats led to the suggestion that aromatizable androgens, e.g., testosterone, are more active behaviorally than nonaromatizable androgens, e.g., DHT. To determine if androgen stimulation of gerbil scent marking was related to androgen aromatization, castrated male gerbils were treated with testosterone propionate (TP) or with 6α-fluorotestosterone propionate (6α-fluoroTP), a nonaromatizable androgen. Two doses of each steroid were used (50 or 150 μg twice weekly) and controls received the vehicle only. TP and 6α-fluoroTP were equally effective at each dose level for reinstating scent marking and for promoting scent gland growth. In contrast, the seminal vesicles were twice as large after TP therapy as they were after 6α-fluoroTP. These data suggest that the failure of DHT to stimulate gerbil scent marking is not related to its inability to be aromatized. The ability of 6α-fluoroTP to maintain mating in castrated male rats is under study.

707 ESTRADIOL-17β AND 5αDIHYDROTESTOSTERONE: TESTOSTERONE METABOLITES RECOVERED IN CELL NUCLEI FROM ADULT RAT BRAINS. Ivan Lieberburg* and Bruce S. McEwen (SPON: D. Micco). The Rockefeller University, New York, N. Y. 10021.

Studies during the past five years have indicated that testosterone (T) metabolites formed in the brain could be causally related to the maintenance of sexual behavior and other sex-related functions in the adult male rat. Since steroid hormones exert a major action in the cell nucleus where specific receptors for them exist, we chose to examine the levels of T and its metabolites present in brain cell nuclei after an injection of tritiated T to adult male and female rats. Adult rats, castrated and adrenalectomized (GX-ADX) 7 days previously were injected intravenously (i.v.) with ^{3}H-7-T (5.7μg/kg). Animals were sacrificed after 2 hr and enriched nuclear pellets were prepared from the following brain regions: pituitary (P), preoptic area (POA), basomedial hypothalamus (H), corticomedial amygdala (A), remainder of hypothalamus (RH), remainder of amygdala (RA), septum (S), hippocampus (HIP), midbrain (MB), and cerebral cortex (C). Levels of T, estradiol-17β (E_2) and 5αdihydrotestosterone (DHT) were determined in whole tissue homogenates and nuclear fractions by double isotope dilution, phenolic separation, methylation or acetylation, thin layer chromatography, and crystallization if possible. In all whole tissue homogenates from both sexes total radioactivity was represented predominantly by T (11-25%), followed by DHT (8-17%), and a variety of other androgens and E_2 (all <6%). However, radioactivity in enriched nuclear fractions was represented almost entirely by T, DHT and E_2. E_2 in POA, H, A and RH nuclei represented 25-80% of total nuclear radioactivity, other regions being much lower. DHT was highest in P nuclei (58-61%) and lower in other regions (8-50%). T was also highest in P nuclei (33-34%) and lower in other regions (6-36%). In males the levels of nuclear E_2 standardized for DNA were A>>S=H>POA>RH>RA>P=HIP=MB=C, whereas the female pattern was A>>POA>H>S>RH>RA>P=HIP=MB=C. The pattern of nuclear DHT was similar in both sexes, being P>>H=S>POA=A=RH=HIP=MB>RA=C.

To ascertain whether the regional pattern of nuclear DHT and E_2 as T metabolites reflected E_2 and DHT nuclear receptor concentrations, GX-ADX adult rats were injected i.v. with either ^{3}H-6,7-E_2 or ^{3}H-1,2-DHT, and nuclear levels of radioactivity were analyzed in the above-mentioned brain regions 2 hr after injection. Since specific, saturable nuclear E_2 receptors have been previously demonstrated, we used a near saturating dose of 2.3μg/kg. In males levels of nuclear E_2 standardized for DNA were P>H>POA=A>RH>RA>S>MB>HIP>C, whereas the female pattern was P>POA>H>A>RH>RA>S>MB>HIP>C. Thus, the levels of E_2 formed from T probably reflect brain aromatizing levels rather than E_2 nuclear receptor levels. In addition, male POA nuclei retained less E_2 than did female using either ^{3}H-E_2 or ^{3}H-T injections. Initially, a saturable, stereospecific nuclear receptor was demonstrated for ^{3}H-1,2-DHT in pooled limbic areas (H, POA, S, A, RH, RA) and P. Subsequently, the regional nuclear pattern of ^{3}H-1,2-DHT retention in both sexes (4.0μg/kg) was P>H=S>POA>A=RH>RA>HIP=MB>C which corresponded well with the pattern observed for DHT as a T metabolite.

These results point to E_2 and DHT as being potentially the two most important T metabolites at the brain cell nuclear level where specific, saturable receptors for them exist. The results also implicate A, H, S and POA as being the most probable sites of action of E_2 formed from T in the adult rat brain. (Supported by grants from USPHS and The Rockefeller Foundation.)

708 ASSYMETRICAL NUCLEAR SIZE DIFFERENCE IN NEURONS IN MAGNOCELLULAR PART OF PARAVENTRICULAR NUCLEUS FOLLOWING UNILATERAL CASTRATION IN RAT. Anthony N. van den Pol, Psych. Dept. Yale University, New Haven, Ct. 06520.

To examine the possibility of neural connections between the testes and the hypothalamus, a testis was removed unilaterally from the left or right side of 11 male rats of different ages (6 pigmented Sherman, 5 albino Sprague-Dawley). The sizes of nuclei from neurons in several hypothalamic areas contralateral and ipsilateral to the side of orchiectomy were measured. Nine sham-operated males (albino and pigmented) served as controls. Three weeks following surgery animals were sacrificed; brains were simultaneously embedded in paraffin. All brains were stained at the same time and coded to prevent experimenter bias during measurement. Coronal nissl-stained sections (10μ) were projected through a Zeiss microscope fitted with a projection arm; nuclear circumferences were traced at a magnification of 750X and measured on a Zeiss Particle Size Analyzer. Two hundred ($\pm$ 15) nuclei from each side of each hypothalamic area investigated were sized.

Neurons in the arcuate nucleus, ventrolateral part of the ventromedial nucleus (VMH) and dorsomedial part of the VMH showed no significant lateralized assymetries in the unilaterally castrated animals or in controls. Similarly, little size assymetry was found in paraventricular nucleus (PVN) in controls (Fig. 1). In contrast, unilaterally castrated animals displayed a size assymetry in magnocellular PVN: nuclei contralateral to the castrated side were larger than those ipsilateral to it (Fig. 1). This difference of 9% (nuclear volume) was significant at $p < .005$ (paired t-test). Since the effect is unilateral, it could not have been mediated by hormonal feedback from the testis to the hypothalamus. A more likely explanation would involve a predominantly unilateral neural connection between testis and PVN. These results are analogous to those of Halasz and Szentagothai (Z. Zellforsch. 50: 297, 1959), who found a nuclear hypertrophy in neurons of the VMH contralateral to the unilaterally adrenalectomized side, and suggest that the peripheral nervous system may play some role in neuroendocrine feedback.

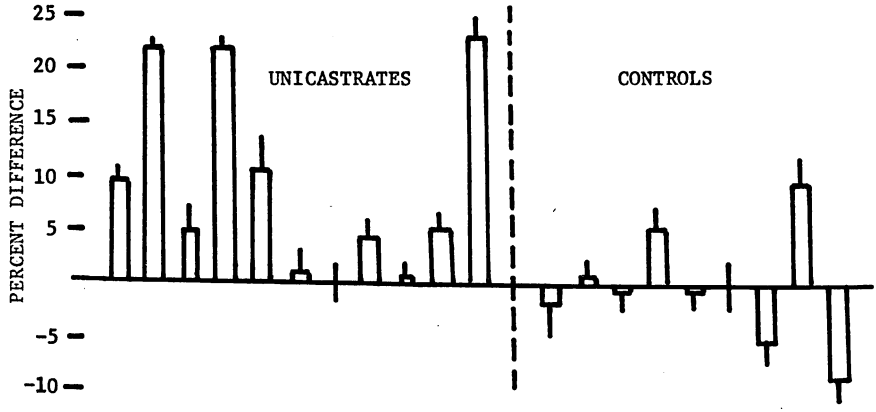

Figure 1 shows, for each animal, the nuclear volume increase in PVN neurons contralateral to the castrated side as compared to ipsilateral PVN neurons. Laterality in controls was arbitrarily assigned on the basis of odd or even numbers.

709 IDENTIFICATION OF ESTROGEN-SENSITIVE NEURONS IN THE PREOPTIC-SEPTAL AREA OF THE NORMAL CYCLIC FEMALE RAT. Martin J. Kelly*, Carol A. Dudley*, and Robert L. Moss. Dept. Physiol., Univ. Tx. Hlth. Sci. Ctr., Southwestern Med. School, Dallas, Tx. 75235

It has been suggested that neurons of the preoptic area involved in the control of the estrous cycle are sensitive to the feedback of estrogen from the ovaries. Preliminary experiments have been conducted to study the effect of iontophoretically applied 17β-estradiol on the electrical activity of preoptic (POA) neurons. One hundred and ten extracellular potentials were recorded from the POA-septal area via glass multi-barreled micropipettes in 4-day cyclic female rats anesthetized with urethane. Seventeen of the 110 neurons were antidromically identified as having their axons terminating in the arcuate nucleus and median eminence (ARC-ME) complex. The mean latency of the antidromic potentials was 14.6 msec. Of these neurons, 12 were non-responsive, 2 were excited and 3 were inhibited by iontophoretically applied estrogen. The average latency of response (<5 sec) was too short to be explained by metabolic changes within the neuron(s). Fourteen of these neurons were tested with cortisol and were shown to be unaffected by its application. The population of neurons which could not be antidromically identified but were localized in the POA-septal area (N=93), were for the most part non-responsive to estrogen (N=52). However, 33 neurons were found to be inhibited and 8 neurons were excited by its application. Cortisol was likewise tested on 76 of these neurons; 56 were unaffected, 17 were inhibited and 3 excited. Further efforts are being made in our lab to elucidate the membrane-steroid interaction and to see if differences exist in response to estrogen as a function of the stage of the cycle. (Supported by NIH-USPHS Grant #NS-10434-END).

710 FACILITATION OF THE LORDOSIS REFLEX BY ELECTRICAL STIMULATION OF THE LATERAL VESTIBULAR NUCLEUS. Doan T. Modianos and Donald W. Pfaff. Rockefeller University, New York, N. Y. 10021.

The lordosis reflex of female rats is characterized by dorsiflexion of the vertebral column: elevation of the head and rump and depression of the thorax. Lesions of the lateral vestibular nucleus (LVN) reduce the frequency of lordosis in female rats (Modianos and Pfaff, Fed. Proc., 1975, 34:396). Therefore we studied the effects of electrical stimulation of LVN on lordosis. Ovariectomized female rats received bilateral, stereotaxically guided implants of nichrome wire electrodes, and following recovery from surgery, were injected with estradiol benzoate and tested for lordosis. Electrical stimulation (200 pulses per sec.) of LVN facilitated lordosis in response to manual stimulation of the rump and perineum and in response to mounting by male rats. LVN stimulation did not facilitate lordosis in the absence of somatosensory input. Stimulation of control sites in the cerebellar cortex and brainstem did not facilitate lordosis. This is the first demonstration of facilitation of the lordosis reflex with electrical brain stimulation.

711 HORMONAL INFLUENCES ON RECEPTIVE FIELD AREA OF SINGLE TRIGEMINAL GANGLION NEURONS. D.A. Bereiter* and D.J. Barker* (SPON: C.L. Prosser) University of Illinois, Urbana, Illinois 61801.

Previous studies demonstrated that facial receptive field areas (RFA) of individual trigeminal neurons from gonadectomized female rats were significantly enlarged following long-term estrogen treatment sufficient to promote female sexual behavior. Estrogen-induced RFA enlargement was not altered by trigeminal root transection, suggesting a peripheral mechanism of action on mechanoreceptor function. The present experiments were designed to study the (a) time course, (b) hormone specificity, and (c) sex specificity of RFA enlargement in single trigeminal ganglion neurons. Gonadectomized female rats were injected with 20μg estradiol benzoate (EB) per day for 2,5, or 10 days. The threshold RFA for well-isolated single neurons was determined using the Von Frey technique. All experiments were done blind. Females given EB for 2 or 5 days showed significant RFA enlargement (without displaying female sexual behavior) when compared to control (propylene glycol only) or testosterone propionate (TP)-treated (500μg/day for 10 days) animals. Females given EB for 10 days showed RFA enlargement equivalent to that of 5-day EB females, but also showed female sexual behavior. Castrated males given EB for 10 days also showed RFA enlargement. TP treatment for 10 days did not promote RFA enlargement in males or females. The phenomenon of RFA enlargement cannot be explained on the basis of gross changes in the mechanical properties of the skin, since force-displacement measurements revealed no differences between treatment groups. Our results suggest that: (1) RFA enlargement is specific for estrogen, (2) occurs independent of and prior to (2 days vs 10 days) the onset of estrogen-induced female sexual receptivity, and (3) occurs in males as well as females. (Supported by USPHS Grant GM 619)

712 Neuroendocrine REGULATION OF SEASONAL REPRODUCTIVE CYCLES IN THE HAMSTER. L. Tamarkin*, S. Brown* and B. Goldman* (SPON: B.E. Ginsburg). Univ. of Connecticut, Storrs, Ct. 06268.

The Syrian hamster is a seasonally breeding species which does not reproduce during fall and winter in the wild. Reduction in photoperiod from 14 hours illumination daily to 10 hours per day results in a long period of gonadal quiescence in both sexes. This effect of short photoperiod is abolished by pinealectomy, or by superior cervical ganglionectomy, indicating that the pineal acts as a neuroendocrine transducer for photic information. In male hamsters the serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are reduced in short photoperiods. This appears to be at least partly due to an increased sensitivity to the negative feedback effect of androgen on the secretion of LH and FSH, as demonstrated by differential responses of long- and short-photoperiod males to various sizes of testosterone implants.

Daily injections of a pineal product, melatonin, results in testicular regression in male hamsters provided that the injections are administered during the late afternoon. An equivalent dose given daily at 10 a.m. had no effect. These results suggest that (1) a diurnal rhythm in the production of melatonin, or possibly a diurnal rhythm in sensitivity to this compound, is important in the pineal regulation of seasonal reproductive cycles and (2) the pineal hormone (melatonin?), which acts to block gonadal function, may not suppress gonadotropin secretion directly but, rather, sensitize the hypothalamic-pituitary axis to the gonadotropin-suppressive effects of circulating steroid hormones.

Injection Regime		Testes Wt. (mg)
10am	4pm	
oil	oil	3,238 ± 141 (7)
25ug melatonin	oil	3,229 ± 85 (6)
oil	25 ug melatonin	1,617 ± 287 (6)

713 NEUROENDOCRINE CONTROL OF PINEAL AND ADRENAL GUANYLATE CYCLASES. S. J. Strada, M. W. Martin* and W. J. Thompson*, The University of Texas Medical School at Houston, Department of Pharmacology, Houston, Texas 77025.

Rat and hamster pineal and adrenal glands had high specific activity guanylate cyclase enzymes as measured by radioisotopic procedures. Pineal homogenate guanylate cyclase showed an apparent Michaelis constant for GTP of ~150 μM, required Mn^{++} as cofactor, and was stimulated by calcium ion (0.1-10 mM) using suboptimal manganese. This enzyme was inhibited by norepinephrine, dopamine, tryptamine, 5-hydroxytryptamine, and histamine in a dose-dependent fashion and the inhibition was also influenced by the manganese concentration. Enzyme activity increased following bilateral superior cervical ganglionectomy indicating a postjunctional localization for the enzyme, while pineal guanylate cyclase activity decreased after chronic adrenalectomy.

Hamster adrenal guanylate cyclase studied in a 12,000 x g supernatant fraction of whole adrenal homogenate, showed an apparent Km for GTP of ~125 μM with 5 mM Mn^{++} and 36° C activity optima; particulate enzyme activity was enhanced by treatment of the 12,000 x g pellet with Triton X-100. Continuous light exposure for 10 days, a condition known to inhibit pineal gland function, increased adrenal supernatant guanylate cyclase activity.

These results suggest that sensory, neural, environmental and hormonal factors may modulate pineal and adrenal guanylate cyclase activities apparently in an interrelated manner. These studies were supported by grants from the U.S.P.H.S. (GM 21361 and HL 16552) and from the Pharmaceutical Manufacturers Association Foundation.

714 HISTOFLUORESCENCE AND ULTRASTRUCTURAL ANALYSIS OF MACAQUE AND HAMSTER PINEAL. Michael N. Sheridan* and John R. Sladek, Jr. (SPON: D. B. Puro). Dept. Anat., Univ. Rochester, Sch. Med. & Dent., Rochester, N.Y. 14642.

Application of the Falck-Hillarp technique revealed yellow fluorescence within a minority of pinealocytes in hamster, but within virtually all pinealocytes in rhesus monkey. Microspectrofluorometric analysis established the identity of this histofluorescence as a serotonin fluorophore (exc. 385, 410 nm; emiss 520 nm). Intra-pineal nerves contained both a catecholamine (presumably norepinephrine) and serotonin in hamster, but failed to demonstrate serotonin fluorescence in macaque, wherein relatively fewer catecholaminergic nerves were observed than in hamster.

At the fine structural level both rhesus and hamster pinealocytes displayed usual cytoplasmic organelles. Vesicles 400-1200 Å in diameter were frequently seen in hamster pinealocyte cell bodies, processes and terminals. Many of the larger vesicles contained dense cores.

Dense cored vesicles were rarely observed in pinealocytes of rhesus pineal.

In view of the present correlation of histofluorescence-spectral data with the fine structural data of the two species examined, we suggest a negative correlation between dense cored vesicles and microspectrofluorometrically detectable indoleamines. (Supported by USPHS Program Project Grant NS 11642 and GRSG RR 05403).

715 PARADOXICAL INCREASE OF N-ACETYLINDOLEALKYLAMINES IN CEREBELLUM AFTER PARACHLOROPHENYLALANINE. G.A. Bubenik* and L.J. Grota* (SPON: L. Spero) Clarke Institute of Psychiatry, Toronto and University of Rochester, N.Y.

Parachlorophenylalanine (PCPA) decreases the activity of tryptophan-5-hydroxylase (TR-5-OHase) in many CNS tissues. This in turn lowers levels of serotonin, a precursor of melatonin. In order to investigate the influence of PCPA on the N-acetylindolealkylamines content of cerebellum, mature male rats treated for five days with PCPA (3 with 300 mg/kg daily and 3 with 150 mg/kg twice daily i.p.) were sacrificed 12 hrs after last injection. N-acetylindolealkylamines (N-acetylserotonin and melatonin) were visualized by immunohistology using a peroxidase labeled double antibody technique. Increase of melatonin in the granule layer of cerebellum was found after either type of PCPA treatment. Specificity of staining was proven by disappearance of reaction products after saturation of antiserum with melatonin. It appears that activity of TR-5-OHase in the cerebellum is not blocked by PCPA and in fact may be increased. In this case, the cerebellum is presumably the second CNS tissue (after septum, Harvey et al. Science 183, 869, 1974) where TR-5-OHase is not blocked by PCPA. The mechanism producing increase of melatonin in the cerebellum after PCPA is not yet known. (Supported by OMHF, MRC and USPHS).

716 ELECTRICAL EXCITABILITY OF CULTURED ADRENAL MEDULLA. Bernard Biales*, Marc Dichter*, Arthur Tischler* (SPON: M. Selzer). Beth Israel Hospital, Harvard Medical School, Boston, Massachusetts 02215.

Cells from adult human and gerbil adrenal medullae were dissociated and maintained in culture for one to seven days. The cells in culture morphologically resembled chromaffin cells and exhibited formaldehyde induced fluorescence characteristic of catecholamine containing cells. They also demonstrated the chromaffin and argentaffin reactions. Intracellular microelectrode recording revealed resting potentials as high as -60 mV. The cells were capable of generating all-or-nothing, short duration, overshooting action potentials. These were inhibited by tetrodotoxin at 10^{-6} g/ml (with occasional very small residual regenerative potentials) and were not significantly reduced by Ca-free solutions or cobalt chloride at 10 mM. Iontophoretically applied acetyl choline depolarized the cells and could trigger action potentials.

717 ORGAN CULTURE AND HISTOFLUORESCENCE OF INDOLEAMINES IN MEDIAN EMINENCE. John R. Sladek, Jr., Celia D. Sladek, and Karl M. Knigge. Dept. Anat., Univ. Rochester, Rochester, N.Y. 14642.

Morphological and biochemical data suggest that ependyma (tanycytes) of the median eminence of the hypothalamus contain and synthesize serotonin (5HT). Examination of fresh and organ-cultured rat median eminence revealed the presence of monoamine histofluorescence within tanycytes, in addition to fluorescence known to exist in the contact and fibrous zones. Tanycyte fluorescence appeared yellow-green and was of sufficient intensity to be detected without the need for monoamine oxidase inhibition. This fluorescence was more readily detectable in 1-3 day cultures than in freshly dissected tissue. Microspectrofluorometric analysis of tanycyte fluorescence indicated the presence of 5HT (exc. 385,410nm; emiss. 520nm).

When median eminence was placed in organ-culture with 3H-tryptophan as substrate, radiolabeled 5HT appeared in the media in increasing amounts during 8 days in culture. Neuronal elements of the median eminence were degenerated and virtually gone by the 4th day of culture. Primary cultures of dispersed cells of median eminence also synthesized 5HT from ^{3}H-tryptophan. In contrast to whole median eminence, indole metabolism of dispersed cells was characterized by relatively little, if any, of the major monoamine oxidase product hydroxyindoleacetic acid.

These data indicate that tanycytes, cells belived to be of marked neuroendocrinological significance, are capable of synthesizing and storing the indoleamine neurotransmitter substance, serotonin. (Supported by USPHS Program Project Grant NS11642).

718 DOPAMINE-CONTAINING CELLS IN RAT DIENCEPHALON: RESISTANCE TO 6-HYDROXY-DOPAMINE. Wm. J. Shoemaker, M. Schlumpf*, E. Battenberg, and F. E. Bloom. NIMH, Saint Elizabeths Hospital, Washington, D. C. 20032.

Hypothalamic dopamine (DA) has previously been shown to be resistant to the depleting action of 6-hydroxy-dopamine (6-OHDA) (Shoemaker, Front. in Catecholamine Res. pp. 815-817, 1973; Cuello, Shoemaker and Ganong, Brain Res. 78: 57, 1974). Bjorklund and Nobin (Brain Res. 51: 1973) have described three other DA-containing cell groups of the rat diencephalon besides the well-known arcuate nucleus-tubero hypophysial system. Using a micro-dissection technique with chemical determination of catecholamines and glyoxylic acid induced fluorescence microscopy, we have determined the degree of depletion of these 4 rat diencephalic DA cells groups as well as caudate nucleus. Adult rats were given two intracisternal doses of 6-OHDA (Br salt; 250 µg; 24 hours apart) after pretreatment with pargyline and DMI. Two control groups received either intracisternal NaBr solution on the same schedule or were uninjected. Two weeks later, the brains were quickly removed and frozen. We dissected 5 regions from 500 µ cryostat sections: the rostral periventricular cell group (A14), the arcuate nucleus-median eminence region (A12), the dorsal hypothalamic group which includes the medial zona incerta (A13), the caudal hypothalamic group (A11) and a portion of the caudate nucleus. DA was measured by a modification of the radioenzyme assay utilizing COMT. Whereas caudate nucleus DA was depleted by 90% (Control rats = 84.1 ng/mg prot. vs. 6-OHDA rats = 9.7 ng/mg prot.; $p<.001$), the DA conc. in the A11, A13, and A14 regions were unchanged or slightly increased. The DA conc. in A12 was about 40% depleted. (Control rats = 18.2 ng/mg prot. vs. 6-OHDA rats = 11.6 ng/mg prot.; $p<.01$). For fluorescence microscopy, animals treated in an identical manner with 6-OHDA (with parg. + DMI) or NaBr solution were perfused with paraformaldehyde and gloxylic acid; 16-20 µ cryostat sections from their brains were immersed in glyoxylic acid (Battenberg & Bloom; Psychopharm. Commun. 1: 3-13, 1975). Serial sections of the mes- + diencephalon in horizontal or sagittal planes permitted positive identification of the cell groups A6, A9, A10, A11, A12, A13 and A14. After this treatment with 6-OHDA, the A6 neurons and ascending axonal systems survived well, but most neurons of the A9 and A10 nuclei were replaced by autofluorescent orange-brown pigmented cells. Terminal fluorescence in caudate nucleus was greatly reduced. Within the hypothalamus, the dorsal catecholamine neuronal clusters of the A11, A13 and A14 groups appeared well preserved with full retention of long dendritic and probable axonal process. Bright fascicles of fine fluorescent axons could be seen in a narrow band just beneath the ependyma of the third ventricle. The arcuate nucleus neurons were only partially intact with approximately half of these neurons being converted to the orange-brown autofluorescent appearance. Fine terminal varicosities within the anterior lateral septum were still visible in abundance in these animals. Thus the histofluorescent view of 6-OHDA treated animals corresponds well with the biochemical determinations: although the substantia nigra-caudate nucleus DA-containing neurons are severely depleted by the treatment, the DA-containing cells of A11, A13 and A14 are unaffected. The reason for these cells' resistance to 6-OHDA is not known but does not appear to be due to lack of exposure to the drug. Catecholamine-containing cells and processes close to the ventricle were unaffected, whereas some regions distant from the ventricular system were greatly depleted. A possible explanation for their resistance to 6-OHDA is that they lack the conventional uptake mechanisms necessary for 6-OHDA to enter the cell. The role of DA in such cells remains unclear, but possible endocrine functions have been suggested.

719 PARALLEL EFFECTS OF PHENYLPROPANOLAMINE AND INSULIN ON HYPOTHALAMIC ELICITED FEEDING, SELF-STIMULATION, AND STIMULATION-ESCAPE.
Luis Hernandez* and Bartley G. Hoebel. Dept. Psychology, Princeton Univ., Princeton, New Jersey, 08540.

Phenylpropanolamine (PPA), an appetite suppressant drug, and insulin, a hormone which may act as a satiety signal, show parallel effects on behavior. Both decreased spontaneous feeding but not drinking and inhibited electrically elicited feeding but not elicited drinking. They also inhibited lateral hypothalamic (LH) self-stimulation and increased stimulation escape at the same electrodes.

In rats that performed operant responses both for food and for water during LH stimulation, PPA selectively inhibited only the elicited feeding when injected either i.p. (5-40 mg/kg) or into the LH as crystals via an electrode-cannula. In rats with multiple electrode assemblies including an LH electrode which elicited feeding and a posterior hypothalamic electrode which elicited copulation, PPA (5mg/kg) selectively decreased self-stimulation and increased stimulation-escape only at the LH site. Insulin (s.c., 1 I.U./kg) decreased self-stimulation in the LH but not in the subcommissural septal region in the same rats. The same dose of insulin increased LH stimulation-escape. These effects were not the result of overall changes in arousal or nonspecific operant responding because the effects were specific to certain electrodes and select behaviors.

The results suggest: 1)that LH self-stimulation is related to elicited feeding; 2)that both are under inhibitory control by processes involved in satiety; 3)that LH self-stimulation and escape are reciprocally related; 4)that these measures of reward and aversion reflect rewards and aversions involved in eating; and 5)that pharmacological and hormonal agents such as we used can distinguish among the various systems underlying hypothalamic elicited behaviors.

720 STRIATAL DOPAMINE: RELATIONSHIP TO WEIGHT LOSS AND REGULATORY DYSFUNCTION AFTER NSB LESIONS.
K. Simansky* and J. A. Harvey. Dept. Psych., Univ. Iowa, Iowa City, IA, 52242.

Lesions of the nigrostriatal bundle (NSB) produce a transient aphagia and adipsia, with consequent weight loss, and permanently impair certain ingestive behaviors. It has been suggested that depletion of striatal DA is related to body weight loss and regulatory dysfunctions such as loss of 2-DG-induced feeding. In the present study, bilateral (BNSB) and unilateral (UNSB) lesions produced significant weight loss which was related to the striatum with the greatest DA depletion in BNSB ($r = .70$) and to the DA content of the striatum ipsilateral to the lesion in UNSB ($r = .50$). The duration of the weight loss in BNSB rats was related to the striatum with the smallest DA depletion ($r = .73$), but not to striatal DA content in UNSB rats. The severity of the weight loss after BNSB lesions was multiply determined by the DA content of the individual striati (the multiple correlation of low and high content DA with weight loss was 0.82 and with duration was 0.85). BNSB rats ate less after 2-DG than controls, but this was not correlated to striatal DA. NSB rats drank less over a 24-hour period than control rats, but this was also uncorrelated with DA content. UNSB rats responded normally to 2-DG, but were hypodipsic under ad libitum conditions or after deprivation and reduced their water intake by 70% when food was removed. They responded normally to injections of 2 M NaCl or renin, but when the renin test was extended to 24-hr, they drank less than controls. This study further indicates which characteristics of the "lateral hypothalamic syndrome" may be attributed to striatal DA and which should be ascribed to other systems. (Supported in part by USPHS MH16841-07 and MH10641).

721 EFFECTS OF GLUCOSE ON HYPOTHALAMIC MULTI-UNIT ACTIVITY IN NORMAL AND OVARIECTOMIZED RATS. Bernard M. Rabin and Donald S. Miller*. Dept. Psychol., Univ. Md. Baltimore County, Baltimore, Md. 21228.

Since the hypothalamus is sensitive to glucose, theories of the hypothalamic regulation of food intake have proposed that glucose functions as the metabolite which signals satiety. In cats, systemic injections of glucose produce consistent decreases in lateral hypothalamic activity and increases in medial hypothalamic activity (Brown and Melzack, Exp. Neurol. 24: 363, 1969). Glucose may not produce similar consistent changes in the activity of the rat hypothalamus (Oomura et al., Nature 222: 282, 1969). To further evaluate this possibility, multi-unit activity was recorded from the hypothalamus of female rats and the population responses to injections of 30% glucose and to saline were observed. The multi-unit response from 34 histologically confirmed recording sites in the hypothalamus was extremely variable, with increased, decreased, or unchanged levels of activity being equally probable in response to glucose injections in different animals. Since these recording sites were adjacent to each other, it was not possible to define an area within the lateral hypothalamus that responded to glucose in a consistent and specifiable manner. To determine if the observed variability in the hypothalamic response to glucose in female rats could be due to an interaction with hormones that have also been implicated in the regulation of feeding, several additional experiments were performed using male and ovariectomized female rats. Neither of these procedures resulted in an increased level of consistency in the multi-unit response to glucose injection. The variability of the hypothalamic multi-unit response to glucose is not consistent with the hypothesis that the hypothalamus functions to regulate feeding behavior by monitoring glucose utilization. (Supported in part by NIH Grant NS 12203).

722 COMPARISON OF HYPERPHAGIA SYNDROMES PRODUCED BY PARASAGITTAL AND CORONAL HYPOTHALAMIC KNIFE CUTS. Anthony Sclafani. Department of Psychology, Brooklyn College, Brooklyn, N.Y. 11210

Bilateral parasagittal knife cuts between the medial and lateral hypothalamus and bilateral coronal knife cuts posterior and lateral to the ventromedial nucleus produced hyperphagia and obesity in female rats. The parasagittal cuts produced greater increases in food intake and body weight than did the coronal cuts, but the coronal cuts produced greater increases in water intake. Both types of cuts produced similar alterations in circadian feeding and drinking rhythms and altered the rat's response to adulteration of their food and water, but the cuts did not affect the anorexia effect of amphetamine injections. The results indicate that the parasagittal and coronal cuts transect a common feeding inhibitory pathway which either ascends or descends in the hypothalamus. This conclusion is further supported by the finding that unilateral parasagittal cuts combined with contralateral coronal cuts either in the posterior hypothalamus or midbrain also produce hyperphagia and obesity. The behavioral evidence indicates that the feeding pathway severed by these cuts differs from the recently identified ascending noradrenergic feeding pathway.

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723 NEURAL SUPPRESSION OF THERMOGENESIS BY STARVATION. Keith E. Bignall and Franklin W. Heggeness*, Dept. Physiol. Sch. Med., U. Rochester, Rochester, N.Y. 14642

Oxygen consumption of 5 and 10 day old rat pups was measured by the volume displacement method at thermoneutral temperatures (5d:35C, 10d:32C) and during cold stress (5d:30C, 10d:27C), either fed or starved for 2-20 hrs. In the cold, fed animals doubled their O_2 consumption, from a basal rate of 27±2 ml/min/kg at thermoneutrality. 8-10 hrs. of starvation abolished this metabolic response to cold stress, without affecting basal rate. Ten U/kg (sc) insulin produced similar results. In both cases blood glucose dropped to about 60% of normal concentration, with O_2 intake in the cold changing in direct and linear proportion. Colonic temperature fell from 36C to 32C. Intragastric feeding of isotonic glucose restored 80% of the response in starved pups. Noradrenalin (NE) or 6-hydroxydopamine (6-OHDA), which presumably stimulated NE release, transiently elevated O_2 uptake in starved pups to values approximating those in the fed condition. Decerebration at the midpontine level caused O_2 uptake to remain high despite starvation, both in the warm and cold, and prevented the drop in T_c and glucose concentration in the cold. The high O_2 uptake was blocked by 6-OHDA (25 mg/kg,sc). The suppression of the metabolic response to cold exposure by starvation thus seems to be an active neural control, linked to blood glucose concentration, and is not due to depletion of available energy stores. The transection may release the starvation-imposed inhibition, resulting in unrestrained high metabolism, analogous to motor rigidity in the decerebrate adult. Decerebrate rigidity was not seen in these neonates.

724 GLUCOSE PREFERENCE AND CALORIC INTAKE IN WEANLING RATS WITH VENTROMEDIAL (VMN) AND DORSOMEDIAL (DMN) HYPOTHALAMIC LESIONS (L). Lee L. Bernardis and John R. Border* Dept. Surgery and Pathology, SUNY at Buffalo, Buffalo, NY. 14215.

Weanling male rats received bilateral electrolytic lesions in the VMN and DMN resp.; Sham-operated rats served as controls. Two weeks thereafter the animals were subjected to a glucose preference test assessing the choice between a 10% (w/v) and a 35% d-glucose solution. Weanling VMNL rats, as their mature counterparts, preferred the stronger over the weaker glucose solution throughout the experiment (16 days). Weanling DMNL rats, however, showed a bimodal response by initially preferring the stronger, but later preferring the weaker solution. The weanling controls behaved different from mature control animals by preferring the dilute solution only during the latter part of the experiment. Caloric intake from glucose alone was similar in all three groups of rats but magnitude and pattern of calories from chow and total calories were only similar in the VMNL rats and the controls.

Three other groups of rats, comparable to the above animals, were injected intraperitoneally with glucose solution to examine the anorexigenic effect of this nutrient. VMNL rats showed a longer depression of food intake than DMNL rats and controls. It appears that in the weanling, as in the mature rat, the VMN are involved in long-term feeding control and do respond to the metabolic signal arising from glucose. The DMN appear to be less involved in this mechanism.

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725 EFFECTS OF NACL INJECTION UPON LATERAL HYPOTHALAMIC NEURONS OF INFANT RATS. C. Robert Almli and Gregory T. Golden*
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Male and female infant rats (8-17 days of age) were prepared for recording from the lateral hypothalamic area (LHA). Basal unit activity (spikes/sec.) was recorded prior to injection of 16% NaCl or 0.87% NaCl solutions. Basal spike rates of 3-7/sec. (for both sexes) did not change following 0.87% NaCl. The 16% NaCl injection resulted in 93% of the units deviating from the basal rates. Altered neuronal firing was operationally defined as ±50% of the basal rate. Using that criterion, nine units increased, four units decreased, and two units both increased and decreased in activity rate following the 16% NaCl injection. The mean latency for altered activity, post-injection, was 2.8 min. These results suggest that as early as eight days of age, rat LHA neurons are osmosensitive. The time-course of neuronal activity change corresponds to temporal patterns of behavioral drinking responses induced by 16% NaCl injections for infant rats.

726 EFFECT OF FEEDING SEQUENCE ON LOCOMOTOR ACTIVITY OF RATS BRED FOR RUNNING. Walter Riss and Elliott Mufson*. Dept. Anat. and Biol. Psychol. Program, SUNY, Downstate Medical Center, Brooklyn, N.Y. 11203.

After 11 generations of brother-sister matings in which the selection criterion was the greatest locomotor response to food deprivation, littermates (equated for sex and weight) were compared for locomotor response to short rations using 2 different sequences of feeding but the same total amount of powdered lab chow. Locomotor activity measurements (with Wahmann type LC-34 activity cages) were begun when the rats were 30 days of age and terminated no more than 45 days later. All rats received 6 gm. of food at the same time every 4th day. Group I received 8 to 17 gm. on days 1 to 3, with increasing amounts to day 3. Group II received 17 to 8 gm. on days 1 to 3, with decreasing amounts to day 3. Group I outperformed Group II throughout the study. The cost of increased locomotor activity revealed itself as a decreased growth rate in Group I.

It is anticipated that such inbred rats will be valuable in the study of short-term neuroendocrine changes which may underlie the deprivation-induced locomotor activity, especially where the energy expenditure can be as pronounced as found under the present conditions. For example, peak locomotor activity often reached 20,000 revolutions/day.

727 EVIDENCE FOR INVOLVEMENT OF THE RENIN-ANGIOTENSIN SYSTEM IN ISOPROTERENOL DIPSOGENESIS. James E. Schwob* and Alan Kim Johnson. Dept. Psychol. Univ. of Iowa, Iowa City, Iowa, 52242.

Isoproterenol (ISOP), a beta-adgenergic agonist, induces hypotension by direct action on vascular smooth muscle, initiates renin release, and causes water consumption. Because the drinking to ISOP is abolished by nephrectomy, Houpt and Epstein (Physiol. Behav., 7, 897) proposed that the dipsogenesis is mediated by the renin-angiotensin (RA) system. However, further analyses employing agents which interfere with the RA system have not supported this proposal. Peripheral application of peptide antagonists of angiotensin II (AII) (Tang & Falk Pharm. Biochem. Behav., 2, 401) and of converting enzyme (Lehr et al. Science, 182, 1031) failed to block ISOP induced drinking.

Perhaps the reason for the failure to demonstrate a blockade of drinking with systemically applied antagonists is that the action of AII on peripheral smooth muscle is also blocked and the kidneys respond to the sustained ISOP produced hypotension by releasing more renin. If so, eventually the blockade would be overridden and drinking would ensue. The work reported here employed intracranial (i.c.) injections of Sar^1, Ala^8 AII (saralasin acetate, Norwich Pharmacal) in order to minimize the peripheral vascular effects produced by direct action of the antagonist. The i.c. route of administration of saralasin has been shown to be effective in blocking peripherally administered AII (Johnson & Schwob, Pharm. Biochem. Behav., in press).

In Exp. 1 Sprague-Dawley derived, male abino rats were implanted with i.c. cannulae. Animals were screened for drinking to i.c. AII and to s.c. ISOP. Twelve animals that passed the screening tests received four treatment conditions in random order: (1) i.c. isotonic saline (ITS) (2 µl) and s.c. ITS; (2) i.c. ITS and s.c. ISOP (Isuprel, Winthrop Lab.) (13 µg/kg); (3) i.c. saralasin (1 µg) and s.c. ISOP (13 µg/kg); and (4) i.c. saralasin (5 µg) and s.c. ISOP (13 µg/kg). Water intakes were recorded at 30 and 60 min. At 30 min there was no significant difference in intake between condition 2 (4.0 ml± S.E.M. .42 ml) and condition 3 (3.7 ml± .48). However, there was a significant ($p<.001$) attenuation under condition 4 (1.2 ml± 4.8) as compared with condition 2 at 30 min. At 60 min, there was no significant difference between condition 2 (4.6 ml± .49) and condition 3 (4.2 ml± .61) or condition 4 (4.7 ml± .68).

To determine if there are any nonspecific disruptive effects of saralasin pretreatment, Exp. 2 examined the effect of i.c. saralasin on drinking to s.c. injections of hypertonic saline (HTS). Rats were implanted and their cannula placements screened as in Exp. 1. The animals then received two s.c. screening treatments with .75 ml 10% NaCl solution. Eight animals received in random order: (1) i.c. ITS (2µl) and s.c. ITS (.75 ml); (2) i.c. ITS and s.c. 10% NaCl (.75 ml); and (3) i.c. saralasin (5 µg) and s.c. 10% NaCl (.75 ml). There was no significant difference in water intake at 30 min between the i.c. ITS-s.c. HTS (3.7 ml± .44) and the i.c. saralasin-s.c. HTS (4.7 ml± .72) conditions.

Exp. 3 tested whether degradation of the central blockade in the face of maintained activity by ISOP could account for the rebound drinking seen in the second ½ hr in Exp. 1. Six male rats, after s.c. screening to ISOP, received in random order: (1) s.c. ISOP (13 µg/kg) with continuous access to water; (2) s.c. ISOP (13 µg/kg) with access to water delayed by ½ hr. There was no difference in intake 60 min. following injection following delayed access (4.1 ml± .91) and continuous access (4.3 ml± .64).

These experiments demonstrate that an AII antagonist applied i.c. will delay the onset of drinking to ISOP. This increase in latency is not due to a non-specific disruption of behavior. Drinking seen in the second half of the hour test following ISOP and saralasin is probably due to the degradation of the central antagonist while the action of the ISOP is sustained. Taken together, these studies provide evidence that the RA system is involved in the mediation of ISOP drinking. (MH25345-01 & MH26571-01 NIMH)

728 ANGIOTENSIN-INDUCED THIRST: ANTEROVENTRAL THIRD VENTRICLE SITE OF ACTION. James Buggy*. (SPON: J.P. Hegmann). Dept. Physiology & Biophysics, University of Iowa, Iowa City, Iowa 52242.

Depletion of either the intracellular or intravascular fluid spaces is sufficient for the arousal of thirst. Angiotensin II (AII) may be importantly involved in the mediation of thirst resulting from decreased blood volume or pressure because a) intravascular thirst challenges result in increased formation of AII, b) exogenous AII induces drinking when administered systemically or intracranially and c) nephrectomy attenuates both AII formation and the drinking response to several intravascular challenges. Receptors sensitive to the dipsogenic action of AII are probably within the central nervous system since intracranial (IC) injections of AII induce drinking at a lower threshold and with a shorter latency than systemic injections. However, the central site(s) and mechanism of action of AII remain in question. Early IC mapping studies implicated a diffuse number of tissue sites. However, a more recent study (Johnson & Epstein, Brain Research 86, 399) indicates such data need reevaluation since mapping with low doses of AII revealed that the critical aspect of the IC injection was that the peptide gain access to the brain ventricular system. Simpson & Routtenberg (Science 181, 1172) proposed that the subfornical organ (SFO) contains the exclusive dipsogenic receptors for AII since lesions of the SFO abolished drinking to lateral preoptic (LPO) injections of AII. The experiments described here assess whether the dipsogenic response to AII depends on ventricular transport to SFO tissue, and if not, what other structures of regions may be involved.

In Exp. 1, the effects on drinking of AII injections into the lateral ventricle (LV), LPO, and anteroventral third ventricle (AV3V) of adult, male rats were assessed before and after SFO lesions. Results showed that although complete SFO lesions attenuated AII induced drinking following injection into the LV or LPO, the response showed partial or full recovery within a few days. Also, there was no decrease in drinking post-lesion from the AV3V placement. Furthermore, intraperitoneal injections of renin still elicited drinking in animals with complete SFO lesions. Thus, SFO cannot be an exclusive receptor site for AII induced thirst. Exp. 2 involved a dose-response study of the dipsogenic response to AII injections into the AV3V placement. Results indicated that drinking could be induced at as low a threshold (0.1 ng) as reported for SFO.

In Exp. 3, the hypothesis was tested that the post SFO lesion deficit in AII induced drinking might be due to some effect of the lesion other than SFO damage. SFO lies at the confluence of the lateral and third ventricle and lesions here could block the interventricular foramen, either by edema or lesion-produced debris, thereby retarding passage of cerebrospinal fluid from LV to the rest of the ventricular system. Such a blockade would seal off injections into lateral placements from access to the AV3V. To mimic such a condition, cold cream plugs were placed in the third ventricle at the level of the interventricular foramen. This preparation is of particular interest since it permits independent stimulation of SFO and AV3V regions, as well as preventing access to either site following injection of angiotensin into LPO or LV placements. Radioactive and dye tracings were used to determine effectiveness of plug barriers and results from successful plugs indicated that a) AII induced drinking from LV and LPO was abolished, b) drinking attributable to SFO stimulation was reduced 65%, and c) drinking to AV3V AII injections was not at all diminished. When plugs were placed in the AV3V region, drinking to AII injections in LV or LPO was abolished despite continued access to SFO. From these experiments it was concluded that AII exerts its dipsogenic effect by spread through the ventricular system to anteroventral third ventricle receptors, and that though SFO may somehow be involved, it is by no means crucial. Following intracranial injection, access of the hormone to the AV3V is both necessary and sufficient for the arousal of thirst.

729 BLOCKADE BY MICROIONTOPHORETIC APPLICATION OF P113 ON SUBFORNICAL ORGAN NEURONS RESPONSIVE TO ANGIOTENSIN II.
M.Ian Phillips and Dominik Felix*. Dept. Physiology, Univ. Iowa, Iowa City, Ia.52240 and Institute for Brain Research, University of Zürich, Switzerland.

There is increasing evidence that circumventricular organs are involved in angiotensin (A II) mediation of thirst and blood pressure. The subfornical organ (SFO) is one such site which contains A II sensitive neurons. The question arises: are these neurons specifically sensitive to A II ? We have used the A II analogue sar^1-ala^8-A II (P113) and found specific blockade of A II sensitive neurons in the SFO.

Five adult cats were anesthetised and the SFO exposed for penetration by a 5-barrel microiontophoresis electrode of 22 units sensitive to A II, 18/19 had their response to A II blocked by P113, whereas only 2/16 units responsive to ACh were antagonised. P113 alone depressed firing rates in 12/22 units, 9/22 had no effect, 1 was excited. The most sensitive antagonism with respect to dose of P113 was on neurons responsive only to A II and not to both A II and ACh. We conclude that there are specific A II sensitive neurons in the SFO (Supported by Swiss NSF 3.822.72 and NIMH Research Development Award).

730 THE ANTIDIPSOGENIC ACTION OF PROSTAGLANDIN E_1 (PGE_1). Nancy J. Kenney and Alan N. Epstein, Inst. Neurological Sciences, U. of Pa., Phila. 19174.

Injection of PGE_1 (5µg in 1µl over 10 sec) into the lateral cerebral ventricle of adult male rats reduces water intake stimulated by intracerebroventricular injection of angiotensin II (AII, 5ng). Prostaglandin A_1 (5µg) and prostaglandin $F_{2\alpha}$ (5µg) have no effect on water intake. PGE_1 produces a severe reduction in water intake in all rats. Intakes are reduced by an average of 60% compared to intakes following control injections. At the lower dose of 1µg, PGE_1 reduces water intake stimulated by centrally-administered AII (5ng) and carbachol (100ng) and by peripherally-injected hypertonic saline (0.75ml 2M NaCl/100g body weight). When the dose is further reduced to 100ng, PGE_1 is ineffective in blocking water intake due to carbachol or hypertonic saline treatment. Blockade of AII-induced intake remains effective with a dose of PGE_1 as low as 10 ng. At this dose, the hyperthermia resulting from PGE_1 treatment is no greater than that resulting from control injections which have no effect on water intake. Thus, the antidipsogenic effect of PGE_1 appears to be independent of its pyrogenic effects.

PGE_1 blockade of ingestion is specific to water intake. Although PGE_1-treated animals do reduce food intake when they must ingest water in order to ingest food, no reduction in food intake is seen when the animals receive water by gavage prior to food presentation. PGE_1 is, therefore, a specific antidipsogen and not a malaise-producing inhibitor of all ingestive behaviors. Since PGE_1 is synthesized in the brain (Kataoka et al., Science, 157, 1967), it may be involved in the satiation of thirst during spontaneously initiated bouts of drinking behavior.

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731 BRAIN AND BLOOD-BORNE ANGIOTENSIN II IN THE CONTROL OF THIRST. Wilson A. Saad, Alan N. Epstein, John B. Simpson, and Luiz A. Camargo*, Inst. Neurological Sciences, Univ. of Pa., Phila., Pa. 19174.

Renin-angiotensin systems are present in both brain and periphery, and angiotensin II (AII) induces thirst by both intravenous and intracranial routes of administration by utilizing receptors in the subfornical organ (SFO). To study how the cerebral and renal renin-angiotensin systems interact in arousal of thirst, the dynamics of cerebral AII were studied by radioimmunoassay (Schwarz-Mann) of CSF samples taken from the cisterna magna of barbiturate anesthetized rats. AII was found in the CSF at higher concentration (~90pg/ml) than in plasma and its concentration rose markedly after nephrectomy. CSF AII therefore originates from brain renin. Dipsogenic treatments that raise peripheral renin-angiotensin (hypovolemia of hyperoncotic colloid, hypotension of isoproterenol), depress CSF AII, as does intravenous infusion of AII (25ng/min/rat, 30min). Blood-borne AII therefore interacts reciprocally with the cerebral renin-angiotensin system. Lastly, approximately 7% of exogenous AII was recovered from the cisterna after injection of dipsogenic doses (1µl of 1, 10, and 100 ng in 10 sec) into the lateral cerebral ventricle. Like exogenous AII injected into the ventricle, AII originating from cerebral renin may contribute to thirst by reaching the third ventricle and stimulating the SFO. Blood-borne AII of renal origin does not enter the CSF and reaches the SFO directly from the blood.

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732 SPECIFIC INHIBITION OF ANGIOTENSIN MEDIATED DRINKING IN RATS BY STIMULATION OF THE SEPTUM. Elliott M. Blass and James S. Moran. Dept. Psychol. Johns Hopkins University, Baltimore, Md. 21218.

Bilateral electrical stimulation of the medial or lateral septal areas of rats inhibited drinking to hypotension and to intraperitoneal injections of hyperoncotic colloid. Drinking to both of these extracellular thirst challenges is thought to be mediated, at least in part, by Angiotensin II. However, drinking following cellular dehydration or following subcutaneous injections of hyperoncotic colloid was not inhibited by septal stimulation. These forms of drinking are thought to be independent of the renin-angiotensin mechanism. Moreover, electrical stimulation did not interfere with feeding a liquid diet, offering additional proof that electrical stimulation did not affect the consumatory response of licking. These findings support the view that elements within the rat septum participate in inhibiting drinking mediated by renin-angiotensin and further suggest that the overdrinking to angiotensin that follows bilateral destruction of the rat septum (Blass, Nussbaum and Hanson, J. Comp. Physiol.Psychol., 1974, 87, 422-439) is not due to ventricular damage which could facilitate angiotensin reaching central thirst receptors. (Supported by Grant NS-09305).

733 REGIONAL DEPLETION OF FOREBRAIN AMINES BY MESENCEPHALIC KNIFE CUTS: EFFECTS ON FOOD AND WATER INTAKE, "SENSORY NEGLECT", MOTOR ABILITIES AND CATALEPSY. Valerie C. Abbott*, and Ernest W. Kent (SPON: J. D. Davis). Dept. Psychol., U. of Illinois at Chicago, Chicago, Ill. 60680.

Following the demonstration (Kent and Grossman, 1973) that interruption of amine and other projections turning laterally from the medial forebrain bundle lead to loss of intake and impairment of performance of learned behaviors, but not to other deficits reported to follow from chemical or surgical lesion of the MFB, nigro-striatal bundle, or lateral hypothalamus, we prepared white rats with stereotaxic wire knife cuts in the mesencephalon to interrupt dopaminergic and serotonergic projections to the striatum and to the basal forebrain. The resulting depletions were confirmed by fluorometric assay (Welch and Welch, 1969) for dopamine serotonin and nor-epinephrine simultaneously by region. The results support the conclusion that the aphagia resulting from injury to these projections is correlated with striatal dopamine loss, but that it cannot be ascribed to "sensory neglect", oral motor dysfunction, or hypoactivity and catalepsy. These latter effects seem rather to be correlated with striatal serotonin and basal forebrain dopamine and serotonin involvement. Other aspects of these manipulations are discussed in a companion presentation (Kent and Abbott).

Kent, E.W., and Grossman, S.P., Elimination of Learned Behaviors After Transection of Fibers Crossing the Lateral Border of the Hypothalamus. Physiol. and Behav., 10, 953-963, 1973.

Welch, A. and Welch, B. Analyt. Biochem., 30, 161-179.

734 EFFECTS OF CENTRAL GREY TRANSECTIONS AT VARIOUS ROSTRO-CAUDAL LEVELS. David Wirtshafter*, Robert Pociask*, and Ernest W. Kent (SPON: K. Asin). Dept. Psychol., U. of Illinois at Chicago, Chicago, Ill. 60680.

Coronal knife cuts were made at various rostro-caudal levels in the region of the central grey substance of rats. Animals with cuts at the level of the meso-diencephalic junction displayed an exaggerated response to quinine adulteration of their water but, unlike VMH or septal lesioned animals, failed to show a supernormal response to addition of saccharine. Food and water intake, body weight gain, open field activity, jump/flinch thresholds, and acquisition of a two way avoidance response were all unaffected. More posterior transections at the level of the dorsal tegmental nucleus, behind the dorsal raphe, failed to produce "finnickiness", but resulted instead in an extreme hyperactivity in the open field. Other measures were unaffected.

735 FEEDING PATTERNS IN THE MONKEY. Benjamin H. Natelson and James C. Bonbright, Jr.* Dept. of Neurology, VA Hosp., E. Orange, NJ and Dept. of Exp. Psychol., Walter Reed Army Institute of Research, Washington, DC.

The study of feeding patterns of rodents has led to conflicting opinions about the role of physiological variables in the control of feeding. To try to resolve this inconsonance, we studied feeding in the monkey over 20 day periods. Five adult male monkeys (Macaca mulatta) were housed in cages kept in closed booths lit 12 hr a day. A 750 mg food pellet was always available. Whenever the pellet was eaten, the time was recorded, and another pellet was made available; water was always present. Monkeys fed in discrete meals and when feeding stopped for 5 min, the monkey almost never took another pellet until its next meal. Using this 5 min pause to delineate meals, we found that monkeys ate significantly more meals in day than night ($p<0.01$; Table) and that 62% of all pellets were eaten in daytime meals. We then determined correlation coefficients between the size of each meal and the time preceding it and following it. This table shows that correlations between meal size and the pause following that meal were highly significant, especially during the 12-hr day period. Correlations between meal size and the pause preceding each meal were poor. These correlations of raw data are the first reported in the monkey to indicate a lawful relationship between meal size and subsequent satiety and are consistent with the idea that food ingestion releases a physiological inhibitor, proportional to meal size, which is responsible for the discrete, episodic pattern of feeding seen here.

	DAY ONLY			NIGHT ONLY		
Monkey	Before	n	After	Before	n	After
M-831	0.131	105	0.225*	0.138	42	0.058
M-297	0.076	74	0.404**	-0.105	45	0.602**
M-447	-0.100	40	0.605**	0.210	10	0.513
L-410	-0.030	71	0.443**	0.229	45	0.505**
M-283	0.284*	79	0.451**	-0.347	26	0.726**

(n = no. of meals; *$p<0.05$; **$p<0.001$)

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736 THE ROLE OF HUNGER IN SCHEDULE-INDUCED POLYDIPSIA. Virginia L. Wolfe*, William J. Freed* and Joseph Mendelson* (SPON: Nancy A. Dahl). Dept. Psych., University of Kansas, Lawrence, Ks. 66045.

When food-deprived rats are intermittently given small morsels of food, they drink enormous quantities of water, sometimes as much as one-half their body weight during a three hour period (Falk, Science 133: 195, 1961). This phenomenon, known as schedule-induced polydipsia (SIP), is characterized by a bout of drinking which normally occurs immediately after each food pellet is eaten. One of the parameters controlling the intensity of SIP is the hunger level of the rat. The more severe the food-deprivation, the greater the degree of SIP. The purpose of the present experiment was to determine the precise role of hunger in the induction of SIP. Specifically, is the drinking a result of the hunger present while the pellet is being consumed or the hunger present during the interpellet-interval (IPI)? To answer this question it was necessary to use a form of hunger which could be turned on or off at any instant. Electrical stimulation of the lateral hypothalamus (ESLH) induces a state which can resemble deprivation-induced hunger (Wise, Brain Res. 67: 197, 1974); therefore, we substituted ESLH for normal hunger.

Seven rats bearing electrodes in the lateral hypothalamus that induced feeding when electrically stimulated (stimulus-bound feeders) were used as subjects. They were deprived of food until they reached 80% body weight and pretrained on one hour daily sessions. One 45-mg. food pellet was automatically delivered each minute and water was continuously available. When drinking occurred on at least 90% of the IPIs the animals were satiated with food and subjected to the same experimental situation using ESLH to induce hunger. Each rat was tested under two conditions: i) simultaneously with pellet delivery ESLH was turned on for three seconds (3 sec. condition) ii) simultaneously with pellet delivery ESLH was turned on for 15 seconds (15 sec. condition). It is known that stimulus-bound (S-B) feeders often become S-B drinkers when only water is present. Two precautions were taken to guard against this: i) a "protection" procedure described by Valenstein (J. Psychiat. Res. 8: 335, 1971) involving extensive pretraining with only food present was instituted and ii) during the 15 sec. condition a second 15 seconds of ESLH was delivered 30 seconds after pellet delivery. This provided a continuous monitor of whether the drinking that occurred was schedule-induced or stimulus-bound.

During the 3 sec. condition, drinking occurred on only 12%, 18%, 12%, 10%, 19%, 34% and 7% of the IPIs (mean=16%). When the 15 sec. condition was initiated three of the rats became S-B drinkers (they drank in response to ESLH after pellet delivery as well as during the second 15 seconds of ESLH, when no pellet was delivered). Therefore, no further data was collected from these animals. The four remaining animals drank on 81%, 86%, 90% and 90% of the IPIs during the 15 sec. condition (mean= 87%). Rebound inhibition and order effects were ruled out by appropriate controls.

This data provides additional evidence to support the contention that the motivational states produced by food-deprivation and by electrical stimulation of the lateral hypothalamus are similar. It is concluded that post-pellet hunger is necessary to generate schedule-induced polydipsia. Hunger only while the pellet is being consumed is not a sufficient condition. (Supported by a dissertation fellowship awarded by the University of Kansas to Virginia L. Wolfe and by a University of Kansas research grant to Joseph Mendelson.)

737 DISORDERED DRINKING AFTER ABDOMINAL VAGOTOMY IN RATS. F. Scott Kraly*, James Gibbs, and Gerard P. Smith. Dept. Psychiatry, Cornell U. Med. Coll. and E.W. Bourne Behavioral Research Laboratory, New York Hospital, White Plains, N.Y. 10605.

After finding that vagotomized (Vgx) rats had a low water to food ratio (Vgx 1.3 ± 0.5 ml/g, sham 1.8 ± 0.1 ml/g, $p < .001$), we analyzed their drinking behavior under a variety of conditions. Bilateral subdiaphragmatic vagotomy (N=12) or sham vagotomy (N=10) was performed in Sprague-Dawley male rats (430-560 g body weight, BW). Verification of vagotomy 4 months after surgery determined that 9 rats had sustained complete vagotomy. These 9 rats exhibited marked drinking deficits: (1) Less drinking during 24 hrs. when no food was available (Vgx 1.8 ± 0.4 ml/100g BW, sham 6.1 ± 0.8 ml/100g BW, $p < .001$). Dysphagia did not account for the decreased drinking because Vgx rats ate the same amount of Purina chow as sham rats when water was not available. (2) Less drinking following 24 hr. water deprivation. (3) Less drinking following cellular dehydration produced by 0.5% and 1.0% BW loads (i.p.) of 2M NaCl (0.5% load: Vgx 0.7 ± 0.3 ml/100g BW, sham 3.0 ± 0.3 ml/100g BW, $p < .001$). Vgx rats did not appear behaviorally depressed, since 4 of 7 Vgx rats drank with a normal latency but drank much less than sham controls. (4) Failure to drink to isoproterenol (0.16 mg/kg BW, s.c.: Vgx 0.4 ± 0.4 ml/100g BW, sham 2.3 ± 0.3 ml/100g BW, $p < .01$). (5) Less drinking in 24 hrs. after polyethylene glycol (PG, 5 ml of 30% w/w soln. in 0.9% NaCl: Vgx 12 ± 2.6 ml, sham 22 ± 2.0 ml, $p < .02$). Vgx rats were capable of drinking because they exhibited normal salt appetite after PG as measured by drinking of 0.9% NaCl. Transection of both vagal trunks was necessary for these deficits, since drinking was normal in 2 rats with verified unilateral vagotomy. We believe these deficits result from loss of vagal afferent stimuli from the abdominal viscera that are important for the neural integration of thirst-motivated behavior.

738 PAIN-INDUCED STIMULUS BOUND BEHAVIOR IN RATS WITH LATERAL HYPOTHALAMIC LESIONS. Elliott Mufson*, Saul Balagura, and Walter Riss. Biol. Psychology Program, Neurosurgery Dept. and Anat. Dept., Downstate Medical Center, Brooklyn, N.Y. 11203.

Among the characteristics of the so-called "lateral hypothalamic syndrome," rats display aphagia, adipsia and lethargy. In order to evaluate a possible relationship between lethargy and unresponsiveness to food, experimental and control rats were prodded to greater arousal by tail-pinching. Ten experimental male rats sustaining lateral hypothalamic lesions bilaterally were compared with five sham-operated controls. During the period of recovery when experimental rats exhibited aphagia, they were tested by tail pinch (TP) and two types of non-tail pinch (NTP) trials. Type I NTP trials consisted of placing a Lab Chow pellet under the rat's snout for 60 sec. Type II NTP trials consisted of holding the pellet under the rat's snout while its tail was grasped gently for 10 sec. In TP trials, the rat's tail was forcefully pinched for 10 sec. No component of feeding was evident in any rat during NTP trials. During TP trials, experimental animals responded by gnawing at the pellet ($X^2=28.8$; $p<.001$; $df=5$); by contrast, controls did not. The response in experimental animals was strikingly similar to stimulus bound behavior elicited by electrical brain stimulation. The result suggests that electrical stimulation of certain neural systems may disrupt normal processing as does hypothalamic damage, and may introduce the same strong neural activation as does pinching. Both disruption and strong stimulation may be required for the display of stimulus bound behavior.

739 TELENCEPHALIC CONTROL OF FEEDING IN THE RAT. Joseph P. Huston, Bert Siegfried* and Masaaki Shibata*. Institute of Pharmacology, University of Zuerich, 8006 Zuerich, Switzerland.

Feeding can be elicited by single waves of KCl-induced spreading depression (SD) in the cortex, caudate nucleus or hippocampus of rats. Electrical stimulation of these structures elicits post-stimulation feeding also by inducing SD. Recent studies on SD-induced feeding can be summarized as follows: (a) In the absence of food cortical spreading depression (CSD) will induce lever-pressing in satiated animals that were pretrained in a Skinner-box under food reinforcement, indicating that the induced eating has "motivational" properties. (b) SD induced in the frontal cortex induces eating sooner than SD induced in the posterior cortex suggesting that either the frontal pole might be critical for this phenomenon, or the structures along the route of the wave of depression into caudate nucleus. (c) Bilateral electrolytic lesions of amygdala nuclei attenuated but did not completely prevent CSD-induced eating. Unilateral lesions of the amygdala attenuated CSD-induced eating from the ipsilateral cortex significantly more than from the contralateral one, suggesting some role of the amygdala in this phenomenon. (d) Unilateral injections of 6-OHDA into the substantia nigra attenuated CSD-induced feeding significantly more from the ipsilateral hemisphere than from the contralateral one, implicating a possible lateralized role of catecholamines in this phenomenon. (e) Slow-potential recording in the hippocampus and cortex of chronic rats showed that eating elicited by injections of norepinephrine into the hippocampus was not caused by a SD in this structure.

740 The effect on feeding of a variety of nutrients in intact and vagotomized rabbits. Donald Novin and Milan Rezek* Dept. of Psychology, UCLA, Los Angeles, California, and Dept. of Physiology, U. of Manitoba, Winnipeg, Canada.

Vagotomized rabbits, unlike controls, show no suppression of food intake following (5% w/v) glucose infusions. (Physiol. & Behav. 13:3, 1974). These results and others on the effects of vagotomy on 2DG induced food intake (Science 181:858, 1973) suggested the importance of visceral processing of glucose and the innervation of viscera in the short-term regulation of food intake. Duodenal or hepatic-portal infusions of glucose (0.5M), glycerol (0.5M), casein hydrolysate or control saline were made in order to see if other nutrients affect food intake by mechanisms similar to those responsive to glucose. Glucose was effective in suppressing food intake when administered duodenally but not portally whereas the other nutrients were effective in both cases. In general, nocturnal administration of nutrients at a time when food intake was elevated was less effective in suppressing food intkae then comparable infusions diurnally administered. Casein, duodenally administered, was an exception in that its effect was enhanced nocturnally. Vagotomy had little or no effect on the response to casein or glycerol but as previously reported eliminated the suppressive effect of glucose. A dose of atropine methyl nitrate (0.25 mg/kg) was established which in the acute preparation blocked the activation of gastric contractions normally induced by stimulation of the distal end of the cut vagus. This same dose was used to pretreat rabbits prior to glucose or saline infusions. Glucose suppressed food intake as well in atropinized rabbits as in non-atropinized rabbits. This result in combination with the results of surgically vagotomized rabbits suggests that it is the afferent portion of the vagal innervation of visceral organs that is critical to satiety induced by glucose. Other, non-carbohydrate, nutrients must operate through mechanisms other than the activation of vagal afferents and implies a special role for glucose in the short-term regulation of food intake.

741 LIMIT CYCLES IN FEEDING RECEPTOR SENSITIVITY. Elizabeth Omand* and Jacob Zabara. Depts. Physiology, Biophysics and Pharmacology, Temple Univ. and C.O.M., Phila., Pa. and Hebrew Univ-Hadassah Med. Sch., Jerusalem, Israel.

Studies were undertaken to examine 'spontaneous' variation of taste receptors to examine the peripherally-based rhythms entering into the fly (Phormia) feeding cycles. These rhythms are apparently limit cycles whose description can be fundamental to a system analysis of fly feeding. The problem of receptor inconstancy resulting in potential system instability is also investigated.

Single unit recordings were made by placing a micropipette containing a stimulating solution and conducting electrolyte over the tip of a perioral taste hair containing minutely exposed terminals of a few chemosensory cells.

In several phases of experimentally induced feeding/deprivation cycle, receptor responses to standard stimulating solutions showed a regular relationship to the degree of deprivation. Average sugar receptor responses increased about 15 impulses in the first second per 24 hours in animals deprived from emergence. Fed flies had an average receptor response of 9 for all ages tested. When flies had both a feeding and a deprivation period the receptor response corresponded to that of the state prevailing at the time of testing.

With repeated test trials, spanning 1 hour in unfed animals a significant number receptor responses showed a regular and sustained increase. Analysis of individual receptor responses showed that increase or sensitization occurred either gradually or occurred more rapidly during the first few tests. Sensitization was observed with a wide variety of carbohydrate test solutions and with 2 different electrolytes and over a range of concentrations. Sensitization occurred most frequently among receptors with an initially low response and least frequently among highly responsive receptors.

When sensitization was analyzed with respect to the absolute change in response, no relationship could be noted between the initial response magnitude and the degree of sensitization. The amount of increase typically fell between 20 and 40 impulses per second during a testing sequence of about 10 trials spread over 30 minutes. When tests were limited to 20 or 30 minute intervals, responses often increased to a degree comprable to that in with more frequent testing/stimulating.

The degree of sensitization in a given animal was great enough to contribute to altered behavior in an intact specimen. This was true if a summation of a number of receptors was assumed in the CNS over the initial period of receptor discharge, or whether a single receptor was considered for a somewhat longer discharge period. The gain of the system is thus directly linked to changes in receptor sensitivity, which therefore contributes to the magnitude of feeding rhythms.

A clear result of this work is that frequency coding of stimulus intensity is not unvarying for a given stimulus category. We offer the possibility of a centerally generated autogenic function which modifies peripheral sensors with respect to physiological variables, e.g. hunger state, and previous recent stimulation.

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742 DISSOCIATION OF SHOCK-MOTIVATED PASSIVE AVOIDANCE AND ILLNESS INDUCED TASTE AVERSION BY HABENULAR LESIONS. Ralph L. Elkins and Jennifer Jerald*, Augusta VA Hospital, and Stephen H. Hobbs, Augusta College, Augusta, Ga. 30904.

These habenular-lesion experiments are part of a similar series directed at limbic system and related structures. They are designed to evaluate the Garcia and Ervin (Commun. Behav. Biol. 1968, 389) hypothesis that neuroanatomically discrete associative mechanisms subserve the acquisition of illness-induced taste aversions and conditioned avoidance of distinctive environments that have been paired with painful cutaneous shock. Dissociation of these two classes of avoidance responses has been observed following septal lesions and lesions of the anterior portions of the olfactory bulbs. Septal lesions disrupt shock-motivated passive avoidance while leaving intact the acquisition of illness-induced taste aversions (McGowan, Hankins & Garcia, Behav. Biol., 1974, 841) while anterior bulbectomies have opposite disruptive effects (Elkins & Hobbs, Society for Neuroscience, 1974, Abst. 183). Since the habenular nuclei are structures having integral connections with the olfactory tubercule and septum, a similar analysis of their contributions was indicated.

Albino rats were randomized to habenular lesion, sham-operated or normal groups. Stereotaxically-placed electrolytic lesions were produced under Equithesin anesthesia. Sham operations consisted of penetrating the dura without activation of the lesion maker. Following surgical recovery subjects (Ss) were 24 hr. fluid deprived and given a novel .05% solution of sodium saccharin in water. This solution was available for at least 10 min. and for 5 min. after the onset of ingestion. Five min. after saccharin removal randomly designated Ss from each group received either a 25 mg/kg intraperitoneal cyclophosphamide injection (Cytoxan^{R}, Mead-Johnson) or an isotonic saline injection. Aversions were evaluated over 40 days with a two-bottle test that provided access to both the saccharin solution and plain water. Preference scores were calculated to reflect saccharin-solution consumption as a percentage of total daily fluid intake. Normal, sham- and habenular-lesioned Ss given cyclophosphamide developed strong saccharin aversions while saline control Ss showed marked saccharin preferences. Statistical tests confirmed that the three conditioned groups failed to differ over the first five days of preference testing. The only indication of a possible lesion effect consisted of significantly ($p<.05$) accelerated extinction by lesioned Ss relative to sham-operated or normal Ss.

Experiment II replicated Exp. I with these exceptions. The drug dose was reduced to 10 mg/kg to determine if the strong aversions of Exp. I might have masked an initial lesion effect. Also, Ss were trained to avoid a shuttle box compartment that was paired with shock. Cyclophosphamide groups again showed initial equivalent aversions, but lesioned Ss failed to show accelerated extinction or any other differential taste-aversion effect. However, lesioned Ss were deficient in shock-motivated passive avoidance. In this task Ss were placed in one side of a two-compartment shuttle box and allowed to explore freely until they left and reentered the original compartment, at which time they were given scrambled footshock (0.7ma) until they escaped. Reentry latency or failure to reenter within 15 min was recorded. Significantly ($p<.05$) more lesioned Ss (61.5%) reentered the shock compartment than sham-operated (33.3%) or normal Ss (28.6%)

Histological verification revealed that lesions of both experiments produced extensive damage that was for the most part confined to the medial and lateral habenular nuclei. Overall these results are interpreted as supporting the Garcia and Ervin (1968) hypothesis of neuroanatomical diversity of associative mechanisms subserving illness-induced taste aversions and shock-motivated compartment avoidance.

743 EFFECTS OF CLONIDINE ON SELF-STIMULATION, EATING, DRINKING AND BODY TEMPERATURE IN THE RAT. James L. Meyerhoff, Gregory E. Martin* and George F. Koob, Div of Neuropsychiatry, Dept of Neuroendocrinology, Walter Reed Army Institute of Research, Washington, D.C. 20012.

Rats with chronic electrodes in the posterior hypothalamus were placed in experimental chambers with access to three levers, one of which activated a brain stimulation circuit from a constant current source, the other two delivered a 90 mg food pellet or a 0.05 ml drop of water. The rats were allowed continuous access to all levers 24 hours per day on a CRF schedule. Stable baseline rates of self-stimulation (ICSS) were established and then each rat (N=8) was subjected to a series of intraperitoneal (i.p.) injections of saline or clonidine HCl, at doses of 12.5, 25.0, 50, 100, 200 and 400 ug/kg. During the six hours following injection, ICSS increased significantly following doses of 12.5, 25.0 and 50.0 ug/kg, with a maximum increase of 82% at 25 ug/kg. At 400 ug/kg, however, ICSS was significantly decreased by 54%. Food and water intake showed maximum increases of 116% and 80%, respectively at 50 ug/kg and maximum decreases of 57% and 91% respectively at 400 ug/kg. At 200 and 400 ug/kg, the decrease in ICSS and in food intake were reflected in the 24 hour totals, while the acute decrease in water intake was not. In a separate group of animals, rectal or intraperitoneal temperature was measured following the i.p. administration of clonidine in a similar dose range. Core temperature increased slightly at 12.5 ug/kg and decreased precipitously by greater than 2^{o}C and 3^{o}C respectively following 100 and 200 ug/kg doses. At the latter dose, the animals appeared lethargic. The data indicate that the dose-response curve for clonidine is biphasic for these behavioral and physiological parameters. Further, clonidine in excess of 100 ug/kg may produce physiological effects which interfere with performance of a behavioral task.

744 EFFECTS OF UNILATERAL NIGROSTRIATAL BUNDLE LESIONS WITH 6-HYDROXYDOPAMINE ON SELF-STIMULATION FROM THE A9 DOPAMINE CELL GROUP. R. M. Clavier, A. G. Phillips and H. C. Fibiger. Div. Neurol. Sci., Dept. Psychiat., Univ. of B. C., Vancouver, Canada.

This study attempted to determine whether stimulation of the dopamine (DA) containing neurons of the nigrostriatal bundle (NSB) was responsible for intracranial self-stimulation (ICSS) from the area of the A9 DA cell group. After stable ICSS baselines were established via electrodes aimed at the A9 cell group in the substantia nigra, pars compacta, male albino rats were anesthetised (halothane) and given single injections of 6-hydroxydopamine (4 µg/2 ml) via intracranial cannulae aimed at the hypothalamic level of either the ipsilateral or the contralateral NSB. Pretreatment with desipramine (25 mg/kg, i.p.) was given 30 min prior to the lesions to protect noradrenergic neurons from the toxic effect of the 6-hydroxydopamine. Neostriatal DA levels indicated the extent of NSB damage. Extensive (>75%) reductions in neostriatal DA ipsilateral to A9 electrodes resulted in cases of reductions, at times greater than 95%, to below the ICSS criterion of 50 responses per 15 min. Similar lesions of the contralateral NSB never resulted in deficits to below criterion. In both groups, however, average performance rates fell by about 50% after one post-lesion week, but then recovered to about 90% of pre-lesion values after three weeks. By contrast, lesions that resulted in minimal (<17%) ipsilateral NSB damage produced only a 25% ICSS deficit after one week, and rates rose to about 70% above pre-lesion levels after three weeks. From these results we concluded that while unilateral NSB lesions may result in non-specific performance deficits, the ipsilateral NSB may be essential for the maintenance of A9 ICSS.

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745 NEUROPHARMACOLOGICAL CORRELATES OF BRAIN STIMULATION REWARD IN THE NEOSTRIATUM. David A. Carter*, Anthony G. Phillips and Hans C. Fibiger. Dept. of Psychology and Psychiatry, Univ. of British Columbia, Vancouver, Canada, V6T 1W5.

In an attempt to provide additional support for the role of dopamine (DA) in brain stimulation reward (BSR), electrodes were implanted throughout the caudate-putamen (CP). Previous mapping studies of this region of the brain yielded inconclusive evidence of BSR, a finding which posed difficulty for the 'DA hypothesis', as biochemical and histochemical studies have provided evidence of high concentrations of DA in the neostriatum. The results of the present mapping study revealed that 87% of the 59 electrodes located in the CP would support intracranial self-stimulation (ICS), and that rates as high as 1200/15 min could be obtained from sites in the anterior region. This finding is of particular relevance, as it has recently been reported that the highest concentrations of DA are found in the rostral aspects of the neostriatum.

Having established that ICS could be obtained from the CP, a second experiment was conducted to determine the effect of catecholamine agonists and a cholinergic antagonist on BSR in the neostriatum. Animals were tested with low intensity current, and both the d- and l-isomers of amphetamine (1.0 mg/kg) produced comparable facilitation of ICS. Specifically, the d-isomer produced a 197% increase and the l-isomer a 180% increase over saline control scores. These equipotent effects of the amphetamine isomers on ICS in the CP confirm and extend the results obtained at other DA sites such as the substantia nigra pars compacta, and the nucleus accumbens. In contrast to amphetamine, the anticholinergic drug scopolamine (0.5 mg/kg) produced a significant reduction in neostriatal ICS, to 51% of saline control. This suppression may be related to the attenuation of activity in cholinergic interneurons in the CP.

746 SELF-STIMULATION IN DORSAL MIDBRAIN OF THE RAT. R.H.Thalmann*(SPON:John H. Perry) Dept.Cell Biol.,Baylor Col. Med., Houston,TX. 77025.

Extensive testing of the region of the locus coeruleus for reward which is produced by electrical stimulation of brain(self-stimulation or SS), together with pharmacological studies, has led several workers to suggest that locus coeruleus cells are involved in SS. To further examine the validity of this proposition, a total of 75 electrodes has been aimed at the midbrain course of the main ascending projection from locus coeruleus, the dorsal noradrenaline(NA) pathway. Each rat was trained to bar press under conditions arranged such that each response delivered a 25 microampere sine wave 250 milliseconds in duration through the electrode being tested. Electrode tip locations which resulted in bar press rates of at least 100/5minutes were considered to be SS loci. First examined were electrode locations within a 1.25X1.25millimeter square whose medial edge was placed on the midline of coronal sections such that the dorsomedial corner of the square was centered in the cerebral aqueduct. Although SS loci were found in the portion of the square which lay outside the central gray and through which the dorsal NA pathway travels, the proportion of positive placements was low (about 10%). By contrast, about 40% of the electrode placements within the central gray portion of the square yielded SS. SS has not been obtained from loci which immediately surround this square, such as the dorsal part of central gray and bordering tegmental areas, or loci within or just dorsal to the red nucleus. Although these results are probably consistent with involvement of structures in the area of the dorsal NA pathway, they also suggest participation of structures in the ventral portion of central gray. Candidates for such a central gray structure would include the periventricular NA pathway, an alternate pathway for ascending locus coeruleus axons(e.g. Acta physiol.scand.Suppl.412 (1974)1-48).

747 DIRECTIONALITY OF NEUROPHYSIOLOGICAL INTERACTIONS BETWEEN BRAINSTEM AND HYPOTHALAMIC SELF-STIMULATION LOCI IN RATS. Richard J. Bodnar*, Steven J. Ellman, Robert F. Ackermann*, Edward R. Greenblatt*, Solomon S. Steiner* (Dept. Psych., CUNY, New York, N.Y. 10031) and Edgar E. Coons (Dept. Psych., New York Univ., New York, N.Y.).

Ellman et al. (JCPP 88:816, 1975) using sinusoidal stimulation found that locus coeruleus (LC) and hypothalamic (HYP) intracranial self-stimulation (ICSS) response rates are enhanced when the two sites are stimulated simultaneously at threshold intensities as compared to the sum of response rates elicited by each site alone at the same intensities, suggesting neurophysiological interaction between the two sites. Farber et al. (Science, in press) demonstrated that LC lesions reduce or abolish ICSS in HYP sites with nigro- and neo-striatal influences, but do not affect medial forebrain bundle ICSS. Ungerleider and Coons (Science 169:785, 1970) using Deutsch's (JCPP 58:1, 1964) C-T technique found that with bilateral HYP stimulation [C(conditioning) pulse in the left HYP, T (test) pulse in the right HYP or vice versa], a neurophysiological interaction occurs such that refractoriness is eliminated. In order to determine the neurophysiological relationship between brainstem and HYP ICSS sites, twelve rats were stereotaxically implanted with one electrode aimed at the HYP and a second at either the LC or periaqueductal midbrain central gray (PMCG). Each rat was trained to bar-press for monophasic square-wave electrical stimulation; a voltage was chosen which would optimally support ICSS rates at C-T intervals outside the refractory period, but yield operant level responding when the T pulse was omitted. To determine refractoriness for each site, nine C-T intervals, ranging from 0 to 5.0 msec., were randomly presented in each of nine days; refractory period duration for each site ranged between 0.5 and 1.5 msec. The C and T pulses were then split between the two sites at their respective voltages; nine days of C-HYP, T-LC/PMCG and nine days of C-LC/PMCG, T-HYP, alternated in an abba manner, were randonly tested over the nine C-T intervals each day. In nine of twelve animals stimulated in this manner, individual site refractoriness was eliminated. The remaining three animals did not self-stimulate at one electrode site and when either the C or T pulse was delivered to their neutral sites, these animals pressed at only operant levels, as though they were receiving pulses only in their ICSS sites. Of the nine animals in which refractoriness was eliminated, four animals had significantly higher response rates for the C-LC/PMCG, T-HYP combination than for the C-HYP, T-LC/PMCG combination, suggesting that the interaction between LC/PMCG and HYP is predominantly an ascending excitatory influence of the LC/PMCG upon the HYP. In the other five animals in which refractoriness was eliminated, response rates were similar for both combinations. However, the C-HYP, T-LC/PMCG combination generated slightly higher rates, suggesting a weak descending excitatory influence of HYP upon LC/PMCG. These results are discussed with respect to the neuroanatomical placement of electrodes.

748 BEHAVIORAL MEASUREMENT OF ABSOLUTE REFRACTORY PERIODS, RELATIVE REFRACTORY PERIODS, AND SUPERNORMAL PERIODS OF NEURONS MEDIATING SELF-STIMULATION WITH UNEQUAL CURRENT PULSE PAIRS. John S. Yeomans* (SPON: C.R. Gallistel). Univ. Pennsylvania, Philadelphia, Pa. 19104.

Many previous studies have attempted to measure the excitability properties of the stimulated neurons which produce various behaviors (notably self-stimulation) by observing changes in behavior at different pulse-pair spacings (C-T intervals). These studies have not been able to distinguish between the different neuronal properties which may account for the results, i.e., absolute refractory periods, relative refractory periods, supernormal periods, and synaptic interactions.

It is shown in this paper that the contributions of these different properties can be separately and quantitatively measured using C and T pulses of different intensity and using the scaling methods proposed by Yeomans (Physiol.Behav. in press). The supernormal contributions were found to be large, so that the relative refractory contributions were consequently small. The absolute refractory categories having the largest effects on hypothalamic self-stimulation were in the range from 0.5 to 1.2 msec with much smaller contributions at longer intervals. Axonal excitability changes account for the results found more adequately than synaptic changes.

749 MODEL OF AFFECT CODING BY INTRALAMINAR THALAMIC NEURONS: SUPPORTING EVIDENCE. J. J. Keene. Dept. Physiol., Sch. Med., Univ. of Puerto Rico, San Juan, P. R. 00936.

In previous experiments on unanesthetized postcollicular cerveau isolé rats, believed rewarding medial forebrain bundle (MFB) and aversive dorsal midbrain reticular (RET) stimulus trains (0.2 sec, 100 Hz, 0.5 msec cathodal pulses at 600 uA) have elicited prolonged opposite responses lasting seconds and up to a minute in single units anatomically localized in intralaminar thalamus (Brain Res., 64: 211-224) and medial pallidum (Exp. Neurol., in press). For a number of reasons, an affect coding model was proposed in evaluation of the possible behavioral significance of the convergence on single cells of the MFB-elicited inhibition and RET-elicited excitation in intralaminar thalamus and of the inversely opposite medial pallidal responses.

Current data from freely moving, chronically implanted cats confirm the earlier reports of convergence of longlasting MFB and RET effects: mostly inhibition of discharge in cerebral cortex and VA-VL thalamus; excitation or inhibition in other thalamic nuclei; and opposite responses in intralaminar thalamus, which summated or cancelled after simultaneous MFB and RET stimuli.

New results supporting the suggested affect coding by intralaminar units have been: 1) Replication in another species, cat. 2) Replication in an intact brain. 3) Behavioral demonstration of the rewarding and aversive properties of the MFB and RET stimuli, in tests of self-stimulation and escape. 4) Similar current thresholds for the behavioral and suspected affect-related unit responses. 5) Elicitation of affect-related responses with stimulus delivery controlled by the animal during self-stimulation and escape tests. 6) Greatly reduced firing after escape, as if the escape was somehow equivalent to a reward, and if so, as predicted by the suggested affect code. 7) Indications that secondary reinforcers with learned emotional significance elicited responses as the proposed code would predict. 8) During slow wave sleep judged by behavioral and EEG criteria, complete abolition of affect-related unit responsiveness recorded when the animal was judged to be awake by both criteria, as shown by the following mean firing rates (± SEM) in spikes per second and percent changes over five second poststimulus periods, for a single well isolated intralaminar unit:

CONDITION	SPONTANEOUS	MFB STIMULI	RET STIMULI	BOTH MFB & RET
Quiet Awake	4.25 ± .70 (0%)	2.62 ± .46 (-40%)	9.35 ± .98 (+119%)	7.67 ± .95 (+79%)
Slow Sleep	1.50 ± .20 (0%)	1.10 ± .17 (-27%)	1.02 ± .20 (-33%)	0.90 ± .12 (-40%)

These findings support the contention that a significant fraction of the variability of activity of single intralaminar units may pertain to an affective or emotional dimension. This concept, which is more inclusive than reward or aversion alone, was invoked because information regarding rewarding and aversive inputs appeared to summate, or be integrated by intralaminar and medial pallidal units. In addition to confirming the belief that intralaminar thalamic units display pain-related activity, these data 1) raise questions about other thalamic nuclei, such as the posterior nucleus, which have also been so implicated, 2) support the idea that these units also show reward-related activity, and hence 3) provide further verification of the proposed intralaminar affect code. Further experiments are required to verify the code with natural stimuli, both primary and secondary reinforcers, positive and negative. Verification of the code, if it is correct, would provide a first definition of an affective dimension on the single neuron level, which could compliment behavioral techniques already in use, in numerous studies of phenomena related to affect.

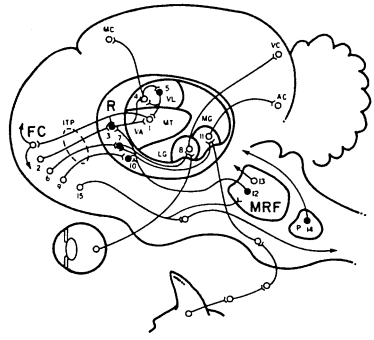
750 THALAMIC RETICULAR NUCLEUS: A SENSORY GATE CONTROLLED BY THE FRONTAL GRANULAR CORTEX AND THE MESENCEPHALIC RETICULAR FORMATION. James E. Skinner and Charles D. Yingling*, Physiology Dept., Baylor Coll. Med., Biology Dept., Rice Univ., and Neurophysiology Dept., Methodist Hospital, Houston, Tx. 77025.

Changes in the degree of EEG synchronization, evoked potential amplitude, and slow potential shifts have been associated with psychological states of attention and arousal in man. These three types of electrocortical activities have been shown to be regulated in animals by the mesencephalic reticular formation (MRF) (Rossi and Zanchetti, 1957) and the mediothalamic-frontocortical (MT-FC) system (Skinner and Lindsley, 1973). The MRF and MT-FC systems converge upon the thalamic reticular nucleus (R), a structure that projects systematically into the thalamus (Scheibel and Scheibel, 1966). These results will suggest that these convergent influences regulate an inhibitory gate in R during control of the three types of cortical activity associated with attention and arousal.

I. EEG synchronization: pathways mediating phasic thalamic inhibition. The recruiting response was used as a model for synchronous activity. Analysis of unit responses in R to medial thalamic stimulation suggests that projections from R mediate the prolonged IPSPs recorded in thalamic nuclei during recruiting responses (Purpura and Cohen, 1962). The EEG desynchronization following MRF stimulation appears to result from inhibition of R by the MRF; R units are silenced for an interval (20-30 sec) similar to the period of desynchronization that follows a brief MRF stimulus. Cryogenic blockade of the inferior thalamic peduncle (ITP), a pathway which interconnects the thalamus with the frontal cortex, abolishes R responses to medial thalamic stimuli as well as cortical and thalamic recruiting responses, findings which suggest that R units are driven by a mediothalamic-frontocortical-R pathway.

II. Slow potentials: cortical shifts reflect dual regulation of R. MRF stimulation produces prolonged negative shifts in frontal cortex and parallel positive shifts in R that are independent of the integrity of the ITP; similar shifts accompany orienting responses to novel stimuli. The positive shifts in R reflect inhibition of R units. Slow potentials of identical polarity but briefer time course are evoked by the warning stimulus in a conditioned expectancy paradigm; these shifts, however, are abolished in both frontal cortex and R by ITP blockade. Again, independent regulation of R by MRF and frontal cortex is demonstrated.

III. Evoked potentials: selective vs. general regulation of thalamic relay nuclei. Brief stimulation of areas of R adjacent to thalamic relay nuclei abolishes the cortical evoked potentials in only the sensory modality served by the adjacent thalamic nucleus. We propose that MRF stimuli, or MRF activation during general arousal, enhances evoked potentials in all modalities by generalized inhibition of R. Selective enhancement by partial ITP blockade results from inactivation of specific regions of R; this mechanism could underlie selective disinhibition of thalamic transmission by the frontal cortex during focussing of attention.



The diagram illustrates the connections between the MRF, the MT-FC system, and R, showing the convergent control of R by both these systems. This model explains the different regulation of cortical input during general arousal and selective attention. Excitatory neurons are shown in white, inhibitory neurons are in black.

751 THE EFFECT OF DESMETHYLIMIPRAMINE ON SELF STIMULATION RATES DURING EXPOSURE TO HYPOXIA. Zoltan Annau. Dept. Environmental Medicine, Johns Hopkins Univ., Baltimore, Md. 21205

Sixteen rats were implanted with chronic electrodes in the lateral hypothalamus. After recovery from surgery they were trained to self stimulate and placed in chambers equipped with three levers. One lever activated the brain stimulation circuit, one a food dispenser and the third a water delivery device. All responses were on continuous reinforcement schedules. After stable baselines had been established, 8 animals were exposed to 8% oxygen for 48 hours. A profound depression of responding on all three levers lasted for the entire 48 hour period. The other 8 rats were treated once a day with 5 mg/kg of desmethylimipramine starting two days prior to the hypoxic exposure and continuing through the exposure. Self stimulation rates were not affected by the drug before the 8% oxygen exposure. During the 48 hour exposure to 8% oxygen self stimulation, rates remained normal while food and water intake stopped. The results suggest that some of the deleterious effects of severe hypoxia can be prevented by altering brain catecholamine levels.

752 BRAINSTEM NEURONS THAT FIRE SELECTIVELY TO A CONDITIONED STIMULUS FOR SHOCK. Robert P. Vertes* and Neal E. Miller. Rockefeller Univ.,New York,NY 10021.

Extracellular potentials were recorded from single brainstem reticular neurons in freely moving rats to determine if they showed a conditioned response to electric foot shock. Eighty-one rats were pretrained over a 10-day period to respond discriminatively to three cues:(a) a cue signaling the delivery of an aversive electric foot shock,(b) a cue signaling the delivery of water, to be used as a control for the arousal effect of a signal for a biologically significant event, and (c) a neutral stimulus not followed by any unconditioned stimulus. After pretraining, under Nembutal anesthesia a small micromanipulator capable of moving a fine-wire microelectrode in a dorsal-ventral direction through the brain was cemented onto the skull of each rat (Vertes,Electroenceph. clin. Neurophysiol. 1975, 38, 681). After recovery from surgery, the rats were presented with the same sequences of CS-US pairings that were used during pretraining while, at the same time, the activity of single brainstem neurons was monitored. The results were the following: In the 81 rats recorded from, 10 neurons were found (one in each of 10 rats) that responded by an increase in activity to a CS paired with foot shock but not to a neutral CS nor to a CS signaling the delivery of water to a thirsty rat. Nine of these neurons showing a conditioned response to stimuli signaling shock (CR-S cells) were clustered in the region of nucleus pontis caudalis, the other CR-S cell was localized to nucleus gigantocellularis. The possibility that the increase in firing to the CS for shock was an unconditioned response to the specific stimulus used as this CS was controlled for. No correlation was observed between the activity of CR-S cells and gross movements of the rat. The firing of these neurons during avoidance learning is being studied. (Supported by USPHS grant MH 13189 and by a grant from the Grant Foundation)

753 DURATION REGULATION OF INTRACRANIAL SELF-STIMULATION: THE EFFECTS OF STIMULATION FREQUENCY, ELECTRODE SITE, AND CATECHOLAMINERGIC DRUGS.
Michael A. Edwards* and Harry M. Sinnamon. Psych. Dept., Wesleyan Univ., Middletown, CT 06457.

Intracranial self-stimulation was studied using a shuttle-box in which rats controlled durations of stimulation (ON times) and intervals between stimulations (OFF times). ON times and OFF times decreased with increasing stimulation frequency (40-160 Hz), whereas the percentage of total time spent ON remained fairly constant. ON times decreased at a faster rate with frequency for ventral tegmental sites (VTA; N=6) and sites on the dorsolateral periphery of the lateral hypothalamus (LHP; N=5) compared to lateral hypothalamic sites (LH; N=6). The LHP group also had lower mean ON times than the VTA group. No group differences were seen for mean OFF times or for the OFF time frequency function.

Within sessions for individual sites, successive pairs of ON and OFF times usually had a slight positive correlation. However, within anatomical groups, ON and OFF times averaged over sessions correlated negatively among LH sites, positively among LHP sites, and were uncorrelated among VTA sites.

The effects of two doses of several drugs were evaluated at each site. The dopaminergic antagonist, haloperidol, and the α-adrenergic antagonist, phentolamine, increased OFF times but had effects on ON times that depended on the electrode site. The β-adrenergic blocker, propranolol, usually had no effect. A few sites were also tested with two catecholamine depleting drugs. Evidence suggests a general involvement of dopamine and noradrenaline in the regulation of OFF times.

The differential effects of stimulation frequency, electrode site, and drugs on ON and OFF times indicate independent mechanisms underlie these self-stimulation measures.

754 NEURAL RESPONSES TO SELECTED FEATURES OF ELECTRIC FIELDS IN THE MIDBRAIN OF THE CATFISH. Eric I. Knudsen. Dept. Neurosciences, Sch. Med., UCSD, La Jolla, Ca. 92037

Catfish can recognize and locate prey using electrical cues alone, as well as orient in weak uniform fields. Features of an electric field that could serve to identify its nature and source are its frequency, spatial distribution, gradient magnitude, gradient vector direction and movement. The torus semicircularis, being a high major central projection area of the electrosensory system, was studied for its responsiveness with respect to these various electric field properties.

A unifying aspect of torus organization is its systematic progression of field vector direction preferences from 0° (measured in clockwise rotation of the field vector from head positive = 0°) in rostral torus, to 180° in caudal torus (Fig. 1). Rotating the field vector out of the best response direction causes a decrease in response amplitude and an increase in response latency. Underlying this progression is a somatotopic representation of contralateral receptive fields in the torus. The frequency responses of torus units to uniform, sine wave fields lie between .01 and 30 Hz (Fig. 2). The units can be categorized into three types based on their frequency responses: #1) low frequency (<2 Hz), #2) high frequency (>2 Hz), and #3) broad band sensitive units (1 to 10 Hz). These units exhibit preferred movement speeds to movements of a dipole field of between .1 and 15 cm/sec; the optimal speed being predictable from their frequency response characteristics. Approximately 40% of each type of unit show directional selectivity to such a field movement in the horizontal plane, while the majority responds to all movement directions provided the stimulus enters the unit's receptive field. Frequency response types and directionally selective units seem evenly distributed throughout the torus, however the electric field vector preference, as a dominant organizational parameter, suggests that the analysis of the spatial distribution of electric fields is a possible important function of the torus semicircularis.

Fig. 1

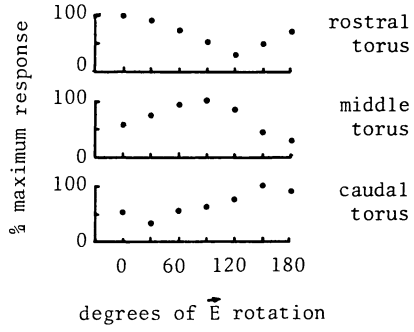
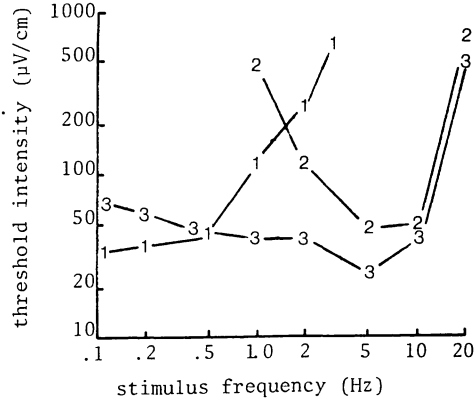


Fig. 2



755 ACTIVATION OF THE ELECTRIC ORGAN MOTOR NUCLEUS BY STIMULATION IN THE REGION OF THE TORUS SEMICIRCULARIS IN ASTROSCOPUS y-GRAECUM.

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The stargazer, Astroscopus y-graecum, a marine teleost, is considered a strongly electric fish. The stargazer normally buries itself in the sand, and observation of its feeding behavior in aquaria indicate that it discharges its electric organ while feeding on small fish which swim above its head. The electric organ is derived from several extraocular muscles. It is innervated by a greatly enlarged oculomotor nucleus which fills the medial mesencephalic tegmentum throughout its entire anterior-posterior extent. A large, short latency antidromic field and intracellular antidromic action potentials can be recorded throughout the nucleus in response to electrical stimulation of the third nerve. Monopolar stimulation in or just ventral to the torus semicircularis with current levels less than 75 μA elicits a large negative field, intracellularly recorded excitatory postsynaptic potentials (EPSPs) and orthodromically triggered spikes throughout the electromotor nucleus. The potentials are best elicited by paired pulses and show additional temporal summation in response to short trains. In contrast, stimulation of the optic nerves or the tectal surface does not elicit fields or intracellular signs of activation of the electromotor nucleus although optic nerve stimuli do produce a negative field in the superficial tectum. The torus semicircularis has been shown to receive afferents from the lateral line lobes. This combined with the observation that stimulation of the torus semicircularis produces strong activation of the electromotor nucleus raises the possibility that the lateral line system or some derivative may be involved in reflex activation of the stargazer electric organ. (Supported by NIH Grant NS 11255, Training Grant NS 05743 and the Moody Fdn. of Galveston.)

756 SENSORY PROPERTIES OF SINGLE UNITS IN CAUDAL RAPHE NUCLEI

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Lesion studies (1) have shown that nuclei of the caudal raphe receive afferent fibers from the spinal cord. This preliminary study was designed to investigate the nature of this input and to observe its effects on the firing pattern of single units in the raphe. Extracellular microelectrode recordings were made in chloralose anesthesized or decerebrate cats immobilized with gallamine triethiodide. Mechanical, thermal and electrical stimuli were effective in discharging units, many of which were multimodal with respect to applied stimuli. Electrical stimulation of the sural nerve evoked a 15-25 msec latency burst of 25 msec duration followed by an inhibitory gap of 50-150 msec which was succeeded by a prolonged discharge. No inhibitory interaction between stimuli applied to contralateral limbs was observed, nor could any somatotopic organization be discerned.

The data presented here are discussed with regard to the suggestion by Taber et al (2) that these midline nuclei might be functional constituents of the ascending activating system. (Supported by NSF Grant GB 44325 and NIGMS Training Grant: 1 TO 2 GM 05010-01 MARC).

Ref.

1. Brodal, A., Walberg, F., and Taber, E. 1960, J. Comp. Neurol 114: 261-279.
2. Taber, E., Brodal, A. and Walberg F. 1960, J. Comp. Neurol 114: 162-187.

757 DISCHARGE PATTERN IN GIGANTOCELLULAR PONTINE NEURONS: COMPARISON OF WAKING AND REM SLEEP. Dennis J. McGinty and *Mary K. Fairbanks, Veterans Administration Hospital, Sepulveda, California 91343.

Giant neurons of the pontine paramedian reticular field of the cat exhibit accelerated unit discharge during REM sleep as compared with slow wave sleep (SWS) and quiet waking (W) and have been proposed to be elements in a neuronal system controlling REM. We compared discharge patterns in REM and various W behaviors to assess the specificity of REM-associated activity. Well-isolated units were recorded during spontaneous and elicited head movements, head restraint, quiet W, SWS and REM. Of 47 pontine neuronal units, 24 were found to exhibit little spike discharge in quiet W or SWS, but accelerated discharge during REM. Each cell also exhibited phasic discharge bursts in relation to specific W head movements such as head extension or turning to the left or right; discharge was suppressed by head restraint. Two minute spike train samples were analyzed to determine burst-pause patterns, inter-spike interval, and auto-correlation functions. Most components of frequency distributions of burst durations, interburst intervals, and intraburst spike frequencies were found to contain similar relative frequencies during waking samples with head movement and during REM sleep, with only long burst (> 720 msec) and short interburst intervals (≤ 200 msec) showing small but significantly higher frequencies in REM. The W and REM interval histograms exhibited similar distribution, although interquartile points were located at shorter intervals during REM in 75% of the cases, indicating faster overall spike activity. Auto-correlation functions failed to reveal short-term periodicity in either W or REM spike trains. These data show that pontine cells exhibiting phasic bursting in relation to voluntary movements show very similar bursting patterns in REM sleep. REM spike trains in these cells may reflect normally-organized but intense voluntary movement.

758 TIME COURSE OF LOCUS COERULEUS UNIT ACTIVITY DURING DESYNCHRONIZED SLEEP EPISODES. R.W. McCarley and J.A. Hobson, Harvard Med. School, Boston, USA

We have proposed that the dynamic and rhythmic alternations of the sleep cycle result from interactions of two populations of cells. One population, the giant cells of the pontine gigantocellular tegmental field, (FTG) reaches maximum discharge activity during the desynchronized phase of sleep and is postulated to control and coordinate events during this phase. Averaged discharge activity of cells from this region during the entire sleep cycle is well approximated by curves derived from our model of sleep cycle control and its Lotka-Volterra equations.

We now examine the time course of activity during, before, and after desynchronized sleep episodes of a second population of cells in the region of the nucleus locus coeruleus and subcoeruleus (LC) to determine if this time course is compatible with our postulate of LC cells' terminating desynchronized sleep episodes through FTG cell inhibition. An increasing inhibitory influence of LC cells should be reflected in an increase in LC discharge activity before the end of the desynchronized sleep period. In our model LC theoretical curves have in common a low point at desynchronized sleep onset and a maximum at the end of desynchronized sleep (see Fig. 1a for an example). The model defines the beginning and end of a desynchronized sleep episode as the points of crossing equilibrium values by the FTG population activity.

The experimental data is derived from 10 cells histologically localized to the LC of cats and with lowest discharge rates in desynchronized sleep. For each cell we measured the discharge rates during 6 equal-duration epochs: 4 quartiles of desynchronized sleep, and the synchronized sleep period preceding and the waking period following desynchronized sleep.

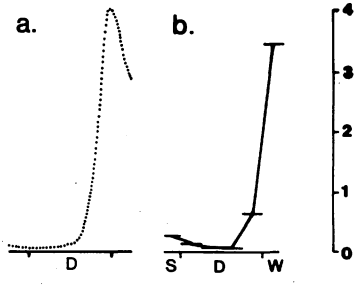


Fig. 1.a. Example of theoretical time course of LC activity during desynchronized sleep (D). b. Time course of actual LC cell discharge rates. S=synchronized sleep, W=waking. Ordinate is impulses/sec.

Fig. 1b displays the geometric mean discharge rates for the pool of 10 LC cells. Note that the discharge levels in synchronized sleep and the first 3 quartiles of desynchronized sleep are very low compared with those in waking, as predicted by the model curves, although the actual data shows discharge rates to be slightly lower in the third, rather than in the first quartile of desynchronized sleep. The data curve also shows the clear rise in LC discharge rates during the last quarter of desynchronized sleep, as is predicted by the model. This important feature was present in each of the 10 records ($p < .001$, binomial test). During the last quarter of desynchronized sleep discharge rates are more than twice as great as during preceding epochs. Continuous, entire cycle recording of LC cells will afford the opportunity for more precise and more extensive point-by-point comparisons of model and actual time curves of activity, but the present analysis of pooled LC data shows results consistent with our reciprocal interaction model of sleep cycle control.

Supported by NIMH.

759 SINGLE UNIT RECORDING IN THE MIDBRAIN OF RATS DURING SHOCK-ELICITED DEFENSIVE BOXING BEHAVIOR. Fred J. Pond*, Harry M. Sinnamon and David B. Adams, Dept. Psychol., Wesleyan U., Middletown, Ct. 06457.

Activity of single neurons was recorded extracellularly from the midbrain of rats during defensive boxing behavior and appropriate control manipulations. The defensive boxing behavior was elicited by foot-shock or occurred spontaneously as a result of prior foot-shock presentations. The animal's behavior was recorded on videotape along with a display of unit activity to provide a more detailed analysis of the data.

Cells were found in the midbrain reticular formation just lateral to the central gray which displayed maximum firing rates during defensive boxing behavior. These cells also fired to a limited extent to some of the control manipulations, particularly movement of the opaque partition normally separating the animals. The most consistent response to a neutral stimulus involved tactile stimulation of the body contralateral to the recording site (particularly vibrissae stimulation). These cells fired phasically during defensive boxing and were correlated to some degree with either the approach or paw-strike of the opponent animal or the resultant response of the recording animal. Shock presentations sensitized the response of these cells both during defensive boxing behavior and control manipulations. Lesions of this midbrain reticular region along with the central gray have been shown to abolish defensive boxing behavior in rats (Edwards and Adams, Physiol.Behav., 13:113,1974).

Cells located in other midbrain areas, such as deep layers of the tectum and the red nucleus also responded during defensive boxing behavior. However, these cells were correlated with body movements and showed similar response patterns to control manipulations eliciting the same movements.

(Supported by NIMH Grant MH 21675-02)

760 SOMATOMOTOR AND VISCEROMOTOR EFFECTS OF LESIONS OR STIMULATION OF ROSTRAL OR CAUDAL ZONES OF THE VENTRAL MESENCEPHALIC CENTRAL GRAY AND ADJACENT TEGMENTUM OF THE RAT. S. T. Huprich* and J. C. Liebeskind, Dept. Psychol., UCLA, Los Angeles, Ca. 90024.

As part of a broader study of the functional organization of the rat mesencephalon (Huprich, Doctoral Diss., UCLA, 1975), the effects of electrolytic lesions or, in the sodium-pentobarbitalized animal, electrical stimulation of this area were measured. Lesions of a rostral zone of the ventral mesencephalic central gray (MCG) and adjacent tegmentum resulted in hypothermia, reduced righting speed, reduced aversive response to handling, hypophagia, hypodipsia, and impaired passive avoidance. Stimulation of this zone with 60 ∿ currents (ca. 50 to 500 μA) resulted in blood pressure increases. Lesions of a caudal zone of the ventral MCG and adjacent tegmentum resulted in mydriasis, hyperthermia, increased nonspecific locomotor activity in the open field, increased aversive response to handling, hyperphagia, hyperdipsia, and enhanced passive avoidance. Stimulation of this zone with lower intensity 60 ∿ currents (up to ca. 250 μA) resulted in blood pressure decreases; stimulation of this zone with higher intensity 60 ∿ currents (ca. 250 to 500 μA) would at some points reverse the blood pressure response from a decrease to an increase, while at other points would continue to result in blood pressure decreases.

For stimulation of the rostral zone, where stimulation elicited a hindlimb flexor EMG discharge as well as a blood pressure increase, the dorsoventral locus at which the maximum visceromotor response could be elicited lay dorsal to the dorsoventral locus at which the maximum somatomotor response could be elicited, with the form[illegible] at the level of the ventral MCG and the latter about 0.5 mm further ventral at the level of the ventral border of the MCG.

In other work (Soc. Neurosci., 1973), we have concluded that the alar plate derivatives of the mesencephalon are involved with sensory function. It is concluded in the present work that the basal plate derivatives of the mesencephalon, including importantly the ventral MCG and adjacent tegmentum, are involved with response organization, with a rostral zone involved in the organization of a myotonic-sympathetic response tendency, involving an increase in tonus in skeletal muscle and a sympathetic response bias in cardiac and smooth muscle and underlying both aversive and appetitive responses, and a caudal zone involved in the organization of an amyotonic-parasympathetic response tendency, involving a decrease in tonus in skeletal muscle and a parasympathetic response bias in cardiac and smooth muscle and underlying both post-aversive and post-appetitive responses. It is further concluded, for at least the rostral zone, that, similar to the organization of the basal plate derivatives of the spinal cord and medulla, there occurs in the dorsoventral dimension of the mesencephalic basal plate derivatives a visceral efferent column dorsal to a somatic efferent column. Finally, it is suggested that activity in the rostral and caudal zones of the mesencephalic basal plate derivatives, along with that in homologous zones at other levels of the neuraxis, determines the organism's overall somatovisceral response tendency, a response continuum extending from myotonic-sympathetic at one pole to amyotonic-parasympathetic at the other pole, and underlying aversive, appetitive, post-aversive, and post-appetitive responses. (Supported by USPHS Grant NS07628)

761 MESENCEPHALIC RETICULAR ACTIVATION REVERSES THE DISRUPTIVE EFFECTS OF LOW FREQUENCY MEDIAL THALAMIC STIMULATION ON DELAYED ALTERNATION BEHAVIOR. G.L.King* and J.E.Skinner (SPON: R.P.Borda). The Methodist Hospital and Baylor College of Medicine, Houston, Tx. 77025.

Cortical recruiting responses (RR) produced by 8 Hz medial thalamic (MT) stimuli are known to be reduced or abolished by high frequency stimulation of the mesencephalic reticular formation (MRF). Purpura (1970) has suggested that RRs produce "functional deafferentation" of the cortex due to inhibition of thalamic neurons. Therefore, MRF activation should reverse the effects on behavioral performance produced by the "deafferentation" elicited by MT stimulation. To test this hypothesis, stimulation electrodes were placed in the medial thalamus (n. centralis medialis) and MRF of cats, and after recovery, the animals were trained to perform the delayed alternation bar press task (single alternation with correction). This task was selected because it is sensitive to ablations of the frontal granular cortex, the cortical region critically involved in the generation of RRs. The results showed that increasing levels of either continuous or intermittent MT stimulation produced a monotonic decrease in delayed alternation performance (ie., increased errors). MT pulses combined with a constant level of MRF stimulation resulted in marked improvement of behavioral performance at all levels of MT stimulation tested. Stimulation (8 Hz) of n. ventralis lateralis (VL), which produces augmenting responses, did not disrupt delayed alternation behavior, except at very high intensities. The resultant tremor produced by this high intensity VL stimulation, however, was also reversed by MRF stimulation.

762 HIGHER MENTAL FUNCTIONS AND ELECTRICALLY INDUCED SEIZURES. Iver F. Small, Fred Malloy*, Victor Milstein, and Joyce G. Small. Indiana Univ. School of Medicine & Larue D. Carter Memorial Hospital, 1315 W. 10th Street., Indianapolis, IN 46202 USA

Repeated grand mal seizures are known to impair cognitive functions whether attacks occur as a result of a convulsive disorder or other brain lesions or are induced for therapeutically intended reasons. However in patients with severe mental illnesses such as psychotic depression and schizophrenia, the most striking effects of serially induced seizures are improvements in thinking, mood and behavior, even though some transient deficits in memory and other mental functions may also occur. From a research standpoint, objective measurement of cognitive and memory functions before, during and after seizures induced in patients with relatively normal central nervous systems affords an opportunity to investigate the mechanisms whereby seizures may produce both clinical benefit and functional impairment.

In the present study 25 patients were evaluated before, during and following a series of grand mal seizures induced with bitemporal electrical stimulation. Measurements included the Weschler Adult Intelligence Scale and the Halstead-Reitan-Wepman Neuropsychological Test Battery which was administered prior to treatment, 24 hours after the fifth seizure and shortly after termination of the series. EEG and neurophysiological studies were accomplished at the same times.

The results indicated improvement in task performance over time, particularly in the Weschler performance subtests. There were decrements in some verbal test scores after the fifth seizure, but these were transient despite increments of more seizures. Other psychological and neurophysiological data suggested that non-dominant more than dominant hemispheric functions were altered with repeated seizures. Comparative data from drug treated patients will also be described.

Plasticity, Memory and Learning

763 PLASTICITY IN THE HIPPOCAMPAL EXPLANT. B. E. Alger* and T. J. Teyler. Dept. Psy. & Soc. Rel., Harvard Univ., Cambridge, 02138.

The hippocampal explant of rat evidenced long-term alterations (up to 6 hrs) following several exposures to a low-frequency (10-15/sec) tetanus. Extracellular population potentials were recorded in the cell body and dendritic regions of CA1 in response to stimulation of the Schaffer collaterals. The synaptic alteration increased gradually as a function of tetanus exposures with the population EPSP increasing an average of 50% and the population spike potential increasing an average of 500%. Equal stimulation density control experiments (0.7-1/sec) and low-frequency control experiments (1/5 sec), while displaying some response variability, failed to alter synaptic efficacy as was seen following low-frequency tetanus.

Short-term alterations in hippocampal explant activity were studied using an habituation paradigm. Neither CA3 nor CA1 evidenced response related decrements to various frequencies of stimulation (from 1/10 sec to 15/sec) of the mossy fiber pathway of the Schaffer collaterals, respectively. The dentate gyrus showed marked response decrements, most evident from 1/sec to 15/sec, to repeated stimulation of the perforant path. Response recovery was rapid (<30 sec) for all frequencies studied. There were no long term effects.

764 THE INFLUENCE OF NEONATAL ANDROGEN ON THE DEVELOPMENT OF CORTICAL FUNCTION IN THE RHESUS MONKEY. Patricia S. Goldman and Roger Mac Brown, Lab. of Psychology and Psychopathology, NIMH, Bethesda, Maryland 20014.

Performance on a cognitive task known to require the integrity of the orbital prefrontal cortex in the adult was studied in normal 75-day old male and female rhesus monkeys and in 75-day old females administered a total of 38.5 mg/kg of testosterone proprionate in multiple injections from birth through 46 days of postnatal development. Normal males performed at significantly higher levels than did normal females. Postnatal injections of testosterone proprionate produced slight but permanent alterations in the morphology of the external genitalia and, on the cognitive task, enhanced the performance of the androgenized females to the level of normal males. Additional groups of males and androgenized females were subjected to bilateral orbital prefrontal lesions at 50 days of age and tested at 75 days of age. These groups exhibited deficits on the cognitive task whereas nontreated females that were given identical lesions and tested at the same ages did not differ from unoperated females. These results, together with previous findings in older monkeys (Goldman, et al., Science, 186: 540, 1974), provide evidence that (1) the orbital prefrontal cortex develops at different rates in males and females; (2) androgen accelerates the development of orbital prefrontal function, suggesting that gonadal hormones may play an inductive role in the differentiation of cortical mechanisms similar to that played in the differentiation of hypothalamic mechanisms; and (3) the critical period for the influence of androgen on functions of the central nervous system in primates extends into postnatal life.

765 PLASTICITY OF RETINOTECTAL PROJECTIONS AFTER PARTIAL LESIONS OF RETINA IN NEWBORN SYRIAN HAMSTERS. D.O. Frost & G.E. Schneider. Dept. Psych., MIT, Cambridge, Mass. 02139

In newborn Syrian hamsters, unilateral partial lesions of the retina were produced alone or in combination with one, two, or all of the following procedures: enucleation of the opposite eye, bilateral ablation of the posterior neocortex, transection of the contralateral brachium of the superior colliculus. When the animals were young adults, the central projections of one or both eyes were traced using the Fink-Heimer or autoradiographic techniques.

Following retinal lesions alone, the zone in the superior colliculus (SC) corresponding to the lesion in the opposite eye was, in some cases, totally or partially filled by the axons of ganglion cells in intact regions of the contralateral retina; in other cases, most or all of this area was completely without contralateral retinal innervation. The filling in of the denervated zone is not critically constrained by anomalous projections to that region from the intact eye or visual cortex; in animals subjected to the destruction of those afferent systems by the procedures described above the variability in the projections of the lesioned eyes was not altered. Plasticity of the retinotectal projection was also not affected by the transection of the optic-tract axons entering SC at the time of the retinal lesion.

Filling in of the denervated zone was found to be correlated with the number of surviving ganglion cells in the contralateral retina. This finding implies that the ratio of ganglion cell number/SC area may be the critical parameter constraining the filling in of denervated tissue. These data indicate that the exact locus of termination of optic axons is influenced by their interaction with each other, although polarity cues may also be present within SC.

766 RETINAL AFFERENTS RELABEL THE TECTUM IN GOLDFISH. John T. Schmidt. Biophysics Res. Div. and Dept. Zoology, Univ. Michigan, Ann Arbor 48104

The theory that neurones carry labels which specify their synaptic connections is widely accepted. However, the labels must change during plastic reorganizations. Specifically, when the optic nerve of a goldfish was crushed and half of the retina removed, the remaining fibers first grew back to their original tectal sites, but later (after 100 days or more) spread retinotopically over the denervated half tectum (Figure 1). When this expanded half retinal projection was interrupted by optic nerve crush, and the projection mapped 50 days later, the expanded projection was reestablished. This implies that the change of labels survived optic nerve crush, but does not distinguish between relabelling of retinal fibers or tectal cells. The next set of experiments does.

In the normal animal, deflection of the optic nerve of one eye onto its ipsilateral tectum results in a positionally normal map. In the experimental animal, after the half retina had formed an expanded map on the contralateral tectum, its optic fibers were deflected onto the normal ipsilateral tectum (Figure 2A). After 40 days it regenerated a normal (not expanded) map on the appropriate rostral half tectum. This indicates that retinal labels had not changed during the earlier expansion. Similarly, the normal eye of an experimental animal was deflected to innervate the tectum with the expanded half retinal projection (Figure 2B). After 40 days both eyes' projections were again mapped. The normal eye's projection was in register with the expanded half retinal projection. Those areas of the normal retina corresponding to the missing areas of the half retina were not represented. Apparently the tectal labels sought by fibers from these retinal areas were not present. This abnormal projection from a normal eye indicates that tectal labels had been changed by the fibers of the half retinal eye. (This work was supported by PHS grants EY-00168(to S.S.Easter) and GM-1355, and by a Parke-Davis Travel Award.)

Figure 1:

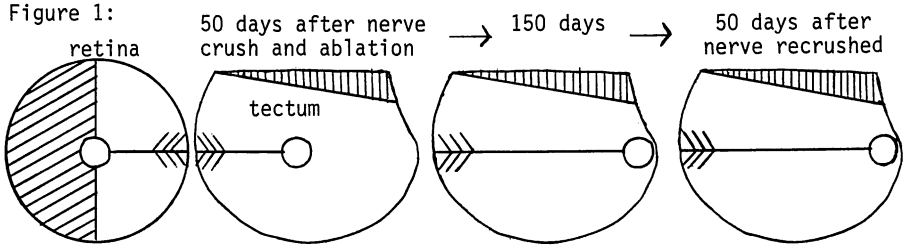


Figure 2A:

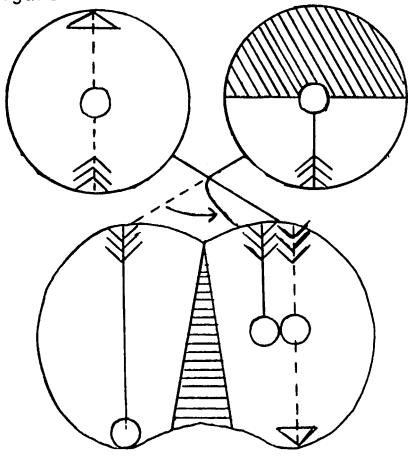
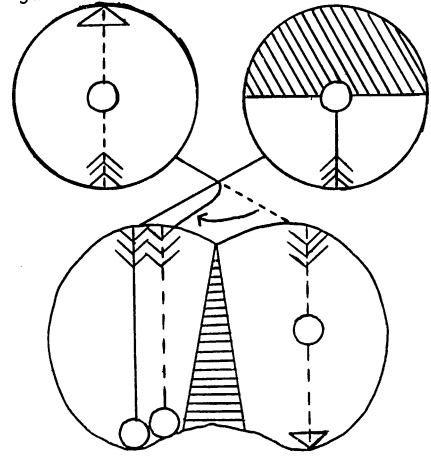


Figure 2B:



767 POLYRIBOSOMES IN VISUAL CORTEX: EFFECTS OF DEPRIVATION AND ANESTHESIA.
Simon LeVay, Dept. Neurobiol., Harvard Med. Sch., Boston, Ma. 02115

Ribosomes of cortical neurons are normally aggregated into polysomes, suggesting active protein synthesis. Neurons in area 17 were examined electronmicroscopically for presence or absence of polysomes, in 21 normal kittens and cats and 26 animals whose eyelids were sutured together at ten days of age. Until the end of the first month of life all cells contained polysomes in both groups of animals. Normal animals in the second and third months showed variable numbers of neurons lacking polysomes ('ribolytic cells') in layer IV, but at later ages ribolytic cells were found rarely if at all. In binocularly deprived kittens, ribolytic cells appeared at the beginning of the second month, formed half the layer IV cell population by 7 weeks, and remained at this level until at least 7 months. Monocularly deprived kittens possessed fewer ribolytic cells, and none in the monocular segment of cortex innervated from the open eye. In the ribolytic cells polysomal disaggregation was remarkably complete, affecting both membrane-bound and cytoplasmic ribosomes. The rough ER was disorganized but other organelles appeared normal. A previous EM study of Golgi-impregnated neurons allowed the two main neuron types of the fourth layer, the spiny and spine-free stellate cells, to be distinguished on the basis of synapse distribution on the perikarya. The spiny stellate cells, which form the principal target of the visual afferents, were the cells susceptible to ribosomal disaggregation; spine-free cells were immune. Pentobarbital anesthesia (30 mg/kg) caused a complete reaggregation of ribosomes in ribolytic cells within 4 hours. The results indicate that at the start of the second month ribosomal aggregation in spiny stellate cells becomes dependent on normal visual input. This dependence may be related to the poor responsiveness of cortical neurons in binocularly deprived kittens and their failure to complete the process of synaptogenesis.

768 ELECTRONMICROSCOPY OF AXONAL GROWTH CONES, AFTER CNS AND PNS LESIONS. Jean Jew and Terence H. Williams. Dept. Anat., Univ. of Iowa, Iowa City, Iowa, 52242.

Earlier studies of growth cones have taken two directions; work on nerve cells in culture, and observations from embryonic and postnatal animals. Although ultrastructural evidence for remodelling in the normal (Sotelo and Palay,Lab. Invest., 25:653-667, 1971) and lesioned (Raisman, Brain Res., 14:25-48, 1969) CNS has been presented, information about growth cones in the mature mammalian nervous system after lesions is inadequate. We are concerned with the practical question of developing criteria for recognizing central regeneration at the fine structural level, and from this point of view, growth cones could provide a much needed parameter in measuring postlesion plasticity in the CNS.

Three different experiments will be reported. Structures having the characteristics of growth cones were observed after lesions, in mature rats and cats, of (a) the vagosympathetic trunk, (b) the septal region, and (c) after redirecting the vagosympathetic trunk into the CNS environment. In experiments (a) and (b), post-lesion survival periods were 3-7 days. In (c), the redirected peripheral nerve trunk was allowed to regenerate within the CNS environment for 3 months.

Degenerative, dystrophic, and growth cone elements were seen in all cases. Growth cones were abundant in the cut vagosympathetic trunk as early as 3 days after the lesion. Axons redirected into the hypothalamus maintained their seeking mode, characterized by many growth cones, even after 3 months. Although many growth cone variants were noted, resemblance was closer to those described for cultured material (Birks et al., J. Neurocytol.,1:311-340, 1973 ; Bunge, J. Cell Biol., 56:713-735) than some described in embryonic and postnatal animals (del Cerro, J. Comp. Neur., 157:245-280). Contents included growth tubules and vesicles, autophagic vacuoles, and few to many mitochondria.

Profiles with fundamentally similar features were seen, although less abundantly, close to the edges of septal and hypothalamic lesions. These central axonal growth cones will be described and compared with examples from the PNS.

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769 ON THE REGENERATION OF CNS AXONS. Carl W. Cotman, Hilary Hyatt*, Pamela Kaups* and Gary Lynch. Dept. Psychobiol., Univ. of Ca., Irvine, Ca 92664

It is generally believed that most mammalian CNS neurons have little, if any, capacity to regenerate damaged axons. We have examined the regenerative capacity of axons in the rat brain after freezing lesions. The fimbria, which interconnects the septum and hippocampus, is an ideal system to study CNS axonal regeneration. For a short distance, the fimbria is isolated from neuropil and consists of a bundle of unbranched, primarily myelinated, axons. We damaged a small section of the fimbria by placing a thin metal probe cooled in liquid nitrogen on a part of it where it runs as a free nerve. Gel foam was inserted into the operated area and the animal allowed to recover for various times. At short time periods the area appeared extensively damaged and the distal processes of axons showed signs of degeneration. Acetylcholinesterase activity, which originates in the septum was markedly decreased in the hippocampus and hippocampal efferents to the opposite hippocampus showed signs of terminal degeneration. No branches, offshoots or other abnormal characteristics beyond the damage itself was observed. At long survival times (60-180 days) we observed a much different situation. In most animals, near the area of the initial freezing lesion, we observed a sizable offshoot or branch which now interconnected the fimbria and the caudate. The branch (or in some cases branches) consisted of numerous myelinated axons and fibrous astrocytes. The simplest explanation of these findings is that the freezing lesion induced the growth of axons across the ventricular space of the brain. The gel foam probably served as a solid support system. Our results indicate a remarkable capacity of CNS neurons to grow in response to damage and form a new and long-lasting fiber plexus. (Supported by NIMH grant MH 19691.)

770 REVERSIBLE REDUCED ACCUMULATION OF TYROSINE HYDROXYLASE ENZYME PROTEIN IN CENTRAL DOPAMINERGIC NEURONS IN RESPONSE TO AXONAL INJURY. D.J. Reis, G. Gilad, and T.H. Joh. Dept. Neurol., Cornell Univ. Med. Coll., New York, 10021.

We have recently observed (Ross et al) that the retrograde reaction in central noradrenergic neurons is characterized by a prolonged (2-3 wk) and reversible reduction in the activities and accumulation of the catecholamine synthesizing enzymes tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH). We have sought to establish if the reversible reduction in a neurotransmitter synthesizing enzyme may also characterize the retrograde reaction in central dopaminergic (DA) neurons. Axons of the nigrostriatal pathway were electrolytically transected unilaterally in adult rats either in the lateral hypothalamus or within caudate nucleus (CN). Thereafter, the ipsilateral CN (containing DA axons)and the ipsilateral SN (containing DA cell bodies) were removed and assayed for TH, DBH and amino acid decarboxylase (AADC), and compared with tissues from the contralateral side and unoperated controls. Changes in amounts of enzyme protein were determined by immunotitration with a specific antibody to TH. Several rats from each group were perfused with formaldehyde for histological verification of lesion size and examination of SN neurons for evidence of cell death. Unilateral electrolytic lesions of the nigrostriatal tract in the lateral hypothalamus resulted in marked changes in the activity of TH distal and proximal to the lesion ipsilaterally. In CN, the activity and amount of TH and activity of AADC fell rapidly between 24-48h to approximately 20% of control where it remained indefinitely. In SN, TH activity increased almost twofold by 24-48h. TH activity and amount gradually fell to approximately 40% of control by 14d where it remained permanently depressed for up to 60d. By 30d cell death in SN was evident. The activity of DBH and AADC in the SN were unaffected indicating that changes in TH reflected a retrograde response in DA neurons and not in noradrenergic terminals. The permanent reduction of TH in the SN appeared to reflect a loss of neurons in the pars compacta. Small lesions of CN resulted in a reversible reduction of the activity and accumulation of TH in SN. TH activity fell gradually to 60% of control by 7d, remained at this level for 2-3w and recovered. No cell loss was observed in SN. Large lesions of CN, however, resulted in a persistent 40-50% reduction in TH activity in SN and cell death.

The present study demonstrates that lesions transecting axons of the nigrostriatal system will initiate profound changes in the activities and amounts of the enzyme TH, distal and proximal to the lesion. In confirmation of others (McGeer and McGeer), lesions result in a rapid loss of TH and DDC in CN concurrent with anterograde degeneration. The retrograde reaction in SN is characterized by a reduced accumulation of TH and may be reversible depending upon the proximity of the lesion to the cell body and/or the amount of damage to the total axonal field. We conclude the retrograde reaction in DA neurons, like noradrenergic neurons, is characterized by a reduced accumulation of enyzme synthesizing neurotransmitter and may be reversible depending upon proximity of lesion to the cell body. A reduced accumulation of enzymes subserving neurotransmitter biosynthesis may be a specific biochemical concomitant of the retrograde reaction in intrinsic dopaminergic as well as noradrenergic neurons of the CNS and may be reversible. Since the retrograde reaction in many other neuronal systems is characterized by an increase in net protein synthesis, the demonstration of a reduction in the amount of TH, a specific protein produced by dopaminergic and noradrenergic neurons, suggests that during the retrograde response the neuron may reorder its priorities for protein biosynthesis favoring accumulation of proteins required for regeneration of axonal membranes at the expense of those required for function of the cell in neurotransmission. Supported by NIH Grants NS 06911 and MH 24285.

771 INTERSTIMULUS INTERVAL EFFECTS ON A CLASSICAL CONDITIONING PARADIGM UTILIZING THE HINDLIMB FLEXION REFLEX OF ACUTE SPINAL CAT. Michael M. Patterson. Dept. Physiol.,Kirksville College of Osteopathic Medicine, Kirksville, Mo. 63501.

In recent studies, we have shown pairing of two shock stimuli delivered to the hind leg of an unanesthetized, spinalized cat to produce increases in the response to the initial, or conditioned stimulus (CS). Presented in an unpaired sequence, the stimuli produced no increases and the unconditioned stimulus (UCS) produced no long lasting sensitization which could account for the results. It was also shown that the pairing effect was dependent upon stimulus order; pairing increases appearing only if CS preceded UCS. These results indicated that the isolated spinal flexion reflex is capable of undergoing modification with a classical conditioning paradigm which is analogous to modifications seen with classical conditioning of intact organisms. The present study evaluated the effects of interstimulus interval (ISI) on the spinal classical conditioning paradigm. Utilizing ISIs from 0 to 4000 msec., groups of animals were given acquisition and extinction trials. The results indicated that at CS-UCS intervals from 0 to 125 msec., little or no response alterations occurred. Intervals from 250 to 1000 msec. produced response increases, maximal at 250 msec., while at 2000 and 4000 msec. ISIs, no changes were noted. The results are in agreement with the majority of classical conditioning ISI studies involving intact organisms, further suggesting that the spinal flexion reflex may be an appropriate model in which to study associative processes.

772 PACEMAKER CHANGES AS A PRINCIPAL BASIS FOR INSECT LEG LEARNING. M. Woollacott, T. Tosney* and G. Hoyle. Biology Department, University of Oregon, Eugene, OR 97403.

A tonically-firing locust motoneuron (AAdC) that is involved in leg-position learning was trained to fire at rates more than twice normal (up-learning) or less than half normal (down-learning). The changes, which were stable for periods exceeding two hours, were achieved by an on-line computer that delivered shocks to the tarsus correlated with statistically-significant trends in mean firing rate. A rate trend reverses when shocks are correlated with it in 71% of animals tested. The neuron has an intrinsic pacemaker rate whose initial value was determined by first injecting high Mg^{++} saline around the integrating region, then replacing normal saline and subjecting the preparation to up or down learning. The firing rate was monitored via muscle excitatory junctional potentials; synaptic activity and motoneuron properties were determined by an intracellular electrode placed in the soma. During up-learning (N=9) the pacemaker rate changed from 9.9 ± 5.2 to 15.6 ± 6.3 (1.52x) when the rate in saline changed from 8.6 ± 2.4 to 21.4 ± 2.0 (2.49x). During down-learning (N=7) the pacemaker rate fell from 16.7 ± 14.0 to 6.2 ± 5.4 (2.7x) compared with 17.6 ± 9.7 to 10.2 ± 5.4 (1.7x) in saline. Motoneuron pacemaker rate changes accounted for more than half the learning change in 87% of the experiments. They were associated with shifts in membrane potential and input resistance. The balance of frequency shift may be accounted for by altered probability that individual synaptic potentials cause impulses, or by shifts in the mean frequency of synaptic potentials. In three experiments in which learning occurred there was no change in pacemaker rate; we attribute the learning in these to pacemaker changes in antecedent interneurons. This research was supported by an award from the Sloan Foundation.

773 A CURARIFORM RECEPTOR AS SUBSTRATE FOR MECHANICAL STIMULUS TRANSDUCTION AND HABITUATION IN Stentor coeruleus. David C. Wood, and Charles S. See. Dept. Psych., Psychobio. Prgm., U. of Pittsburgh, Pa. 15260.

The trumpet-shaped protozoan, Stentor coeruleus, is being employed as a model system for the study of stimulus transduction and the molecular basis of habituation. These organisms contract in an all-or-none fashion in response to suprathreshold mechanical, photic and electrical stimuli. Microelectrode recordings indicate that mechanical and photic stimuli elicit receptor potentials which, in turn, initiate all-or-none propagated action potentials. An action potential triggers each contraction.

The probability that an animal will respond to a given mechanical stimulus decreases progressively as the stimulus is applied repeatedly, i.e., the animal habituates. Concurrently, the amplitude of the receptor potential generated by the mechanical stimulus decrements progressively. Thus, in stentor, an alteration in the mechanism involved in transducing the mechanical stimulus into a receptor potential appears to be instrumental in the habituation process. For this reason a search was initiated for molecules, presumably located in the plasma membrane, involved in the transduction process.

Of 34 drugs tested including α and β adrenergic blocking agents, muscarinic blocking agents, local anesthetics, sedatives, etc., only drugs categorized as nicotinic cholinergic blocking agents (decamethonium, d-tubocurarine, succinylcholine, gallamine and TEA) markedly reduce the animal's sensitivity to mechanical stimuli. The desensitizing effect of these drugs is specific to mechanical stimuli; sensitivity to light and electrical thresholds are not affected by these nicotinic agents. However α-bungarotoxin, the α toxin of Naja, acetylcholine and carbachol do not alter the animal's response to mechanical stimuli.

The molecular site of action of these nicotinic blocking agents was studied by characterizing the binding of C^{14}-dimethyl-d-tubocurarine to stentor. It was found that labelled d-tubocurarine is competitively displaced from its binding sites by unlabelled d-tubocurarine, gallamine and decamethonium in that order of effectiveness, whereas acetylcholine and neostigmine were only very weak competitors. This pattern of competition is also the pattern of inhibition of mechanical stimulus sensitivity by these agents. The curariform receptor was localized to the plasma membrane of the cell or to immediately adjacent structures such as the pellicle or pigment granules by autoradiography with C^{14}-d-tubocurarine and H^3-decamethonium. A distinct pattern of exposure is observed in the autoradiograms indicating that curariform receptors are to be found in those areas of the cell surface pigmented strips and not in the clear strips. From these studies it was concluded that the curariform receptors are involved in mechanical stimulus transduction but that they are not cholinergic receptors.

Since the curariform receptors appear to be involved in mechanical stimulus transduction and since habituation appears to occur because of an alteration in mechanical stimulus transduction, the ability of habituated and control animals to bind curare was studied. Differences between the saturation curves for these two groups suggest that the equilibrium constant of the curare-curariform receptor binding reaction is changed as a result of habituation. This suggests that habituation in stentor is associated with an alteration in molecular conformation at the site of stimulus transduction.

774 ACTIVITY OF INFEROTEMPORAL UNITS IN A VISUAL MEMORY TASK.

Joaquin M. Fuster and Richard H. Bauer. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, Calif. 90024.

Anatomical and electrophysiological studies in the monkey have identified the inferior temporal cortex as a substrate for visual information processing beyond the geniculostriate system. Psychological evidence from lesion studies has implicated inferotemporal regions in the neural mechanisms of visual discrimination and visual memory. By combined application of behavioral and microelectrode methods, the present research explores the possible involvement of inferotemporal cortex in visual short-term memory.

Rhesus monkeys were trained to perform a delayed matching-to-sample task requiring the perception and temporary retention of color information. A trial is initiated by presentation of the sample stimulus, either red or green illumination of a translucid circular button in the center of a panel. Upon seeing the colored light, the animal presses the button, thus turning the sample off and initiating an automatically timed delay. At the end of delay, both colors appear simultaneously on two buttons situated in the lower part of the panel. The animal is then rewarded for pressing the lower button that matches the sample. The color on each button is randomly changed between trials.

Fully trained animals were surgically prepared for extracellular microelectrode recording of single-unit activity during performance of the task. In recording sessions the monkey's head was fixed and the sample stimulus appeared at eye-level subtending a visual angle of 11^{o}. Reaction time to the sample averaged about 1 sec. Delays of 16 sec. were generally used. The results reported here are based on the study of 150 units in the anterior inferotemporal cortex of two monkeys. The units were located in the cortex of the middle temporal gyrus and posterior bank of the superior temporal sulcus.

All units were spontaneously active. A majority of them (about two-thirds) were excited by presentation of one or both sample colors. This excitatory response had a minimum latency of about 60 msec. and usually consisted of a brief augmentation of discharge confined to the period when the sample was on. Some units were excited by the two colors but showed a preponderant reaction to red or to green, while others were excited by one color only. A minority (about one-tenth) were briefly inhibited by one or both colors.

Average firing frequency was higher during the delay than during intertrial periods in approximately one-half of all units investigated. In the other half, delay frequency was the same as between trials or, occasionally, slightly lower. Often the firing level of a given unit in the delay was significantly higher after one sample color than after the other. In some units this color-related difference in firing during the delay reflected a similar difference in initial reaction to the sample.

The observed differences in unit-discharge related to sample-color suggest that some inferotemporal neurons are engaged in the coding and retention of color information.

(Supported by NIH grant MH-25082 and NSF grant GB-41867.)

775 OPERANT CONTROLLED CENTRAL EVOKED POTENTIALS IN UNRESTRAINED ANIMALS. B. E. Hetzler, J. P. Rosenfeld*, Paul Birkel*, Northwestern University, Evanston, Ill., A.P. Rudell, State University of New York Downstate Medical Center, Brooklyn, N. Y.

Cats and rats were trained to increase and decrease field potential components evoked by central stimuli. In cats, the shocks were applied to the lateral geniculates and the potentials recorded in striate cortex. In 2 groups of rats, the potentials were applied at 2 respective loci, the optic chiasm and the nucleus subcoeruleus of the pontine reticular formation. The conditioned potentials evoked by chiasm stimulation were recorded at striate cortex. The conditioned potentials evoked by nucleus subcoeruleus stimulation were recorded in nucleus intercollicularis of the midbrain reticular formation. All rats were run in total darkness and were stimulated at random intervals. Non-timelocked body and limb movements were monitored throughout conditioning in rats. Also studied was the effect of conditioning the late (presumably arousal-related) visual component on measures of cortical desynchronization. The changes seen in evoked potentials due to conditioning were in all cases typically well-localized to the pre-specified component. In cats, systematic head movements were associated with conditioning. In rats, timelocked movements were obviated with random intertrial intervals and the other rat movement data indicated that the neural conditioning is probably a novel operant involving an output performance system independent of familiar skeletal muscular activity.

776 ACTH-ANALOGUES ON MOTOR BEHAVIOR AND VISUAL EVOKED RESPONSES IN RATS. O. L. Wolthuis and D. de Wied. Med. Biol. Lab. TNO, Rijswijk and Dept. of Pharmacology, State University, Utrecht, The Netherlands.

It has been demonstrated that ACTH-analogues, devoid of corticotrophic activity, are involved in acquisition and extinction of conditioned avoidance behavior in rats. These peptides delay (7-l-phe $ACTH_{4-10}$) or facilitate (7-d-phe $ACTH_{4-10}$) extinction of conditioned avoidance responses in rats (de Wied, The Neurosciences, 3rd study progr., 653, 1974). These phenomena point at specific effects on the CNS. Recently published (Brain Res. 69, 361, 1974) and yet unpublished results by Urban et al suggest that $ACTH_{4-10}$ causes an elevated excitability in the hippocampal theta generating system. Hippocampal theta is considered to be an EEG concomitant of vigilance and both theta and increased vigilance are associated with suppression of visual evoked response (VER) afterdischarges (Fleming and Bigler, Physiol. Behav. 13, 757, 1974). Therefore the effects of both peptides on the VER in area 17 of the rat were measured. In addition spontaneous motor behavior was analysed to assess whether more generalised arousal occurred. The results show that the latencies of all VER components remain unchanged, as did the amplitudes of the primary VER. Measured at a wide variety of light intensities, however, the amplitudes of the VER afterdischarges were significantly and very similarly diminished by both peptides, the effects of 7-l-phe $ACTH_{4-10}$ being somewhat stronger than those of 7-d-phe $ACTH_{4-10}$. These results support the notion that these peptides have an effect on a CNS vigilance regulating system, yet do not explain the opposite behavioral effects of the two related peptides. The effects seem specific, since spontaneous motor behavior (as indices of changes in generalised arousal) is unaffected by these peptides.

777 AUTONOMIC NERVOUS SYSTEM CONTROL OF THE CARDIAC CONDITIONAL RATE RESPONSE IN THE RHESUS MONKEY. W.N. Schoenfeld, R.M. Kadden,* and P.R. Bindler*. Queens College of the CUNY, and FDR VA Hospital, Montrose, N.Y. 10548.

Pavlov's delay conditioning procedure, in which a conditional stimulus (CS) is presented and is terminated together with an unconditional stimulus (UCS), was applied to rhesus monkey subjects. In our case, CS was a visual stimulus, and UCS was an aversive electric shock. The conditional response (CR) which developed from training in which CS and UCS were paired, was a biphasic one in which a cardiac rate increase was followed by a decrease. When activity of the beta-adrenergic receptors of the sympathetic nervous system was blocked by propranolol hydrochloride, the CR's initial rate increase was eliminated or reduced, but the subsequent heart rate decrease was spared for the most part. On the other hand, block of parasympathetic nervous activity by atropine sulfate left the initial rate increase largely intact but eliminated or reduced the subsequent descending portion of the CR. Injection of both substances together eliminated the whole CR. In addition, a number of other drugs were employed which share the common action of "blocking" either the sympathetic or parasympathetic nervous systems. It was found that those drugs which affect the sympathetic nervous system also affect the initial accelerative phase of the cardiac CR, while those which affect the parasympathetic system also affect the decelerative phase of the CR, and those drugs which affect both types of innervation eliminate both parts of the biphasic CR. In view of these results, it is suggested that a significant input is made into the initial acceleratory wing of the biphasic cardiac CR by sympathetic nervous activity, and into the subsequent deceleratory wing by parasympathetic activity.

778 INFANT'S ELECTROPHYSIOLOGICAL RESPONSE IN A CLASSICAL CONDITIONING PARADIGM. Martin J. Hofmann*, Bernard Z. Karmel and Michael Lester*. Dept. Psychology, University of Connecticut, Storrs, Conn. 06268.

Infants, 14 weeks old (N=8), were tested using a Pavlovian conditioning paradigm consisting of preconditioning, conditioning and extinction trials. The brain electrical potential was recorded from occipital (O1, O2), parietal (P3, P4) and temporal (T3, T4) regions of the scalp. A steady state 2-sec. compound visual and auditory stimulus was used as the CS while a similar compound stimulus lasting 4 secs. square-wave modulated at 6 Hz was used as the UCS. During conditioning a negative voltage shift (Mean = 14uv) prior to the UCS as compared with the voltage prior to the CS was found in the occipital but not in the parietal or temporal regions. During preconditioning no evidence of this shift was found in 5 infants who rendered artifact-free analyzable data. Trials during extinction could not be analyzed due to movement artifacts. A negative shift was recorded also from the occipital region of control Ss (N=3) who were presented random pairings of varying durations of the CS and UCS. Thus, stimulus sensitization cannot be discounted. However, stimulus sensitization would not account for the absence of this negative shift during preconditioning in the experimental Ss when just the CS was given alone.

The negative shift during conditioning may relate to the contingent negative variation (CNV) which correlates with expectancy or anticipation in older subjects. An interaction of sensitization and CNV effects could explain effects found for control Ss since for a majority of trials the UCS was paired with the CS on a partial reinforcement schedule and since no evidence of a negative shift appeared in preconditioning with the CS for the experimental Ss. These results provide the first evidence that the infant as young as 14 weeks can form expectances for which electrophysiological correlates can be obtained.

779 ON THE SEARCH OF THE NEURONAL CIRCUITRY RESPONSIBLE OF MOTOR CONDITIONED RESPONSES. H. Sancho-Ugalde*, B. Prieto-Gómez* and H. Brust-Carmona. Dept. Fisiol., Fac. de Medicina, Univ. Nacl. Autónoma de México, México 20, D.F.

Nowadays, it is generally accepted that motor conditioned responses depend of a neuronal circuitry. Thus, we decided to search for the structures involved and the way in which they participate. It was postulated that the necessary information to press a lever reaches the caudate nucleus (CN) through the medial thalamic nuclei (MTh), and that the excitability of CN neurones is influenced by septal nuclei (S).

We studied in cats the rate of lever pressing (LP) when a light was "on" (reinforced by 0.5 ml of milk) and not to press when the light was "off" (not reinforced). A daily session consisted of 12 series of light on (1 min) and light off (20 sec). After three sessions electrolytic lesions in MTh, or S, were performed. Cats were tested for 20 sessions after the lesion. Afterwards, the acquisition of a passive avoidance response (PAR) was tested.

Large bilateral thalamic lesions (CM, MD, PV) decreased the lever pressing rate which was restablished at a lower level after a few days. Smaller lesions did not affect the responses. In contrast, anterior septal nuclei lesions increased the lever pressing rate in both situations. After the posterior septal lesions the lever pressing decreased when the light was off. These results compared with those of intact cats were statistically (t student test) different at the level of 0.05. All cats acquired the PAR. The results suggest that the anterior septal nuclei have inhibitory actions, which seem to be tonically active while the posterior part has a facilitatory action related to the obtention of reinforcement. The results of lesions in the thalamic nuclei suggest that the information which is necessary to respond (LP or PAR) does not course only through those nonspecific thalamic nuclei.

780 AUDITORY DISCRIMINATION OF THE CLASSICALLY CONDITIONED EYEBLINK RESPONSE IN CHRONIC DECEREBRATE CATS. Robert Norman, Jennifer Buchwald, and Jaime Villablanca. Dept. Physiology, BRI, Mental Retardation Research Ctr., UCLA Med. School, Los Angeles, 90024.

In a continuing investigation of the behavioral capabilities of the truncated mammalian brain, we have studied the acquisition of a classically conditioned eye-blink response in chronic decerebrate cats. Using sterile surgical techniques, the brainstem was transected at the midcollicular level by aspiration. Completeness of the lesions was confirmed visually during surgery and at the end of the experiment by histological examination of the lesioned area. Eleven animals have been studied to date and various degrees of conditioned performance have been obtained in nine of these. The conditioning paradigm was a 350 ms tone CS coterminus with a 1 ms shock pulse US delivered to the eyelid. Reflex and conditioned responses were monitored by EMG recordings from the orbicularis oculi. Intermittent training was carried out until responses to the CS began to appear at three to four weeks post-operatively. Thereafter 100 trials per day were given. The conditioned response was characterized by more than a 100 ms latency, by its appearance to the reinforced CS^+ but not to a neutral tone (CS^-) of equal intensity and duration, and by its absence prior to conditioning, its absence during random presentations of CS^+ and US and by its disappearance during extinction. Conditioned performance was less stable than in normal cats, although at times CS^+ response levels of 80%, with CS^- response levels of 20%, persisted over blocks of 25 to 50 trials. These data affirm and extend an increasing body of evidence that the brainstem reticular formation is capable of supporting the acquisition and retention of simple learned behaviors. (Supported by USPHS Grant NS-05437).

781 THE STORAGE CAPACITY AND RECALL CAPABILITIES IN A STATISTICAL THEORY OF MEMORY. Gordon L. Shaw and W. A. Little*, Physics Depart., Univ. of California, Irvine, Ca. 92664; Physics Dept., Stanford Univ., Stanford, Ca. 94305.

We developed (Little, Shaw, Behavioral Biol. 14, 1975) a theory of short, intermediate and long term memory of a neural network incorporating the known statistical nature of chemical transmission at the synapses. Correlated pre- and post-synaptic facilitation (related to Hebb's Hypothesis) on three time scales are crucial to the model. Considerable facilitation is needed on a short time scale both for establishing short term memory (active persistent firing pattern for the order of a sec) and the recall of intermediate and long term memory (latent capability for a pattern to be re-excited). Longer lasting residual facilitation and plastic changes (of the same nature as the short term changes) provide the mechanism for imprinting of the intermediate and long term memory. We had speculated that the storage capacity was related to the number of synapses rather than the much smaller number of neurons. We have now examined the storage capacity of the network and the access to this memory (or recall capabilities) in our theory. The results of both computer simulation and analytic treatment will be presented. (Supported by the Research Corporation.)

782 EVOKED POTENTIAL CORRELATES OF SEMANTIC INFORMATION PROCESSING. Robert W. Thatcher. Dept. Psychiat., Brain Res. Labs., New York Med. Coll., New York, N.Y. 10029

Averaged evoked potentials (AEPs) were recorded from 11 scalp derivations in human subjects (N=10) viewing sequences of computer generated displays. A trial involved presenting a variable number of random dot displays (controls) followed by a word (first word) followed by another variable number of random dot displays (inter-test interval displays, ITIs) followed by a second word. There were 36 different first words and 12 different second words per session. The second words formed 3 different semantic relationships with respect to the first words. For 1/3 of the trials the second words were synonyms, for 1/3 of the trials they were antonyms and for 1/3 of the trials the second words were neutral. Each subject was run on 4 sessions and semantic relationships within a session were unpredictable and counterbalanced. All displays were foveal, equated for averaged luminance, 20 msec in duration and presented at a repetition frequency of 1/sec. Analyses of AEPs revealed statistically significant differences between word AEPs and random dot AEPs and between first word AEPs and second word AEPs. All subjects exhibited a significant enhancement of late positive components to synonyms and antonyms in comparison to the neutral condition. The differences were most pronounced in occipital, parietal and temporal derivations. Hemispheric amplitude and morphology asymmetries were also noted.

783 ENHANCED RETENTION OF ROUGH-SMOOTH DISCRIMINATION FOLLOWING OVERTRAINING OR SERIAL LESIONS OF SS CORTEX IN RATS. D. Simons*, S. Finger, and A. Baldinger*. Washington University, St. Louis, Missouri 63130.

At the 1974 Neuroscience Meetings we reported that lesions of the somatosensory cortices, whether produced in one or two stages, resulted in severe deficits in retention of a ridge-smooth discrimination in rats. It was hypothesized that capacity differences among the lesion groups may have been obscured by the use of a very difficult discrimination for animals with relatively limited previous training. We now have replicated the earlier study using an easier discrimination (rough-smooth). The new data can be summarized as follows: (1) Animals with successive unilateral lesions of the SS cortices with 4 wks between surgeries do not differ from sham operates in relearning the task; (2) Performance after serial lesions is not further enhanced with interoperative training; (3) Animals with one-stage lesions show deficits relative to sham operated rats and to rats with serial lesions; and (4) Rats given retraining to criterion ("over-training") prior to one-stage lesions do not show deficits.

All of the control animals and many of the rats that had relearned the discrimination in the absence of SS cortex then sustained lesions anterior and posterior to the SS areas. This surgery minimally affected the control animal performance but resulted in retention deficits in most rats that previously had SS lesions. Animals that previously had one-stage and two-stage lesions did not differ from each other in this phase of the experiment.

The data thus show that serial surgery and preoperative overtraining may minimize retention deficits under some conditions. The results further imply that non-SS cortex may be involved in relearning after SS cortical damage.

784 EFFECTS OF ENRICHED EXPERIENCE ON RECOVERY OF RATS FROM CORTICAL LESIONS: PROBLEM-SOLVING SCORES AND BRAIN MEASURES. Mark R. Rosenzweig, Bruno E. Will*, Edward L. Bennett. Dept. Psychology and Laboratory of Chemical Biodynamics, University of California, Berkeley, 94720.

A series of experiments investigated how performance of rats in the Hebb-Williams maze was affected by lesions of occipital cortex and by subsequent experience in enriched (EC) or impoverished (IC) environments. Lesions or sham operations were made on the day of birth in 2 experiments, on day 30 in 2 experiments, and on day 120 in one experiment. Assignment to differential environments lasted 60 days in 4 experiments and 40 days in one. In one experiment EC was given for only 2 hr/day for 60 days. In each experiment, both differential experience and brain lesions had significant effects on maze scores; that is, EC overcame in part the effects of lesions. This effect was achieved with shorter periods of EC than in the few previous experiments of this kind. One of the 30-day-lesion experiments had a companion experiment in which animals were sacrificed at the end of the 60-day EC-IC period for measurements of brain weights and RNA and DNA. The environmental treatments and the lesions both had significant effects on weights of cerebral cortical samples and on the RNA/DNA ratios of certain brain regions. Excluding the region of the lesion from consideration, lesioning significantly decreased the cortical/subcortical weight ratio, whereas environmental enrichment significantly increased it. Dimensions of the cerebral hemispheres were measured in the two experiments with neonatal lesions. Length and width of the hemispheres were found to be significantly decreased by lesions; among the lesioned rats, cerebral dimensions were increased by enriched experience.

(Supported by NIMH grant MH26327 and in part by ERDA; Dr. Will received a fellowship from the French government.)

785 RECOVERY OF LOCOMOTION FOLLOWING CORTICAL LESIONS IN RATS: A CINEMATOGRAPHIC ANALYSIS. A. Gentile*, A. Nieburgs*, W. Schmelzer*, and D. G. Stein. Dept. of Psychology, Clark University, Worcester, Mass. 01610.

In prior experimentation, marked impairment in locomotion of rats was produced specifically by lesions of medial parietal cortex and was not evident following removal of frontal or lateral parietal cortical areas. The present study investigated the effects of sequential removals of this medial parietal area and examined the nature of recovery following one-stage and two-stage lesions. Rats were trained preoperatively to traverse a narrow elevated runway. Cortical removals were made in one-stage or serially with a five week interval between the two-stage lesions. All animals with lesions and sham-operated controls were tested seven weeks after initial surgical intervention. Postoperatively, both one and two-stage animals were impaired in comparison to controls. However, two-stage animals, although more impaired than one-stage subjects during initial postoperative testing, demonstrated more rapid recovery to preoperative performance levels. Response substitution has been suggested as one mechanism underlying recovery following brain damage. Thus a detailed analysis of locomotion after recovery in surgically treated animals was undertaken. High speed photography had been used to record locomotion on the elevated runway during pre and postoperative training. Frame by frame analysis of film data was then used to quantify various characteristics of the movement patterns. Postoperative deviations in response strategies were evident; however, for many movement measures, recovery of the brain damaged animals, especially those with two-stage lesions, were quite similar to controls.

786 EVIDENCE FOR CORTICAL REORGANIZATION AFTER SERIAL LESIONS OF THE VISUAL CORTEX. S. W. Scheff*. (SPON: D. L. Glanzman). Dept. Psychobiology, UC Irvine, California 92664.

Multiple stage (MS) and single stage (SS) ablations of the visual cortex were performed on rats trained to discriminate black and white. Following bilateral SS removal of the visual cortex (sham operation-SS removal or SS removal-sham operation), rats required as many trials to relearn the task as required originally (no savings), regardless of the fact that some training was given between the operations. Following MS bilateral lesions of the visual cortex (unilateral-unilateral removal) rats show no savings in relearning if no training is given in the interval between the two operations, but show positive savings if some training is given during the two unilateral ablations.

Interpretations of reorganization/recovery of function following visual cortex lesions typically stress subcortical mechanisms. While this interpretation may be accurate for SS lesioned animals and MS animals with no inter-operative-training (IOT), reorganization of function may occur cortically in MS animals given appropriate IOT. The role of cortical reorganization was examined by exploring the effects of SS and MS lesions of the visual cortex given in conjunction with removal of cortex either contiguous (C) or non-contiguous (NC) with the visual cortex lesions, and by mapping the C cortex in SS and MS groups with visual evoked potentials.

MS+C lesion animals show a deficit in a second retention problem while MS+NC animals show a savings. SS animals which relearn the habit, show a positive savings after C or NC lesions. Electrophysiological data indicates a possible reorganization of function for animals with positive savings on the first retention task with quantitatively and qualitatively different potentials evoked in the contiguous cortex.

787 FUNCTIONAL CAPACITIES OF RAT SENSORIMOTOR CORTEX

Blair H. Turner, Dept. Anat., Sch. Med., Howard Univ., Wash., D.C. 20059

Intact rats were tested with a variety of measures to assess simple placing and hopping reflexes, reaction to touch and painful stimuli, and use of voluntary musculature. In addition, they were trained to localize very light tactile stimuli on all parts of the body surface. A variety of large and small aspiration lesions were made in what is thought to be sensorimotor cortex. Rats were tested postoperatively up to a year, and results are given only for behavioral effects which had stabilized and were long lasting. Rats with large sensorimotor cortex lesions were unable to localize tactile stimuli anywhere on the contralateral side of the body, and contralateral proprioceptive and visual placing reflexes were absent. This condition lasted for the life of the animal. Small lesions produced more limited behavioral effects, depending upon their placement. Lesions 1-2 mm lateral to the midline at the level of the bregma resulted in a permanent inability to localize tactile stimuli on the contralateral hindlimb; lesions 3-4 lateral resulted in this deficit being limited to the contralateral trunk; lesions 4-6 mm lateral caused effects limited to the mustacial pad. The pattern of these deficits corresponds to the topographical organization of the rat somatosensory cortex observed by electrophysiological recording.

788 TWO ECONOMIC TESTS OF COGNITIVE AND SOMATOSENSORY FUNCTIONS FOR DETECTION OF CEREBRAL DYSFUNCTION.

Aaron Smith and Carmen C. Centofanti*. Neuropsychological Lab., Univ. of Mich. Med. Sch., Ann Arbor, Mich. 48104.

Studies of effects of age and education on written and oral Symbol-Digit Substitutions (SDMT, Smith, 1973) and Single and Double Simultaneous (face-hand) Stimulation (SDSS) tests of 420 normal adults 18-75 years old provided norms for clinical applications of these two tests in diagnostic studies of patients with suspected and others with confirmed brain insults. SDMT performances showed systematic effects of age and education. Written and oral SDMT scores systematically declined with age. However, in each age-differentiated group, both written and oral SDMT scores increased with education. SDSS performances showed no differences as a function of age or education.

The sensitivity of these two tests (requiring 10 minutes for administration and scoring) to the presence of "brain damage" was tested by applying the SDMT age norms to written and oral scores of 100 adults with various types of confirmed brain insults. A cut-off point of -1.5 standard deviations below SDMT age norms, and three or more somatosensory errors correctly identified 93% of 100 patients with chronic brain lesions and 91% of the normals.

The greater sensitivity of the SDMT than the SDSS test to the presence of brain damage reflects significant differences between the nature of the functions involved. Smith, A. Symbol Digit Modalities Test. Los Angeles: Western Psychological Services, 1973.

789 RECOVERY OF LIMB PLACING REACTIONS IN CATS WITH FRONTAL OR CAUDATE NUCLEI ABLATIONS. Jaime R. Villablanca, Robert J. Marcus*, Charles E. Olmstead and David L. Avery. Dept. Psychiat., Mental Retardation Research Ctr., UCLA, Los Angeles, 90024.

The following groups of adult, chronic, cats were studied: 9, with bilateral ablation of all frontal areas rostral to A22 (Snieder-Niemer Atlas); 11, with bilateral caudate nuclei removal through a midline approach; 10, with unilateral caudate removal and 4 sham operated (bilateral midline cortico-callosal lesions). The limb placing reactions periodically examined (preferentially in paws) were: contact placing (CPR) of dorsal and lateral aspects; proprioceptive, visual and chin placing; frontal and lateral hopping; plank walking and retrieval of legs (passively abducted or off edges). The main observation in frontal cats was a progressive recovery of CPRs. Signs of this were clear only after 90-120 days and complete recovery occurred in 5 cats only after 125-400 days. The defects remaining in the other 4 cats (followed for 89, 125, 128, and 175 days respectively) were coarse or absent CPRs on lateral aspects of the paws, absence of lateral hopping and defective plank walking. Various defects, particularly of the CPRs, were observed in cats with extensive caudate lesions; these were, consistently, fully recovered within about 50 days. Such defects could not be readily accounted for by subcortical surgical damage to frontal cortical fibers. Furthermore, the impairments were virtually absent in the sham operated cats. These findings suggested that the caudate nuclei might have a role in CPRs integrity. The results in frontal cats extend Bard's observations and provide an example of very late CNS recovery processes. The caudate nuclei and/or cortical areas caudal to the frontal removal might be responsible for the compensation. (Supported by USPHS Grants HD-05958, MH-07097 and HD-04612).

790 CNS RESPONSE TO INJURY IN ENRICHED AND IMPOVERISHED ENVIRONMENTS: A BEHAVIORAL AND NEUROBIOCHEMICAL STUDY IN PLASTICITY. Jaime Diaz*, Gaylord Ellison*, and David Masuoka* (SPON:Jennie Shamey). Dept. Psych., UCLA, Los Angeles, 90024.

Rats with small central lesions made with intraventricular injections of 6-hydroxydopamine (3X25ug) were allowed to recover in either an enriched "colony" environment or in isolation cages for periods of either 2, 15, or 45 days after lesioning. Social behaviors of the colony animals were recorded daily, and following each recovery period all colony and isolate animals were tested in a novel open field test. At sacrifice, histofluorescent smears of the hypothalamus were prepared, and the uptake of labelled norepinephrine (NE) was studied both using autoradiographs of sagital slabs of the entire brain and using counts from discrete areas. Behaviorally, the colony animals showed on many measures an initial lesioning effect (viz. increased activity, increased aggressivity, decreased dominance) followed by a return to baseline levels which was completed approximately 12-15 days after the lesion. This 2 week latency of recovery resembles the time course of sprouting found by many other investigators. Two effects will be discussed: 1) in the long term non-lesioned animal, housing in the enriched environment leads to enhanced NE uptake in frontal cortex whereas isolation leads to enhanced NE uptake in the brainstem, and 2) following lesioning uptake of NE recovers differently in colony and isolated animals. The behavioral changes measured in both the colony environment and in the novel open field test will be correlated with the histochemical and autoradiographic data.

791 PUROMYCIN-INDUCED RETENTION DEFICIT IN GOLDFISH AS A FUNCTION OF EXTENT OF TRAINING. Alan D. Springer and Bernard W. Agranoff. Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48104

In a factorial study, goldfish received either 20 or 50 active-avoidance training trials, an intracranial injection of puromycin or no-injection immediately following training, and 10 retraining trials either 1 or 7 days following training. While the 50 trial groups made significantly more avoidances than the 20 trial groups, the puromycin-induced retention deficit observed was independent of number of training trials. In addition, a progressive decay of memory to similar levels over the 1-7 day train-retrain interval was evident in puromycin injected groups irrespective of degree of training. These data are viewed to be consistent with the hypothesis that the amnestic agent interferes with a post-training fixation signal, rather than by direct degradation of memory as has been inferred in studies with other species. (Supported by research grants NIMH-12506 and NSF-GB32409)

792 INTRACEREBRAL PROLINE MAY IMPAIR MEMORY RETENTION IN CHICKS. Arthur Cherkin, Michael Eckardt*, and Mary Garman*. Psychobiology Research Lab., VA Hosp., Sepulveda, CA 91343 and UCLA School of Medicine, Los Angeles, CA 90024.

IV injection of L-proline (PRO), but not L-isoleucine (ILE), impaired 45-min retention of one-trial avoidance learning in 12-hr old chicks, consistent with the hypothesis that glutamate release is involved in short-term memory (Van Harreveld and Fifkova, Brain Res. 81, 455, 1974). The present study assessed the effects of PRO on retention 24 hr after training. We injected 44-hr old chicks intracerebrally with 10 µl per hemisphere of 300 mM PRO or 300 mM ILE, 1 or 63 min after one-trial training to avoid pecking a 3-mm steel bead coated with an aversive liquid. Controls were "trained" with a non-aversive uncoated bead. The 24-hr control peck response was similar to that observed in non-injected chicks in prior studies, therefore PRO and ILE had no effect on the control response. PRO, injected 1-min after aversive training, impaired avoidance compared to 1-min and 63-min ILE-treated groups but the 1-min PRO group did not differ significantly from the 63-min PRO group. These results indicate that PRO produced a retroactive amnesia. Current studies are exploring a possible retrograde effect of PRO, at longer intervals between training and injection.

		Avoidance score (%)[a]	
Training	Injection	1 min	63 min
Aversive	PRO	47[b]	60
	ILE	77	76
Control	PRO	7	0
	ILE	3	3

[a]Percent of chicks not pecking in 10 sec. N = 28-30 per group.
[b]Differs from ILE and control groups ($p<0.04$; χ^2 test).
(Supported by Veterans Administration Research Project 1387-02.)

793 ANISOMYCIN IMPAIRS LONG-TERM MEMORY IN GOLDFISH. Robert C. Hermann*, Vincent J. Aloyo, and William L. Byrne. Brain Research Institute and Dept. of Biochemistry, Univ. of Tenn. Center for the Health Sciences, Memphis, Tennessee 38163.

The effect of Anisomycin (ANI) on learning and memory of an active avoidance task was investigated in the Goldfish. ANI is a potent, but relatively short acting, reversible inhibitor of protein synthesis. 25 µg of ANI injected intracranially resulted in >90% inhibition of protein synthesis for at least 2 hours. Inhibition had declined to 65% 6 hours after injection, and no inhibition could be detected at 24 hr. If injected 30 minutes before an initial 10-trial session of dark-avoidance training in a shuttlebox, ANI-treated fish performed poorly compared to saline injected fish when trained for an additional 10 trials three days later. No effect was observed during the initial acquisition training. Partial, but less marked, amnesia was also observed in ANI-treated fish injected immediately after initial training, while an injection of ANI 24 hours after initial training had no effect on subsequent performance. In addition, injections prior to testing indicated that the effect was not state-dependent, although pre-testing injections of ANI slightly impaired the performance of fish independently of initial training treatment. These data indicate that protein synthesis, while important for at least one stage of memory, is not necessary for learning to occur.

794 MEMORY IMPAIRMENT FOLLOWING CYCLOHEXIMIDE INJECTIONS INTO THE AMYGDALA. Robert F. Berman* and Raymond P. Kesner. Dept. Psychol., Univ. of Utah, Salt Lake City, 84112.

Water deprived rats were trained to enter a goal box in order to lick a tube containing water. After reaching a predetermined latency criterion, rats were given a single 3 sec, 3 mA footshock and then tested for retention of the footshock experience 24 hr later. Retention was evaluated as an increase in latency to approach the lick tube relative to pretreatment latency. Immediately or 12 hours after the footshock rats were injected bilaterally via cannulas chronically implanted into either the amygdala or frontal cortex with either 10 or 20 ug of cycloheximide (CHX), a protein synthesis inhibitor, dissolved in 1 ul isotonic saline. Unoperated and implanted saline injected animals were also tested. Rats that were injected with 20 ug CHX immediately, but not 12 hours after treatment exhibited amnesia for the footshock. Ten ug of CHX produced similar but less reliable effects. In contrast, rats injected immediately after the footshock with 20 ug of CHX into the frontal cortex showed retention similar to both the unoperated and saline injected control rats. EEG's taken from the amygdala injection site showed no seizure activity following an injection of 20 ug of CHX. These results suggest that inhibition of protein synthesis in specific neural regions via relatively localized injections of CHX may disrupt normal neural function and normal information processing resulting in retention deficits.

795 REVERSAL OF DIETHYLDITHIOCARBAMATE INDUCED AMNESIA BY NOREPINEPHRINE. John A. Meligeni*, Sandra A. Ledergerber* and James L. McGaugh. Dept. Psychobiology, School of Biological Sciences, University of California, Irvine, 92664.

These experiments investigated the effects of the dopamine-beta-hydroxylase inhibitor, diethyldithiocarbamate (DDC), and of norepinephrine (NE) on retention in rats. Previous studies indicated that DDC impairs retention at doses which decreased brain levels of NE and that the amnesia is attenuated by posttraining administration of NE. In these experiments animals received DDC 30 min prior to training on a 1-trial inhibitory avoidance task and were given intracerebroventricular or subcutaneous doses of NE either immediately or several hours after training. Two footshock levels and several doses of NE were used. Retention was tested several days later. With lower footshock training, immediate posttrial injections of NE attenuated the amnesic effects of DDC. With higher footshock training, amnesia was attenuated only with low doses of NE. No effects were produced by delayed NE injections. These findings provide further support for the view that NE may be involved in the modulation of memory storage processes.

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796 EFFECTS OF CORTICAL SPREADING DEPRESSION ON PASSIVE AVOIDANCE AND SUBCORTICAL ELECTROGRAPHICAL ACTIVITY. R.A. Prado-Alcalá, G.A. Gómez*, M.F. López* and H. Brust-Carmona. Dept. Fisiol., Fac. de Medicina, Univ. Nacl. Autónoma de México, Ap. Postal 70250, México 20, D.F. México.

Impairment of passive avoidance performance (PA) has been produced by subcortical or cortical spreading depression (CSD). To assess the interactions between these structures during CSD behavioral as well as electrographical measurements were made. PA was studied in rats (Ss) in a two compartment box. Ten seconds after being put into the safety compartment, Ss were allowed to step into the gridded compartment where all, except one group, received a footshock. Their latency to step into the gridded compartment was measured 24 hr later (retention).

Each of the 116 Ss was assigned to one of the following groups: unilateral KCl or saline, bilateral KCl or saline, no treatment and no treatment-no footshock. Both KCl and saline were applied on the dura mater overlying the parietal cortex, 10-15 min before the first training session.

No differences in retention were found among the saline, the no treatment and the unilateral KCl groups. In contrast, the bilateral KCl and the no treatment-no footshock groups had retention scores significantly lower than the former groups (lack of passive avoidance).

In 20 additional rats the application of KCl on the dura mater over the parietal cortex produced depression of the spontaneous electrographical activity of the cortex (CSD) as well as in the caudate nucleus, amygdala and thalamic nuclei.

These results suggest that "normal" cortical electrical activity is necessary for the acquisition of passive avoidance behavior. Nevertheless, the fact that subcortical structures are also altered during CSD leads to reconsider the question of whether the behavioral deficits may be due to such subcortical functional alterations.

797 ENHANCEMENT AND IMPAIRMENT OF MEMORY PROCESSES WITH POSTTRIAL HORMONAL INJECTIONS. R.B. Van Buskirk* and P.E. Gold. Dept. of Psychobiol., Sch. Biol. Sci., Univ. of Calif., Irvine, 92664

Recently we found that posttrial subcutaneous injections of several hormones, including adrenocorticotrophic hormone (ACTH) and epinephrine, can enhance or impair later retention of avoidance training if administered shortly after training. In the present studies, rats were trained in a one-trial inhibitory (passive) avoidance task using various footshock intensities and durations. Immediately after training, all animals received posttrial injections of epinephrine, ACTH, or saline. The effects of these hormones on memory varied with the dose and with the footshock severity. For example, after training with a low footshock, a posttrial injection of a low dose of ACTH had no effect on later retention, moderate doses enhanced retention, and higher doses impaired retention. Furthermore, there was an interaction between footshock level and the effect on retention of a single hormone dose. A single posttrial dose of epinephrine (0.1 mg/kg) or ACTH (3.0 I.U./rat) enhanced later retention of training with weak footshock, but impaired later retention of training with a high footshock.

These findings are consistent with the view that normal hormonal consequences of a training experience may modulate memory processing in untreated animals. In addition, the results suggest that other posttrial treatments which affect memory, i.e., centrally acting drugs and electrical stimulation of the brain, may act via hormonal alterations.

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798 EFFECT OF NONCONTINGENT REMINDER FOOTSHOCK ON EXPERIMENTAL AMNESIA INDEXED BY AUTONOMIC AND SKELETAL RESPONSES IN RATS. Ralph R. Miller, Jeffrey N. Kraus* and Alvin M. Berk*. Dept. Psychol., Brooklyn Coll. CUNY, Brooklyn, N.Y. 11210.

Rats received a single conditioned suppression training trial (tone-footshock pairing) followed immediately by 200 mA., 0.3 sec. electroconvulsive shock (ECS), parameters previously found to induce amnesia as measured by autonomic indices of memory as well as skeletal indices. Retrograde amnesia was observed in lick suppression, bolus count and bradycardia. Noncontingent footshock produced evidence of recovery from amnesia in both between-subjects and within-subjects designs. Control groups indicated that the effect was due to specific memory of the training trial. Although the indices of memory used in these studies are known to have different ECS-intensity thresholds for disruption, autonomic and skeletal indices yielded the same reminder shock-intensity threshold for restoration of memory. The data demonstrate that recovery from ECS-induced amnesia does not require the presence of residual memories available to autonomic response systems. Apparently qualitatively similar processes underlie skeletally indexed and autonomically indexed memories.

799 RETINOTECTAL PLASTICITY AFTER HALF-TECTUM ABLATION IN ADULT FROGS. Susan B. Udin. Research Lab. of Electronics, MIT, Cambridge, MA 02139.

The effects of unilateral caudal half-tectum ablation in adult frogs (Rana pipiens) have been investigated by electrophysiological and behavioral techniques. When optic nerve transection is combined with caudal tectum lesions, the retinotectal projection compresses and achieves both normal lamination and retinotopic order of normal polarity. Receptive field positions of postsynaptic tectal cells deep to the optic terminals reflect the retinotectal compression. Tectally mediated orienting behavior also reflects the altered topography: in normal frogs, prey stimuli in the caudal half of the visual field activate the caudal half of the tectum and elicit turns toward the caudal field, but in frogs with caudal tectum lesions, prey stimuli in the caudal half field stimulate the remaining rostral tissue and elicit turns toward the rostral field.

After caudal half-tectum ablation without optic nerve transection, the optic axons which normally arborize in caudal tectum sprout new terminals in the remaining rostral tissue; these terminals interdigitate among the normal undisplaced rostral terminals in the appropriate superficial laminae. However, the new terminals fail to establish clear retinotopic order even after as long as 707 days, and the undisrupted rostral terminals do not compress. Postsynaptic recording and behavioral tests indicate that axons displaced by the caudal lesions make functional connections.

In some cases, a small population of displaced optic axons terminate in the intact ipsilateral tectal lobe where they form a mirror-image map and mediate orienting responses directed to mirror-image positions in the visual field.

It is proposed that retinotectal topography is determined both by polarity cues inherent to the tectum and by mutually-repulsive fiber-fiber interactions among optic axons.

800 DISTRIBUTION OF SPINAL AFFERENTS IN THE CEREBELLUM OF THE REELER MUTANT MOUSE. Dennis A. Steindler. Dept. Anat., Univ. of Wisc., Madison, 53706.

The trajectory and terminal distribution of afferent axons may be affected by disoriented cortical neurons, areas of an ectopic granule cell layer, reduced numbers of granule cells, and lack of foliation in the undersized reeler mouse cerebellum. The distribution of a major cerebellar afferent in the reeler mouse was examined using the spinocerebellar-mossy fiber system. Following spinal cord lesions in normal and heterozygous reeler mice, anterograde degeneration techniques show that spinocerebellar axons terminate in and surrounding the midplane in the anterior lobe vermis (primarily culmen, lobulus centralis, and lingula). In experimental homozygous reeler animals, degeneration is also confined to a midplane area, the majority within a rostral region of the cerebellum. Degenerating mossy fibers follow a characteristic course in the cerebellar white matter and terminate rather diffusely within normal and displaced granule cell areas. A few degenerating fibers appear to terminate in the shallow molecular layer and near clusters of Purkinje cells, and it is possible that anomalous contacts are made in sparse granule cell areas. The data presented here suggest that spinocerebellar mossy fibers distribute within a topographically appropriate area of the abnormal reeler cerebellum, and the factors which govern anomalies in the cerebellum are apparently independent of those which control the projection of axons of the spinocerebellar tracts. (Supported by NIH training grant 5-T01-GM00723-14 and grant NS-09167 from NIH to Dr. Henry J. Ralston III.)

801 SYNAPTIC REINNERVATION IN THE INNER MOLECULAR LAYER OF THE DENTATE GYRUS FOLLOWING PARTIAL DEAFFERENTATION: AN ELECTRON MICROSCOPE STUDY IN THE RAT. J.R. McWilliams*, G.S. Lynch and C.W. Cotman (SPON: S.A. Deadwyler). Dept. Psychobiol., Sch. Biol. Sci., Univ. of Calif., 92664.

In adult rats the inner one-third of the dendritic region of the dentate gyrus granule cells receives projections primarily from the commissural fibers of the contralateral hippocampus and the associational fibers of the ipsilateral hippocampus. Following complete contralateral hippocampal aspiration, 25% of the terminals in the proximal 80 microns of the dendritic zone above the granule cell layer degenerate by 4 days. We felt this would be an excellent model system to investigate possible synaptic reinnervation since (1) the experimental lesion is easily reproducible, (2) the same dendritic region can be located reliably between animals, (3) the synaptic counts are highly consistent in normal animals and (4) no measurable shrinkage occurs in this region following commissural deafferentation.

Complete photomontages of the zone were examined and quantified for three normal, five 2-4 day, and five 50-75 day post-lesioned animals. At 2-4 days the mean synaptic count for intact synapses dropped to 64% of the normal count. It returned to 97% by 50-75 days. The number of terminals showing multiple contacts increased slightly in the long term animals. Measurements of terminal volume for the three groups showed no increase in average terminal volume for the 50-75 day group. These results indicate that this dendritic region is reinnervated following commissural deafferentation and is due primarily to the formation of new terminals rather than the expansion of pre-existing terminals. It is believed that the associational fibers are responsible for this reinnervation.
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802 ELECTROPHYSIOLOGICAL EVIDENCE FOR PLASTICITY IN THE RABBIT OLFACTORY BULB IN SITU. Anne Marie DeLuca* and Stephan L. Chorover, Dept. of Psychology, Massachusetts Institute of Technology, Cambridge, Mass. 02139.

Sustained seizure activity can be induced in rabbit olfactory bulb by daily low-level stimulation of brief duration. The olfactory bulbs of Belted-Dutch rabbits are isolated in situ at the level of the anterior olfactory nucleus. A complete neural transection is made by blunt dissection without penetrating the dura mater. A semi-microelectrode is chronically implanted in the isolated or the contralateral bulb. Recordings are made before and after stimulation. At the current levels used (10uA, biphasic square wave pulses, 100 Hz, 5 sec duration) several successive stimulations are necessary to evoke a change in unit or slow-wave activity in the bilaterally intact preparation. In the isolated olfactory bulb, however, a single stimulation was usually sufficient to produce such a change, which often persisted for several days. In recordings made for up to six months postoperatively, the isolated olfactory bulb retains an ability to exhibit long-term changes in unit and slow-wave activity following a brief period of low-level electrical stimulation. The influence of more caudal brain regions is excluded by the transection, but no retrograde chromatolysis is apparent in the mitral cell layer and the isolated bulb retains an intrinsic capacity to show altered responsiveness. We conclude that the functional changes observed in the isolated bulb are due solely to local events occurring within the tissue itself and suggest that the rabbit olfactory bulb is an attractive object for studies of electrophysiological plasticity.

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803 EEG AND EVOKED POTENTIALS DURING RECOVERY FROM SENSORIMOTOR CORTICAL DAMAGE. DOES THE BRAIN HAVE A SYSTEM FOR ONGOING MAINTENANCE?

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Since regeneration of lost brain regions does not occur, recovery must be due either to compensatory changes or to receding of temporary "shock" in remaining tissue (Eidelberg & Stein, NRP Bull., 1974). The knowledge that brains must undergo adaptive changes during learning provides impetus to explore the former possibility, and some researchers have reported interesting anatomical changes following brain damage. Evolutionary considerations suggest that there would not be special systems for recovery from brain damage (Glassman, Behav. Sci., 1974). Since complex systems require maintenance against entropic factors, e.g., homeostatic mechanisms in living systems, it is speculated that recovery from brain damage is a by-product of a process of ongoing maintenance in information bearing structures. Because EEG activity is spontaneous and involves orderly relations among masses of tissue, it is suspected of participating in maintenance functions.

A previous chronic study of damaged SI cortex of 4 cats showed gradual recovery of gross potentials evoked by forelimb stimulation, concurrently with recovery of sensitivity to brain stimulation and of measures of motor behavior (Glassman, Exp. Neurol., 1971), but no shift in distribution of potentials to indicate compensation. These results are most consistent with a "shock" interpretation of recovery. New data to be presented are from a study of 15 additional chronic preparations with various sizes and placements of sensorimotor cortical lesions. Though many cells of SII have broader connections than those of SI, and SII serves different behavioral functions in cats, there was no redistribution, or convincing increase over prelesion amplitude, in evoked potentials, with recovery in SII. The findings also indicate that while EEG activity is often resistant to loss of brain tissue, in general, slowing, most severe soon after damage, was directly related to size of lesion and proximity of recording point to lesion. This phenomenon is familiar from past experimental (Kennard & Nims, J. Neurophysiol., 1942) and clinical work. Other EEG sequelae of damage, seen in some preparations, e.g., 30-35 Hz orbital cortex spindles, may also be related to the above speculations. No other clear patterns of findings were seen in these animals, for example, no special EEG responsiveness in SII following SI lesions.

It is intriguing to recall other kinds of events associated with synchrony: There is a gradual evolution towards more regular, and then faster, activity in ontogeny (Kiloh, McComas & Osselton, Clinical Electroencephalography, 1972). Alpha rhythm and sensorimotor spindles are each associated with inactivity of the respective functions (Howe & Sterman, EEG clin. Neurophysiol., 1972). (In this regard, present results showed that rhythmic slow activity due to damage was blocked by cutaneous stimulation--at a point which had also been responsive to stimulation before damage.) Slow rhythms are present in sleep. They have been reported to follow frustrations (Walter, The Living Brain, 1963) or rewards (Clemente, Sterman & Wyrwicka, EEG clin. Neurophysiol., 1964). These findings all may be consistent with the idea of inhibitory and also restorative, growth, or readjustment functions of slow activity. Becker, Katzberg and others (Ann. N. Y. Acad. Sci., 1974) have discussed electric field participation in healing and cellular orientation. A prediction is that orderly regenerative processes known to occur in amphibia might be guided not only by chemical signals but by an active electrical process. Supported by a grant from the Illinois Department of Mental Health and Developmental Disabilities.

804 ROLE OF VISION IN RECOVERY OF FUNCTION FOLLOWING FORELIMB DEAFFERENTATION IN THE MONKEY. John G. Gianutsos* and A. J. Berman. V. A. Hospital, Bronx, N.Y. 10468 and Mount Sinai School of Medicine, New York, N.Y. 10029.

A striking degree of functional recovery following dorsal rhizotomy has been reported in the primate literature. In many cases conditioned responding with deafferented forelimbs was carried out by subjects even when vision of the responding extremity was prevented during the test period. In other cases various categories of movement have been observed in situations where the deafferented monkeys were either blindfolded or fitted with displacement prisms. Taken as a whole such studies are of theoretical import, and have led some to theorize that complex behavioral interaction with the environment is possible even in the absence of all feedback. However, in these studies subjects were able to observe their forelimbs at times, especially during the period when they were recovering from surgery and while in their home cages between experimental sessions. These "extra-experimental" visual experiences may have been the critical factor for subsequent recovery of function. Such a hypothesis is supported by experimental studies on normal (non-deafferented) subjects deprived, during early ontogeny, of visual feedback which systematically accompanies self-produced movements. Such animals exhibit deficits in the performance of motor acts directed toward extracorporeally located objects. Although the monkeys utilized in the deafferentation studies referred to above were either juveniles or adolescents having considerable motor experience prior to dorsal rhizotomy, it was deemed possible that recovery of function would have been severely limited or altogether changed had they been prevented from viewing their extremities subsequent to dorsal rhizotomy. It was the purpose of this study to systematically investigate the hypothesis that visual feedback which accompanies voluntary movement is essential to the recovery of certain categories of movement following deafferentation.

Recovery of function was compared in six juvenile ferally-reared male Macacca mulatta. To preclude patterned vision, three subjects had their eyelids sutured closed one month prior to dorsal rhizotomy. All animals were bilaterally deafferented by intradural section of dorsal roots C2 to T3. Hence two groups of monkeys - one with vision intact, the other deprived of patterned vision - were compared for functional recovery after deafferentation. Systematic behavioral observations were begun once the animals were ambulating in an upright position. Animals were placed unrestrained in a special chamber with food and water freely available. Each animal was simultaneously rated by two independent observers for 10 minutes on each of 18 days, and its behavior was recorded on a checklist containing 50 response categories. The observer-observer reliability was very high (r = .99).

Vision intact subjects used their forelimbs to grasp both near and distant objects, for self-grooming and grooming of others, for digging into and picking up individual pieces of straw, and for climbing. In contrast, vision-deprived deafferents were never observed using their extremities to perform acts directed away from their bodies, and while they did use their forelimbs to locate objects on their own bodies and to scratch themselves, they used their mouths to perform functions for which the vision intact group used their forelimbs. Exploration of the environment, obtaining food, and grooming was accomplished by means of the mouth.

These results support the contention that some feedback is necessary for the occurence of outward directed behavior following dorsal rhizotomy. In all observed instances of purposive movement there is some identifiable source of feedback. The high degree of functional recovery attained by deafferented subjects with vision intact illustrates the adequacy of vision as a source of compensatory feedback in instances where somatosensory feedback is lacking.

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805 NEURAL PLASTICITY IN ADULT CAT: TIME COURSE FOR LOSS OF X-CELLS IN THE DORSAL LATERAL GENICULATE NUCLEUS FOLLOWING MONOCULAR PARALYSIS. Dawn L. Brown and Walter L. Salinger. Dept. of Psychology, Univ. of N. Carolina at Greensboro, 27412.

In a previous report we have shown that chronic (more than two weeks) monocular paralysis leads to a selective, but substantial loss of X-cells in the binocular segment of the lateral geniculate nucleus (LGNd) in adult cats. In this experiment, we examined the time course for the onset of the effect of monocular paralysis in the LGNd by recording from six monocularly paralyzed cats during the first two weeks of paralysis. During recording sessions animals were sedated, but not anesthetized. Cells were sampled only from the central 10^{o} of visual space. For the first three days of monocular paralysis X-cells constituted about 50% of the cells sampled. This percentage is comparable to that obtained with systemically paralyzed acute subjects. By day 8, roughly half of the total X-cell loss had occured. Little change occurred from day 9 to day 15. After day 15, however, a second major reduction in the percentage of X-cells was observed, leaving only 16% X-cells as compared with about 50% recorded in the acute stage of paralysis (three days or less duration). The rapidity of this process suggests that monocular paralysis produces an extremely powerful insult to the visual system and that the visual system of the adult cat has a greater degree of plasticity than hitherto reported.

806 CORTICAL "PRUNING" DOES NOT AFFECT PLASTIC CHANGES IN THE DENERVATED SEPTUM. Lucy L. Brown* and Robert Katzman. Dept. Neurol., Albert Einstein Col. Med., Bronx, N.Y. 10461.

Moore et al. (Brain Res., 33: 13, 1971) demonstrated increases in catecholamine (CA) fluorescence in the rat septum after suction lesions of the fimbria, an effect indicative of synaptic reinnervation after denervation. Because our initial studies of the septum following discrete electrolytic lesions of the fimbria did not result in readily observable increases in fluorescence, we based a second series of studies on the hypothesis that either extensive destruction of CA collaterals in the hippocampus and cortex due to suction lesions caused the increase in CAs observed by Moore et al. or that in our initial studies lesions of the fimbria affected discrete septal areas and required more detailed observations. Suction, radio-frequency, and knife-cut lesions were made in 35 rats. The lesions were placed to include the fimbria and large or small amounts of the hippocampus and cortex. Thirty days post-lesion brains were treated according to the Falck-Hillarp technique for histological examination. Fluorescence increases did not vary with the size of hippocampal and cortical damage and, thus, were not dependent upon injury to noradrenergic locus coeruleus collaterals in those areas. However, a change was detected after discrete fimbria lesions. Highly localized, sometimes bilateral, increases in fluorescence intensity and/or area were observed in anterior and lateral nuclei of animals with fimbria lesions and/or small lesions of the hippocampus. Additional posterior nuclei were affected by damage to the stria terminals. These effects are largely dependent upon denervation, although damage to collaterals in the fimbria and stria terminals may still play a role.

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807 PLASMA CATECHOLAMINES DURING TRANSCENDENTAL MEDITATION (T.M.) R. Michaels, Ph.D., M. Huber*, B.S., D.S. McCann, Ph.D., Wayne County General Hospital, Research Department, Eloise, Michigan 48132

It has been hypothesized that alterations in the state of consciousness may be accompanied by changes in plasma norepinephrine (NE) levels.

We are testing the concept with volunteer subjects from the Transcendental Meditation (TM) Society. Control subjects are volunteers not practiced in the art of T.M. matched in age, sex and race to the test subjects: specimens are obtained ca. 2 hr. post prandial in the afternoon via a butterfly needle inserted into the anti-cubital vein, irrigated with heparin and left in situ for approximately 1-1/2 hrs. Seven ml of blood is withdrawn after 20 min. (habituation period); after a further 15 min. (control period), then three samples are obtained during the next 20 min. (meditation period) and a final sample at the termination of the experiment 10 min. later (post meditation period).

Epi and NE are measured by an enzymatic, single isotope derivative proceedure (Passon & Peuler, Analyt Biochem 51:618, 1973).

Preliminary results indicate that control values for test subjects fall well within the range of normal values established in our laboratory (Epi .05±.02*ng/ml; NE .37±.04 ng/ml). Nor has it been possible to date to establish any real differences between meditators and control subjects during the post control period which represents a meditation period for the TM subjects and a rest period for the control subjects.

*SEM

808 EFFECTS OF BLOOD PRESSURE AND US INTENSITY ON CLASSICAL CONDITIONING AND SENSITIZATION IN THE SPINAL CAT. A.R. Light* & R.G. Durkovic (Spon: D.L. Blank) Dept. Physiol., Upstate Med. Ctr., Syracuse, N.Y. 13210.

Acute, T-10 spinal cats were immobilized by either midcollicular transection (MCT) or ligation of the vertebral and carotid arteries (Anemic Decapitate). Mean arterial pressure of 36 MCT animals (125±3.5 torr) were significantly greater than those of 36 anemic decapitate preparations (76 ±2.5 torr). These 72 cats were assigned to conditioning or sensitization experimental paradigms. In the conditioning paradigm, shocks to the cutaneous saphenous nerve (CS=10/sec for 1.5 sec) were paired with shocks to the cutaneous superficial peroneal nerve (US=30/sec for 0.5 sec) such that the US overlapped the last 0.5 sec of the CS. A one-minute intertrial interval (ITI) was utilized for all conditioning animals. CR's and UR's were flexion reflex tensions recorded from the isolated distal tendon of the tibialis anterior muscle. Sensitization control animals received either an alternating schedule (n=18) or a pseudo-random schedule (n=18) of CS and US presentations, all with a 30 sec ITI. Conditioning and sensitization animals were divided into 3 US intensity groups which were assigned Aα, Aαδ or AαδC cutaneous fiber US's (Neur. Sci. Abstr. #403, 1974). The only significant finding was that animals receiving high US intensities (Aαδ and AαδC) showed much greater reflex facilitation in the conditioning paradigm than in the sensitization paradigm. Low US intensity (Aα) did not produce facilitation during either the conditioning or the sensitization paradigms. These results illustrate that the spinal cord may mediate classical conditioning-like behavior when the US intensity is strong enough to excite both Aα and Aδ cutaneous fibers, a condition which produces pain in human subjects (Arch. Neurol. 3:381, 1960). Also, since the results were similar for both blood pressure groups, it appears that spinal conditioning is independent of this physiological factor over the range observed.

809 ABSOLUTE VISUAL THRESHOLD IN THE GOLDFISH OBTAINED BY CLASSICAL CONDITIONING. Maureen K. Powers and S. S. Easter. Departments of Psychology and Zoology, University of Michigan, Ann Arbor, Michigan, 48104, USA.

Absolute visual threshold was obtained by classical conditioning of heart rate in restrained but unanesthetized goldfish. This experiment is the first successful attempt to use classical conditioning of an autonomic response at threshold levels of light. Heart rate decreased following presentation of a visual stimulus that had been previously associated with an electric shock to the tail. A modified staircase method was used to present flashes (λ = 538 nm) of different intensities. Threshold was defined as the intensity that was detected on 50% of the trials. We found that for threshold detection, taking into account visual pigment density and quantum efficiency, there was only a single effective quantum of light per 1800 rods. About ten times as many quanta are required to elicit action potentials in single ganglion cells in the isolated retina of the goldfish, but this may be due to the non-physiological condition of the preparation. The absolute threshold of a human observer in the same apparatus was also about ten times higher than the goldfish's behavioral threshold. This difference may be due in part to the larger size of receptive fields of ganglion cells in the goldfish's retina. We believe that the absolute threshold we have measured is an accurate determination of the goldfish's visual sensitivity.

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810 LESIONS OF THE SUPRACHIASMATIC NUCLEUS (HYPOTHALAMUS) ALTER THE NORMAL OSCILLATION OF RETENTION PERFORMANCE IN THE RAT. Richard A. Wansley* and Frank A. Holloway. Department of Psychiatry and Behavioral Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, Okla. 73190

The relationship between biological rhythms and the retention performance of rats was investigated. One-hundred and fifty rats were assigned to one of three surgical treatment groups: bilateral lesions of the suprachiasmatic nucleus (hypothalamus) (SCN), sham-operated controls (SOC), or non-operated controls (NOC). At seven days following surgery, examination of gross activity over a 24 hr period indicated a normal circadian rhythm in spontaneous activity for both NOC and SOC animals. The SCN animals showed no temporal variation. On the second day following this observation, animals were trained in a one-trial passive avoidance task, and retention performance was examined at five training-testing intervals (TTI) (N = 10 Ss/TTI). The retention performance of the NOC and SOC groups was found to be good at 15 min, 12 hr, and 24 hr, while performance was significantly poorer at 6 hr and 18 hr. Such results replicate previous findings in normal rats trained and tested in a variety of tasks. The retention performance by the SCN groups was similar to the NOC and SOC groups, except that no deficit was evidenced by the 18 hr group. These findings support the proposal that the suprachiasmatic nucleus is an important anatomical location for the maintenance of bio-behavioral rhythms. Secondly, these data suggest that the normal oscillation in retention performance may reflect two overlapping processes. The first may be related to the familiar Kamin-like effect (6 hr deficit), while the other reflects the interaction of retention with a rhythmic physiological state (18 hr deficit).

811 KINESTHETIC FEEDBACK AND RECOVERY FROM ECS-INDUCED AMNESIA. Terry G. Shaw *and Douglas L. Chute. Dept. Psychol., University of Houston, Houston, Texas 77004

Electroconvulsive shock (ECS) produces a robust amnestic effect in retention testing. Recovery from such retrograde amnesia (RA) has been reported but the recovery effect may be due to incomplete interference with the consolidation processes or the addition of extra, though non-contingent, learning trials. Following acquisition (150V, 2ma, 2 sec, foot shock (FS)) in a one trial passive avoidance "step-down" task rats in the experimental group were given a high level, 220ma ECS, presumably resulting in complete RA. In the 24 hr retention test animals were placed on the top of a two level platform. A group that received FS but no ECS in acquisition had long latencies before stepping off the first level, indicating remembering. A group which received FS and ECS had short latencies stepping off the first level, not significantly different from untreated controls. However step down latencies from the second level were significantly longer than controls implicating a reminder effect for kinesthetic cues. It's unlikely that the availability of the kinesthetic cues associated with the two level platform provide an additional learning trial; if anything, extinction should be occurring thus loading the situation against the observation of a reminder effect. The partial reversal of ECS induced amnesia with a kinesthetic reminder suggests part of the ECS effect is due to disruption of retrieval mechanisms.

812 FLUCTUATION IN RECOGNITION MEMORY ACROSS SUCCESSIVE TEST TRIALS. Walter H. Riege, VA Hospital, Sepulveda, California 91343; and Dept. Psychiatry, School of Medicine, University of California, Los Angeles.

Human subjects are not consistent in their repeated recognition of items; a certain proportion of items is normally lost and recovered from one test trial to the next. In an auditory (44 different bird songs) and a visual (44 different geometric art patterns) continuous recognition task, each using a study-test-test-test paradigm, groups of staff volunteers (N=20), students (N=12) and alcoholic patients (N=16) were asked to remember and recognize 8 nonlinguistic items randomly intermingled (p=0.4) with 12 different distractor items in each of the 3 successive test trials. As estimated in sequential dependencies of inter-trial forgetting and spontaneous recovery of items, small but reliable fluctuations in item recognition occurred in all groups. These often derived from a trade-off among items newly forgotten and those spontaneously recovered across test trials, so that recognition level remained relatively constant.

Table 1
Mean Percent of Items
Recognized correctly after recognition failure on preceding trial(s).

	Visual Task		Auditory Task	
	Trial 2	Trial 3	Trial 2	Trial 3
Staff	8	13	3	11
Students	12	14	5	10
Alcoholics	17	29	6	13

(The study was supported by Veterans Administration Project 7452-01.)

813 THE EFFECTS OF PIRACETAM ON AUDIOGENIC PRIMING AND ON SOUND-INDUCED SEIZURES IN C57BL/6J MICE. Robert A. Schreiber and Nelson N. Santos, Brain Research Institute, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163.

Piracetam (UCB 6215; 2-pyrrolidone acetamide), a cyclicized GABA derivative, is devoid of any of the usual psychopharmacological side effects, and shows no toxicity up to 2 mg/kg. Yet, it facilitates learning and memory in many different learning tasks, and protects from hypoxia-induced amnesia. Its pharmacological effects have been proposed by various authors to reside at the level of either 1) an enhanced reception of relevant stimuli, 2) an increased efficiency of consolidation, or 3) an enhanced mobilization of recall mechanisms. An experiment was designed to test these alternative hypotheses using audiogenic priming of C57BL/6J mice as a model system of biological "learning" without formal training. Groups of mice were injected i.p. with 100 mg/kg piracetam in 0.9% saline (P), or else vehicle (S), either 30 min prior to, or else 1 min after a 60-sec exposure to intense noise on day 16 (birth=day 0). All mice then given a second injection of either P or S 30 min prior to testing for susceptibility to sound-induced seizures on day 21 (N=20/group; Σ=160 mice). There were no significant differences on day 21 in the incidences of seizures between either P-30 and S-30 mice or between P + 1 and S + 1 mice. Thus, with this bell intensity (127±2 db) and duration of exposure, no changes in either piracetam-induced sensory reception or in consolidation could be detected. Piracetam injection 30 min prior to testing day 21 significantly reduced the incidence of lethality (Piracetam: 14/80 succumbed; Saline: 27/80. 2x2 $\chi^2 = 4.72$; $p < .05$), which may be attributed to the previously demonstrated piracetam-induced protection from the effects of hypoxia. This research was supported in part by MH 24781 and by GRS Grant #RR05423 to UTCHS.

814 **PASSIVE AVOIDANCE RETENTION DISRUPTION BY UNILATERAL LESIONS OF EITHER THE MEDIAL AMYGDALOID NUCLEUS OR THE LATERAL MEDIAL FOREBRAIN BUNDLE.** **Elaine L. Bresnahan, Psychology Dept., Essex Community College, Baltimore, Md., 21237.**

Albino rats were used to evaluate memory disruption during a retention test given 24 hours after passive avoidance (PA) learning. Small lesions occurring in the medial amygdaloid nucleus, either bilaterally or unilaterally, resulted in disruption of PA retention. Similarly, small unilateral lesions falling along the lateral aspect of the medial forebrain bundle (MFB) resulted in retention disruption, whereas, more medially located lesions were not followed by such disruption.

Both regions, the medial amygdaloid nucleus and the lateral aspect of the MFB were previously investigated by this author using low level electrical stimulation, a procedure resulting in PA retention disruption. It appears that either lesioning or stimulating these discrete areas has a disruptive effect on retention. This effect may be related to amygdaloid and nigro-neostriatal systems that may be normally involved in processes related to the animal's ability to withhold a response and/or to the retention of that event.

815 SUCROSE AS AN AVERSIVE STIMULUS. Harvey J. Grill. The Rockefeller University, New York, N. Y. 10021.

It has been hypothesized that the CS+ in the gustatory avoidance paradigm does not provide a signal for impending illness, but actually reinstates the aversive stimuli which accompanied the illness. In other words, the gustatory stimulus becomes aversive as a result of the association. One approach for testing this hypothesis would be to observe the animal's immediate reaction to the gustatory stimulus before and after pairing with illness.

To test this hypothesis we have devised an apparatus for introducing small quantities of different taste stimuli into the oral cavity of freely moving rats without touching the animal. The gustatory stimulus (50 µl) is delivered through fine polyethylene tubing which is guided into the oral cavity by a larger intraoral fistula. A second intraoral fistula on the opposite side is used to deliver water rinses. We have examined the taste reactivity of rats to a series of 4 concentrations of sucrose, NaCl, HCl and QHCl. The independent variables are tongue movements, mouth movements, swallowing and whole body movements. Initially observations were verbally described on a tape recorder, but more recently trials have been videotaped. The reactions to sucrose and QHCl differ markedly. One molar sucrose first produces low amplitude high frequency mouth movements, then swallowing and lateral tongue deflections. Subsequently, these components alternate for the duration of the response (12-20 sec). Quinine (.003 M) elicits high amplitude low frequency mouth movements and lip retraction (gapes) followed by chin rubbing on the substrate.

With this data as a baseline we performed the following experiment. Seven rats, 5 with previous sucrose experience and 2 without, were given two applications of 50 µl of 1.0 M sucrose each separated by two 50 µl distilled water rinses. Immediately following the second sucrose stimulus the animal was injected IP with 8 cc of .15 M LiCl (Day 1). A second identical LiCl injection was administered after the second sucrose stimulus on Day 2. All responses for 3 animals were videotaped for later analysis. Each animal exhibited the typical quinine-like response to a sucrose stimulus after the second poison pairing, that is by Day 3. On Day 7 all rats were water deprived for 10 hrs, then given access to distilled water and 1.0 M sucrose. The rats gapped on contact with the sucrose spout and subsequently consumed only water. Naive controls tested under identical conditions consumed just under half of their total fluid intake from the 1.0 M sucrose bottle.

816 EFFECTS OF D-AMPHETAMINE AND STRYCHNINE ON CYCLOHEXIMIDE AND DIETHYLDITHIOCARBAMATE INDUCED AMNESIA. Elton E. Quinton. Neuropsychopharmacology Program, Univ. of Louisville, Louisville, KY 40208.

C57BL/6J mice were administered cycloheximide or diethyldithiocarbamate 30 min. before training on a passive avoidance task and tested 72 hrs. later. Some groups were administered strychnine or d-amphetamine immediately after training or shortly before testing. Post-training administration of 5 mg/Kg d-amphetamine prevented amnesia in cycloheximide treated mice, but strychnine or lower doses of d-amphetamine, or pretest administration of 5 mg/Kg d-amphetamine or strychnine, did not prevent or reverse the amnesia. Diethyldithiocarbamate induced amnesia when injected shortly before or within three hrs. after training. Post-training administration of d-amphetamine or strychnine did not prevent amnesia in diethyldithiocarbamate treated mice, but pretest administration of d-amphetamine induced a partial recovery of memory. These results, in conjunction with the results of a subsequent study, suggest that cycloheximide induces amnesia by impairing adrenergic systems during the initial post-training consolidation period. Post-training d-amphetamine administration prevents the amnesia by reducing the effect of CYC on these adrenergic systems. Diethyldithiocarbamate induces amnesia by impairing noradrenergic dependent retrieval mechanisms.

817 INHIBITION OF CEREBRAL PROTEIN SYNTHESIS: RETENTION AT DIFFERENT TIMES AFTER TRAINING. Hasker P. Davis*, Curt W. Spanis* and Larry R. Squire. VA Hosp. San Diego, Ca. 92161 and Dept. Psychiatry, UCSD, Sch. Med., La Jolla, Ca. 92037.

Inhibition of cerebral protein synthesis, by subcutaneous or intracerebral injection, impairs "long-term" memory. Recently, it was reported that subcutaneously injected cycloheximide impaired retention measured 10 min after training in a passive avoidance task. This finding raised the possibility that inhibition of cerebral protein synthesis may sometimes disrupt "short-term" as well as "long-term" memory. To examine this possibility, mice were injected subcutaneously with cycloheximide (120 mg/kg), bitemporally with cycloheximide (100 μg/side), or subcutaneously with anisomycin (150 mg/kg). Mice were then given one training trial in a step-through, passive avoidance box. Subcutaneously injected cycloheximide reduced step-through latencies 10 min after training, confirming previous findings. However, anisomycin and bitemporally injected cycloheximide did not affect performance 10 min after training. Anisomycin caused an improvement in performance 45 min after training. Since the results obtained at short intervals after training varied depending on the drug and the route of injection used to establish inhibition of cerebral protein synthesis, the impairment of performance produced by subcutaneous cycloheximide at 10 min after training cannot be attributed to inhibition of protein synthesis. Apparently, performance at short intervals after training in this task need not reflect the effects of drugs on "short-term" memory. Instead, performance measured at these times appears to be easily influenced by side effects of drugs on step-through latencies. By contrast, the impairment consistently obtained 1-5 days after training is in agreement with all previous studies and supports the hypothesis that cerebral protein synthesis is required for formation of "long-term" memory.

818 DEVELOPMENT OF LEARNING AND RETENTION IN NEONATAL KITTENS. M.S. Levine, T.I. Lidsky, C.D. Hull, N.A. Buchwald. Mental Retardation Res. Ctr., Brain Research Institute, UCLA, Los Angeles, 90024.

As part of a set of experiments designed to study the development of learning in neonates, we have been investigating the ability of kittens to locate their mothers in a two compartment enclosure. The results indicate that although very young kittens display evidence of learning, their ability to retain information over relatively long time periods may not develop until the second postnatal week.

Kittens (2-7 days old) learned to locate an aperture which led to their mothers in 2 to 3 trials. When first placed on one side of an enclosure these kittens rarely found the mother on the 1st trial. After they were pushed through the aperture to gain access to the mother, most kittens found their ways through on a 2nd or 3rd trial. When retested 24 hours later, these kittens typically were unable to find the aperture on the 1st trial, although again they readily performed the task on the 2nd or 3rd trial. The kittens were retested 1 week later and the results compared with those of a control group receiving initial experience on the task during the 2nd week. The performance of the 2 groups was similar indicating that training during the 1st week had little effect upon subsequent performance. Moreover, both of these groups demonstrated evidence of retention of learning on the 24 hour retest indicating that 2 week old kittens were able to benefit from previous training. At 3 weeks of age, these 2 groups were compared with a 3rd, "naive" group. The performance of kittens in the previously trained groups was significantly better than that of animals in the "naive" group, i.e., the previously trained animals had retained information from prior testing a week earlier. These results indicate that the ability to retain information for as long as 1 day in kittens may require a neuronal maturation ordinarily not reached until at least the 2nd week of life. (Supported by USPHS grants MH-07097, HD-05958).

819 PREFRONTAL ABLATIONS AND SHORT-TERM MEMORY IN DOGS. S. Soltysik* (SPON: J. E. Norvell). Mental Retardation Res. Center, UCLA, Los Angeles 90024.

Brown and Soltysik (Acta Neurobiol. Exp. 31: 69-100, 1971) found that delayed differentiation of pairs of tones (go-no go, bar press rewarded when the 2nd tone is different; Low tone 200 Hz, High tone 1200 Hz) is solved differently depending on the task design. In a 3-pair design (L-H, L-L, H-H) dogs learn conditioned switching based on differential reward contingencies. The 4-pair task (L-H, H-L, L-L, H-H) is solved by remembering the 1st tone and comparing it with the 2nd. Bilateral orbital or proreal lesions were made in 8 dogs trained in 3-pair or 4-pair task. The intertone interval (a delay) was 20 sec in the easier 3-pair task and 10 sec in the 4-pair task. All dogs were overtrained and served as subjects in the previous study. Proreal lesions in all 4 dogs were comparable but orbital ablations were more extensive in the 3-pair dogs. Performance (% correct responses) in 25 sessions (200 trials) prior to surgery and in 2 blocks of 25 sessions post-surgery showed (table below) that the difficult memorial 4-pair task was unimpaired or slightly improved ($p<.01$ for inhibitory trials L-L and H-H) in orbital dogs. The 3-pair dogs showed a temporary deficit restricted to H-H trials ($p<.02$) after orbital lesion. The data indicate that neither orbital nor proreal areas are essential for solving a task based on short-term memory of tones (4-pair design).

PERCENTAGE OF CORRECT RESPONSES FROM ALL TRIALS BEFORE AND AFTER OPERATION

Task & Subjects	Pre	Post I		Post II		Lesion
3-pair dogs (N=2)	91.5 (74)	83.5	(51)	83.0	(66)	orbital
3-pair dogs (N=2)	97.5 (93)	98.0	(98)	98.5	(98)	proreal
4-pair dogs (N=2)	93.0 (87.5)	98.25	(97)	99.0	(99.5)	orbital
4-pair dogs (N=2)	96.0 (93.5)	96.0	(96)	93.75	(91)	proreal

Percentages of correct responses on inhibitory trials in parentheses: H-H in 3-pair dogs, and both L-L and H-H in 4-pair dogs. (This study was performed in the Inst. Psychoneurology, Warsaw, Poland).

820 EFFECTS OF VISUAL OR AUDITORY CORTICAL LESIONS ON SPECIFIC CROSS-MODAL TRANSFER IN THE RAT. E. H. Yeterian* (SPON: W. A. Wilson, Jr.). Dept. of Psychology, Univ. of Connecticut, Storrs, Ct. 06268

Cross-modal transfer (CMT) may be demonstrated in rats, ie, rats trained to press a lever only in the presence of one of two alternative intensities of light (or sound) transfer the acquired response pattern when later exposed to two different intensities in the other modality (Over and Mackintosh, Nature 224: 918, 1969). Yeterian, Waters and Wilson have shown that large posterior cortical lesions result in a loss of transfer in the vision to audition situation. The present study investigated the effects of smaller visual and auditory cortical removals on CMT from vision to audition (V-A) and from audition to vision (A-V). Thirty-six male hooded rats served as subjects, 18 in the V-A situation, and 18 in the A-V situation. Testing was carried out in an operant chamber controlled by conventional relay equipment. Prior to training or testing of either the V-A or A-V group, 6 animals received aspiration lesions of visual cortex, another 6, lesions of auditory cortex, while the remaining 6 served as sham-operated controls. Within each of the lesion subgroups, half of the animals underwent direct transfer (same positive stimulus, either high or low intensity, in both modalities), while the other half underwent reversal transfer. In either the V-A or A-V situation, only the sham-operates clearly showed CMT ability; both the visual and auditory subgroups were impaired relative to the shams. While operated animals did not show CMT ability, they acquired the intensity discrimination in the first modality as well as the sham-operated controls. Thus, the functional integrity of visual and auditory cortical areas appears to be particularly essential to CMT, since lesions that did not preclude discrimination learning in either modality alone obliterated transfer ability.

821 EFFECTS OF LOCUS COERULEUS LESIONS ON THE SUSCEPTIBILITY OF LABILE MEMORY TO DISRUPTION. Steven F. Zornetzer and Mark S. Gold. Dept. Neuroscience, Coll. Med., Univ. Fla., Gainesville 32610.

The locus coeruleus (LC), located in the lateral pontine tegmentum, has been implicated in the production of paradoxical sleep (PS). Although the function of PS is not known, Moruzzi has speculated that one function of PS might be related to "plastic processes" which occur continuously in organisms interacting with their environments. A number of recent studies suggest that PS increases as rodents learn difficult tasks and conversely, that PS deprivation interferes with acquisition of new information. The present experiments look at the effect of direct LC manipulations and their interaction with memory processes.

Three experiments were done to look at the effects on memory of discrete electrolytic lesions in the LC of mice. In exp.1 mice received lesions of the LC through previously implanted electrodes immediately following training on a 1-trial inhibitory avoidance step-through task. Retention of this response, measured 48 hr. later was normal, suggesting that LC lesions per se do not interfere with the performance of this rather simple response.

In experiments 2 and 3 mice were treated exactly as in exp. 1 except that a transcorneal ECS (15mA, 200mS) was administered 40 hr. after initial training and LC lesions. Only mice with LC lesions were found to be amnesic following such a delayed ECS treatment when tested either 8 hr. or 24 hr. after ECS. These data are interpreted to suggest that the LC is normally involved in the temporal delineation of the susceptibility period of newly formed or labile memory. The possible role of the LC and its norepinephrine connections to widespread regions of forebrain are discussed in relation to memory fixation.

822 THE DORSAL TEGMENTAL NORADRENERGIC PROJECTION: AN ANALYSIS OF ITS ROLE IN LEARNING. David C. S. Roberts*, Marion T. Price* and Hans C. Fibiger. Div. Neurol. Sci., Dept. Psychiatry, University of B. C., Vancouver, Canada V6T 1W5.

The hypothesis that the noradrenergic projection from the locus coeruleus (LC) to the cerebral cortex and hippocampus is an important neural substrate for learning was evaluated. Maze performance was studied in rats receiving either electrolytic lesions of the LC, or 6-hydroxydopamine (6-OHDA) lesions of the dorsal tegmental noradrenergic projection. LC lesions which reduced telencephalic NE to 29% of controls did not disrupt the acquisition of a running response for food reinforcement in an L-shaped runway. Greater telencephalic NE depletions (to 6 percent of control levels) produced by 6-OHDA also failed to disrupt the acquisition of this behaviour or impair the acquisition of a food reinforced position habit in a T-maze. Neither locomotor activity nor habituation to a novel environment was affected by the 6-OHDA lesions. Rats with such lesions were, however, found to be significantly more distractible than controls during the performance of a previously trained response. In another group of rats with identical 6-OHDA lesions neither the acquisition of a passive avoidance step-down task nor the establishment of a lithium chloride conditioned taste aversion was affected by the lesions. The hypothesis that telencephalic NE is of fundamental importance in learning was not supported. The data suggest that this system may participate in attentional mechanisms.

(Supported by the Medical Research Council of Canada)

823 NORADRENERGIC NEURONS IN A MEMORY ACCESS PATHWAY. Richard P. Cohen*, Andrew Kerr* and Martin D. Hamburg. Dept. Anat., Cornell U. Medical College, New York, New York 10021.

A temporary depletion of brain norepinepherine in rats produced by injection of a dopamine beta-hydroxylase inhibitor, diethyldithiocarbamate (DDC), 30 min prior to testing, prevented performance of a trained passive avoidance response 1, 3, 5 or 7 days after training. Subsequent recovery in performance indicated that the memory itself was not destroyed, but rather that the process of memory retrieval was affected. Animals treated with DDC up to 3 hrs before training were capable of learning the passive avoidance task and of avoidance performance for a few hrs after training. However, these animals failed to produce a long-term memory of the trained response.

Injection of a beta-adrenergic blocker, propranolol within 5 min of passive avoidance training produced similar storage amnesias again with a brief post-training period of adequate performance most likely based on short-term stores. If propranolol injection was postponed until 1 or 3 days after training and testing was conducted 2 hrs later, again poor avoidance performance was obtained.

Injection of a direct norepinepherine precursor, dl-threo 3,4,-dihydroxyphenylserine (DOPS) 60 min prior to DDC treatment prevented both the storage and retrieval amnesias.

The results of these experiments suggest that adrenergic neurons contribute significantly to an access pathway of long-term memory. If learning occurred during a period of depleted norepinepherine, training scores were normal and adequate performance was possible for a short period following training when, perhaps, a short-term memory storage was active. However, the formation of long-term memory was prevented and short-term stores were inadequate to support performance a few hours after training. If training occurred in a normal animal and a long-term memory was produced, then norepinepherine depletion prevented retrieval of that memory for a period of hours after injection. However, the memory remianed intact and complete recovery of performance appeared.

824 EFFECTS OF VARIOUS KNIFE CUTS IN THE REGION OF THE BASAL GANGLIA ON LEARNING. Joseph Kelly*, George Alheid* and Sebastian P. Grossman. Behav. Sci. Dept., Biopsych., U. of Chicago, Illinois, 60637.

Several tests of learning were studied in rats following knife cuts interrupting nigrostriatal and/or pallidofugal (or pedal) pathways in the region of the basal ganglia. These included: a) a cut between the caudate and the globus pallidus (Caud); b) a parasagittal cut (Parasag) along the lateral border of the lateral hypothalamus, about 1.5mm shorter in its AP extent than that reported by Kent & Grossman (1973); c) a cut separating the globus pallidus and the internal capsule (G Pall); d) a cut ventral to the globus pallidus (V Pall). The longer parasagittal cut described by Kent & Grossman (1973) produced deficits in pre- and post-operatively learned instrumental tasks (e.g., lever pressing and running for food, water or brain stimulation and escape and avoidance tasks). With our cuts the following behavioral and monoamine results were obtained: a) Caud--some facilitation in acquisition of a conditioned avoidance response (CAR) concomitant with hyperactivity in a stabilimeter; median percent of control for caudate dopamine (DA) (84%), norepinephrine (NE) (110%), hypothalamic NE (98%), 5HT (118%); b) Parasag--CAR acquisition deficit, but no effect on retention, PA deficit and deficit in sucrose rewarded behavior; caudate DA (23%), NE (60.5%), hypothalamic NE (72%), 5HT (140%); c) G Pall--CAR acquisition deficit, some PA facilitation concomitant with open field hypoactivity, sucrose runway and swimming (cold) deficits; caudate DA (43.5%), NE (84%), hypothalamic NE (87%), 5HT (121%); (d) V Pall-CAR retention deficit concomitant with stabilimeter hyperactivity and deficits in PA and swimming (warm); caudate DA (47%), NE (84%), hypothalamic NE (106.5%), 5HT (158%). These results suggest that fibers entering or leaving the globus pallidus may be important for acquisition of appetitive behaviors as well as active and passive avoidance produced by the parasagittal cuts reported here and by Kent and Grossman (1973).

825 INTER VS. INTRAHEMISPHERIC LEARNING IN DYSLEXIC AND NORMAL READERS. Frank R. Vellutino, William L. Bentley*, Louis DeSetto*, Forman Phillips*. Dept. Ed. Psy., Sch. Ed., SUNYA, Albany, 12222 and Dept. Ped., Sch. Med., AMC, Albany, 12208.

In pursuing the possibility that dyslexia is due to dysfunction in some aspect of verbal learning, two alternative hypotheses were assessed: (1) that the disorder is caused either by language deficiencies (intrahemispheric dysfunction) or (2) by difficulties in associating visual and verbal equivalents that may be stored in each of the brain hemispheres (interhemispheric transfer). In order to evaluate these alternatives, dyslexics and normal readers in second and sixth grades, were given tachistoscopic presentations of novel visual stimuli (Chinese idiographs), randomly presented to the left and right visual fields, and to the central fixation point. Each stimulus was paired with a common English word, orally presented, and subjects were asked to associate the two. A transmission (interhemispheric) deficit hypothesis predicts that poor and normal readers would be differentiated in the case of presentations to the right hemisphere (left visual field), but would not differ significantly in the case of presentations to the left hemisphere (right visual field). However, a linguistic deficit hypothesis suggests that reader groups would be differentiated under all three conditions, since the response words were presented auditorily and were thus represented in the language (left) hemisphere. The results favor the linguistic deficit hypothesis. Normal readers in both second and sixth grades performed significantly better than poor readers in those grades, the findings being consistent under all three experimental conditions.

826 CLASSICAL CONDITIONING IN PARAMECIUM. Todd M. Hennessey*, Wm.B. Rucker, and Colin G. McDiarmid* Department of Psychology, Mankato State College, Mankato, Minnesota, 56001

Single Paramecium caudatum were captured in a capillary tube attached to a audio speaker. Shock (2 sec, 5 volt AC) was initiated during the last two seconds of tone (4 sec, 350 Hz). Paramecia reliably exhibited spinning (unconditioned) responses to the shock, but not initially to tone alone. When spinning occurred during the tone-alone period, this anticipatory conditioned response (ACR) was assigned a liner coefficient for that trial, the sum of which translated responses into linear trends to test for conditioning. ($t = 5.17$, $p < .001$). Other subjects trained with a tone of 300 Hz conditioned faster than subjects trained with a tone of 500 Hz ($t = 2.11$, $p < .05$). Animals given 15 trials with 350 Hz tone and then stored individually for 24 hours showed no improvement on Day 2, implying memory of their conditioning. Naive subjects stored 24 hours and tested for the first time in the blind on Day 2 did show significant increase in ACRs over trials. Control subjects given alternating tone-alone (4 sec) and shock-alone (2 sec) showed a transient increase in spinning during tone-alone (pseudoconditioning), but did not show the same sustained improvement in performance demonstrated in the experiments pairing the two stimuli ($t = 1.11$, $p > .05$). Thus it appears that classical conditioning has been demonstrated in this protozoan.

Media used in the sound-shock experiments was compared to the same media without training. No changes were found for the concentration of hydrogen, sodium, potassium or calcium ions (tests accurate to 1 ppm). Animals shocked in deionized distilled water also showed spinning and ACR before rupturing. The conditioning is not a function of changes in the subjects environment. Supported by M.S.C. Faculty Research Grant.

827 LEG FLEXION RESPONSES IN CHRONIC SPINAL AND NORMAL KITTENS DURING INSTRUMENTAL CONDITIONING. R. G. Durkovic. Dept. Physiology, Upstate Medical Center, Syracuse, N. Y. 13210

Leg flexion avoidance responses were studied in kittens with low thoracic spinal cord transections (1-10 wks. post-op). Experimental animals received foot shock (5/sec, 10 ma) whenever they lowered a particular hind leg, interrupting a photocell circuit. Shocks were terminated whenever the leg was flexed 6 mm or more above the resting (extended position). None of these kittens developed tonic leg flexion in order to avoid foot shock over a 40 min. period. Instead they received a gradually increasing number of foot shocks. Yoked control animals received the same foot shocks as experimentals during the initial 20 min. of the conditioning period regardless of leg position. In general experimental animals spent more time in the flexed position than did yoked control animals during this period. Church (Psych. Bull. 62:122, 1964) has pointed out such differences could result either from the temporal relationship between response and event or from various sources of random error not associated with learning behavior.

During the second 20 min. of the conditioning period in which both experimental and previously yoked controls (PYC) received shock contingent on lowering their own leg, the experimental animals received fewer shocks than PYC animals. These results suggest the presence of learning-like behavior (shock-avoidance) superimposed on habituation or fatigue processes. PYC animals which received the same amount and distribution of shocks as experimentals during the initial 20 min. period were apparently modified in ways which hindered the acquisition of avoidance during the second 20 min. period. Normal kittens exhibited qualitatively the same behavior on this paradigm as spinal kittens. Supported by S.U.N.Y. Res. Found. Grt. No. 67226.

Limbic System and Cerebral Cortex

828 VASCULAR PATTERNS IN THE RAT HIPPOCAMPUS. Peter Coyle, Dept. Anat., Sch. Med., The University of Michigan, Ann Arbor, 48104

Conventional brain lesion and ablation techniques, chemical injection cannulae, electrodes, and probes when inserted into the brain damage blood vessels with consequent neuronal injury or death. The locations and extent of neuronal injury depend on the vessels involved, their field distribution sizes, collateral sources, and requirements of the neurons. This study investigated the course and field distribution of vessels supplying and draining the rat hippocampus. One hundred adult Wistar strain rats were anesthetized with nembutal and perfused via the arterial or venous sytem with India ink, silicone rubber, fluorescent paint, bioplastic or agar gelatine or a combination of the agents. Arterial supply to the hippocampus is via transverse hippocampal vessels that stem from the longitudinal hippocampal artery, a branch of the posterior cerebral artery. The transverse hippocampal arteries course in the hippocampal fissure, supply few branches to the adjacent blade of the fascia dentata, subiculum, prosubiculum, and Cornu Ammonis (CA) field 1, but enter CA3 fields, branch profusely and supply these and the area dentata. Other vessels stem from the longitudinal artery to supply the remaining structures. Transverse hippocampal veins course in the fissure, alternate in position along the longitudinal hippocampal axis with the transverse arteries to CA3 and drain into a longitudinal vein. Other deep transverse veins exist on the alvear surface. Field sizes of the distributing arteries are being investigated by fluorescent and optical dissection technique. To date, the data suggest a highly segmental arterial and venous organization exists for the hippocampus.

829 REGIONAL DISTRIBUTION OF SEROTONERGIC NERVE ENDINGS WITHIN THE RAT HIPPOCAMPUS. Kenneth J. Kellar, William R. Mehler and Joan Vernikos-Danellis.* NASA-Ames Research Center, Moffett Field, Ca. 94035.

The distribution of nerve terminals containing serotonin (5-HT) within the hippocampus has not been fully described. This may be due, in part, to the instability of the fluorescence obtained with this amine using histochemical techniques and the difficulty of quantitating histofluorescence. We have examined the regional distribution of serotonergic nerve endings in the hippocampus by measuring the uptake of 5HT and tryptophan into the rostral, middle, and caudal thirds of the hippocampus of the rat and by measuring the enzyme tryptophan hydroxylase in these same areas. The uptake of 5HT (1×10^{-8} M) into the caudal third of the hippocampus is approximately 128 percent and 70 percent greater than into the rostral and middle thirds respectively ($p < .01$). The uptake of tryptophan (1×10^{-6} M) into the caudal third of the hippocampus is approximately 88 percent and 64 percent greater than into the rostral and middle thirds respectively ($p < .05$). Lesions studies are under way to determine the relative percentage of nerve endings in the hippocampus which project from the midbrain raphe nuclei.

830 THE TOPOGRAPHICAL ORGANIZATION OF THE PROJECTIONS FROM SUBICULUM TO MAMMILLARY BODIES. Richard C. Meibach* and Allan Siegel. Depts. Anatomy and Neuroscience, N. J. Medical School, Newark, New Jersey 07103.

We have recently demonstrated that fornix fibers which project to the mammillary bodies (Mb) arise from the subiculum (Brain Research, 88:508, 1975). In this study we have utilized both retrograde {horseradish peroxidase (HRP) histochemistry} and anterograde {^{3}H-leucine radioautography} neuroanatomical tracing methods to determine more precisely the organization of this pathway. Injections of HRP (0.08-0.1 µl of a 35% conc.) were placed in different portions of Mb in the rat while leucine injections (10µCi/µl) were made into all levels of dorsal hippocampus, dorsal subiculum (DS), ventral hippocampus, and ventral subiculum (VS) in the same species. A topographical projection from the subiculum to the anterior half of the pars posterior (pp) of the medial mammillary nucleus was observed. Fibers from anterior DS pass through the dorsal fornix and terminate throughout the dorsal aspect of the pp. Fibers originating from VS project through the fimbria and terminate in the ventral aspect of the pp while fibers which originate from posterior subiculum project to the central portion of this nucleus. Further, more medial aspects of the pp receive fibers from regions of the DS closest to field CA1 of hippocampus while more lateral aspects receive fibers which arise from cells in the DS which lie closest to retrosplenial cortex. No evidence was found to support the view that hippocampal pyramidal cells project directly to Mb.

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831 THE MIDLINE SEPTUM TELENCEPHALI: A RAPHE. R. B. Chronister*, J.F. DeFrance, B. Srebro and L.E. White, Jr., Department of Neurobiology, Univ. of South Alabama, Mobile, 36688; Department of Anatomy, Wayne State Univ., Detroit, 48201; Institute of Physiology, Univ. of Bergen, Bergen, Norway; Div. of Neuroscience, Univ. of South Alabama, Mobile, 36688.

Morphological and electrophysiological observations have been undertaken in rats and cats to elaborate the functional anatomy of the medial septal-diagonal band region. Using modified rapid Golgi impregnations, cells can be seen on the midline itself. These neurons, which are characteristically fusiform in shape, have dendritic domains restricted to the immediate bilateral midline area. Fiberarchitectural observations indicate that these cells are influenced by the ascending midline projection of the medial forebrain bundle (MFB) or Zuckerkandl's bundle. Electrophysiological studies have verified that these cells respond to bilateral subthreshhold stimulation of Zuckerkandl's bundle, or MFB, to produce action potentials. On the other hand, these cells do not respond to fornix-fimbrial stimulation. Cells lateral to the midline do respond to fimbrial stimulation. The axon distributions of the cells are difficult to determine but some have been followed to the more lateral portions of the medial zone and some appear to descend in Zuckerkandl's bundle. Histochemical observations (AChE) have shown that some of these midline cells are cholinergic in nature. All of these observations lead to the conclusion that the midline septal area may be a functionally distinct area of the septum telencephali. (Supported by NIH Grant No. NS-10809 and NSF Grant GB 35532).

832 ELECTROPHYSIOLOGICAL STUDY OF THE DESCENDING PROJECTIONS OF THE HYPOTHALAMUS TO THE MIDBRAIN TEGMENTUM. Henry M. Edinger, Stanley Z. Kramer* and Allan Siegel. Depts. Physiology, Anatomy and Neuroscience, New Jersey Medical School, Newark, New Jersey 07103.

The purpose of this experiment was to study the electrophysiology of the efferent outflow of the hypothalamus to the midbrain ventral tegmentum in the anesthetized cat. Tungsten microelectrodes were used to record single unit responses in the ventral tegmentum following electrical stimulation of the lateral hypothalamus, preoptic area, and septum. Stimuli were delivered by means of stainless steel semi- microelectrodes and were 50-900 μamps in intensity. Responsive units were found only at levels rostral to the exit of the oculomotor nerve. The lateral hypothalamus was found to be the most effective site from which neurons in the ventral tegmentum were activated. Antidromic activation of units in the medial portion of the ventral tegmental region were prominent and indicated that the conduction velocity of the ascending fibers was approximately 1-2 m/sec. Monosynaptic driving of units in the medial and lateral portions of the ventral tegmentum was observed. Monosynaptic and antidromic activation was frequently followed by a suppression of activity lasting 40-120 msec. Other units in these regions as well as units in the central grey and midbrain reticular formation exhibited mainly inhibition of short duration in response to shocks delivered to the preoptic- hypothalamic area. Few units in the ventral tegmentum responded to stimulation of the prefrontal cortex. (Supported by NIH grant #NS 07941-06.)

833 AN ELECTROPHYSIOLOGICALLY IDENTIFIED PROJECTION FROM DORSAL FORNIX TO THE CEREBELLUM IN THE RAT. J. A. Saint-Cyr and D. J. Woodward. Depts. Psychology, Physiology and Center for Brain Research, Univ. of Rochester, Rochester, N.Y. 14642

In view of known widespread connections between the neocortex and the cerebellum through pontine and brainstem reticular relays, the question arose as to whether parallel projections to cerebellum might exist from the hippocampus via the fornix. Activity of Purkinje cells was recorded in halothane-anesthetized albino rats of both sexes which received bilateral electrical stimulation of the dorsal fornix, anterior-dorsal hippocampus, fore- and hindlimb muscles and snout. Stimulation of dorsal hippocampus or fornix elicited short latency, 5-6 msec, and long latency, 15-16 msec, simple spike responses (via mossy fibers) as well as complex spike responses at 18-20 msec (via climbing fibers). In addition, longer latency oscillations of climbing fiber activity with a period of about 80 msec were commonly observed. The strongest mossy fiber responses were recorded from the vermis of lobules 4, 5 and 6a. Climbing fiber responses were observed over a similar but slightly larger area. Mossy and climbing fiber responses elicited by snout and forelimb stimulation overlapped these areas to a great extent. Possible pathways from fornix to cerebellum may involve relays in the paramedian pontine nucleus and adjacent tegmentum. The convergence of hippocampal, somatosensory, and teleceptive information in these areas could play a role in the execution of behaviors related to spatial orientation. (NSF Grant GB-43301).

834 HYPEREMOTIONALITY AND INCREASED IMMOBILITY AFTER SEPTAL LESIONS IN THE RABBIT. Michael L. Woodruff and Walter Lippincott*. Dept. Psychol., Middlebury College, Middlebury, Vt., 05753.

Hyperemotionality subsequent to septal lesions has been seen in rats and mice, but apparently occurs only rarely in cats and not at all in guinea pigs, golden hamsters or monkeys (see Fried, Psych. Bull. 76: 292, 1972, for a review). On the other hand, dysfunction of the ability to withhold responses which have a high probability of occurrence has been a consistent observation in a variety of species after septal damage (McCleary, Prog. Physiol. Psych. V. 1, 210, 1966). The present experiment was designed to test the effects of septal lesions on tonic immobility (TI) in rabbits. TI is a state of profound motionless induced by sudden inversion and restraint. It has fair species generality, having been demonstrated in amphibians reptiles, birds and mammals (Gallup, Psych. Bull. 81: 836, 1974). A previous study indicated that hippocampal lesions prolonged TI (Woodruff et al. J. comp. physiol. Psych., 88: 329, 1975) and because the effects of hippocampal and septal lesions are similar on many tasks it was thought that septal damage would also potentiate TI. Cathodal electrolytic lesions were produced in the septal area of 8 albino rabbits (Group S). Eight rabbits served as sham operated controls (Group C) and 7 rabbits were unoperated controls (Group N). Immobility was induced in each rabbit by inversion and restraint both preoperatively and 30 days after the operation. Preoperatively the mean TI duration for the rabbits in Group S was 146 sec. The The rabbits in Group C had a mean duration of 80 sec and the rabbits in Group N had a mean of 68 sec. These means did not differ statistically. Postoperatively Group S had a mean duration of 866 sec, Group C a mean of 53 sec and Group N a mean of 37 sec. The postoperative mean of Group S was significantly longer than the means of either of the other two groups (Kruskal-Wallis 1-way ANOVA, $p<.001$; Mann-Whitney U, p .01). A within sample comparison indicated that only the postoperative mean for Group S was significantly different from the preoperative mean (Wilcoxon Matched Pairs Signed Ranks Test $p<.01$). Because TI is a response which involves absolute motionless these results are interpreted as indicating that septal lesions not only produce a deficit in the ability to inhibit a response which involves movement, but also in the ability to inhibit a response which involves the absence of movement, if as McCleary (ref. cit.) proposed, that response has a high probability of occurrence within a given paradigm.

In addition to the increase in TI duration a very definite increase in emotionality was exhibited in all 8 rabbits with septal lesions. This hyperemotionality was exhibited in three separate responses. As the rabbit's cage was approached it would begin to thump its hind feet. This is a normal response for the rabbit, but simply the approach of a person was not observed to produce this reaction in the control rabbits in this study. Second, the rabbits in Group S were almost impossible to catch and, finally, once they were caught by the scruff of the neck they began to vocalize, emitting a continuous high pitched scream. This is the normal distress call of the rabbit, but once again it was not evoked in the control rabbits when they were handled in a similar manner. This series of responses was observed from immediately after the operation until the rabbits were sacrificed 35 days postoperatively. This observation suggests that the form of hyperemotionality exhibited after septal lesions will depend upon the species under consideration, a proposal previously made by Slotnik et al. (Brain, Behav. Evol. 8: 241, 1973).

835 DIURNAL VARIATION PATTERNS IN LIVER GLYCOGEN LEVELS IN NORMAL, HIPPOCAMPECTOMIZED, ADRENALECTOMIZED AND HYPOPHYSECTOMIZED RATS. Cyrilla H. Wideman, Helen M. Murphy and Thomas S. Brown. John Carroll Univ., Cleveland, Ohio 44118 and DePaul Univ., Chicago, Ill. 60614

Cyclic glycogen levels in animals are dependent upon a number of factors. Among these are diurnal variation, hypothalamic-pituitary-adrenal hormones and food intake. This study dealt primarily with the first two factors. Diurnal variation entailed 12 hrs. of light followed by 12 hrs. of dark. Variations in hormone levels were achieved by manipulating body parts which have an effect on the hormones under consideration. Previous studies have shown that the level of glucocorticoids increases in hippocampectomized animals and decreases in adrenalectomized animals. With the removal of the hypophysis ACTH and glucocorticoid levels were lowered. With increased levels of glucocorticoids, the process of gluconeogenesis is accelerated and storage of liver glycogen is enhanced. In this study, 96 male rats were randomly divided into 4 groups or 24 animals each. The four groups included normal, hippocampectomized, adrenalectomized and hypophysectomized rats. Three animals from each group were sacrificed at 3 hr. intervals. The experiment demonstrated that all groups of animals had different levels of liver glycogen throughout a 24 hr. period. Despite fluctuations in the specific amount of glycogen in these animals, the diurnal variation cycle was the same for all groups. The general pattern of the hippocampal lesioned and normal groups showed a gradual increase and decrease in liver glycogen levels. Thus both groups appeared to possess a regulating system which gradually increased or decreased the liver glycogen level. In the adrenalectomized and hypophysectomized animals there was a sharp fall and rise in liver glycogen levels throughout the 24 hr. cycle, indicative of a lack of regulation. In addition, the peak and trough of the hypophysectomized animals appeared three hours prior to the other three groups.

836 HIPPOCAMPAL FORMATION RESPONSES TO ELECTRICAL STIMULATION OF THE OLFACTORY BULB AND TRACT IN THE SQUIRREL MONKEY. Garl K. Rieke and Jody P. Corey*. Dept. Anatomy, Hahnemann Medical College and Hospital of Philadelphia, Pa. 19102.

Biphasic responses were evoked from the cortex of the parahippocampal gyrus of anesthetized (pentobarbital, 35 mg/Kg) male squirrel monkeys, following electrical stimulation of the ipsilateral olfactory bulb or lateral olfactory tract. The responses had a short onset latency (4-6 ms), could not be driven above 35-50 Hz, and demonstrated little or no post-tetanic changes. Stimulation of the cortex of the parahippocampal gyrus evoked small amplitude, short onset latency responses from the ipsilateral olfactory bulb.

A long onset latency (45-50 ms) monophasic positive polarity response was evoked in the dentate gyrus following tract and bulb stimulation. This response was maximal in the molecular layer of the dentate gyrus, although its growth in amplitude was apparent as the recording electrode was withdrawn from the surface of the parahippocampal gyrus. This response could not be driven beyond 10 Hz, however, at low frequency stimulation (1 Hz) of the tract or bulb, it increased in amplitude and became biphasic. This configuration was maintained for up to 60 seconds and then decayed to the original monophasic positive polarity response. This is suggestive of recruitment, with the spreading excitation reflected by the enhancement of the magnitude of the extracellularly recorded response.

In conclusion it is apparent that direct stimulation of the olfactory bulb and tract does elicit responses from the ipsilateral deep structures of the hippocampal formation. Recent observations in the rat, cat and ferret have clearly shown that there is a direct olfactory input to the entorhineal cortex via the lateral olfactory tract or collaterals of the tract. The data presented here suggests that the entorhineal cortex of the squirrel monkey receives a direct olfactory input through the tract.

837 RELIABILITY OF THE RELATIONSHIP BETWEEN HIPPOCAMPAL UNIT ACTIVITY AND BEHAVIOR IN THE RAT. Phillip J. Best and James B. Ranck Jr. Dept. of Psychol. Univ. of Va. Charlottesville, Va. 22901 and Dept. of Physiol. Downstate Med. Center, Brooklyn, N. Y. 11203.

The firing pattern of hippocampal units in the freely behaving rat is related to movement, to appetitive and consummatory behavior and to the cessation of those behaviors (Ranck, E. Neurol. 40:461,1973). Some hippocampal cells fire maximally when the animal is in a distinct place in his environment (O'Keefe and Dostrovsky, Brain Res. 34:171,1971). The complex nature of the relationship between hippocampal neural activity and behavior is very difficult to objectively quantify. We are therefore left with the perhaps unreliable subjective evaluation of the experimenter.

In the present study the reliability of the relationship between cellular activity and behavior was assessed by making TV tapes of recording sessions and presenting these tapes to a number of observers. The recording sessions lasted about 20 minutes, during which time a variety of behaviors was sampled, following the procedure of Ranck(1973). During each session the oscilloscopic display of the cellular activity was pictured on the top 1/4 of the screen and the behaving animal was pictured on the bottom. The output of a discriminator was connected to the audio channel of the tape. Tapes were made from 19 hippocampal and one thalamic unit. In addition, three control tapes were made in which the cellular activity from one session was superimposed upon the behavior from another session. The tapes were presented to 20 undergraduates at the University of Virginia who had recently completed a course in neuropsychology, but who had not been exposed to the literature on hippocampal neural activity in awake animals. The observers were instructed to keep a running account of the behaviors corelated with fast and slow cell activity, to draw conclusions about the relationship between the cell's activity and behavior, and to state their confidence in their conclusions.

The observers showed virtual unanimity and high confidence on 12 of the 23 cells and virtually no confidence or agreement on 5 cells. In general the greater the agreement between the observers, the greater the confidence of each observer. The 5 cells on which no confidence or agreement was found included the three control tapes, the thalamic cell and one true hippocampal cell. Of the remaining 18 cells, 9 were judged to have the spatial properties described by O'Keefe and Dostrovsky(1971). Four of these were purely spatial, that is they increased in firing when a rat was in a certain place in the environment independant of the behavior at that place. The other five were judged to fire fastest when the animal made a particular movement at or towards a specific place. Seven other cells showed some relationship to movement. In two of these cells the relationship was very specific, that is the cell fired at the termination of a specific movement, such as turning. The other five increased in activity during any movement. These latter cells fired much faster in general than the other cells and belong to the class of "theta" cells described by Ranck(1973). One of the remaing two cells was judged to be related to drinking, the other was related to sniffing behavior. No cells were clearly related to feeding. However, some increase in firing was seen by some observers in some spatial cells if the animal ate in the significant place.

The data show that despite the complex and frequently loose relationship between hippocampal unit activity and behavior in the freely behaving rat, the assessment of that relationship by different observers is highly reliable. Although the particular frequency of the different behavioral types of cells seen in this study differs somewhat from those seen in previous studies, this study basically confirms the presence of the variety of behavioral types of cells reported before.

838 HIPPOCAMPAL THETA RHYTHM: TOPOGRAPHIC PATTERNS IN CURARIZED AND FREELY MOVING ANIMALS. Jonathan Winson. Rockefeller Univ., New York, NY 10021.

Studies have been done on the depth profiles of theta rhythm in the curarized rabbit (Green et al., J. Neurophysiol. 23: 403, 1960) and in the freely moving rat (Winson, Electroenceph. clin. Neurophysiol. 36: 291, 1973). The profiles have been found to be different in the two preparations. The present investigation was carried out to determine the source of the difference. Hippocampal electrical activity was recorded from movable microelectrodes and fixed reference electrodes in rats and rabbits. Data were computer-analyzed for mean values and standard errors of the mean of theta rhythm phase and amplitude. Rats were tested in both freely moving and curarized states. Rabbits were tested during three behavioral conditions: REM sleep, voluntary movement, and the application of sensory stimulation to the motionless animal. The results of these studies were the following:

1) In all three behavioral states observed in the rabbit, the profiles of theta rhythm were the same. The common profile showed a sudden phase reversal in the stratum radiatum of CA_1 and a maximum point of theta amplitude at the level of the dorsal blade of the dentate gyrus. These findings imply that in the freely moving rabbit, as in the freely moving rat, there are two generators of theta rhythm, one in the dentate gyrus and the other in the overlying CA_1 layer. The data also indicate the existence of a species difference in generating systems between the rabbit and the rat.

2) Systemic injection of curare changed the profile seen in the freely moving rat to a profile similar to the type seen in the rabbit. In addition, curare altered the relationship between the amplitudes of the two phase-reversed components of the theta rhythm. The change in theta rhythm brought about by curare outlasted the paralytic effect of the drug.

839 HIPPOCAMPAL THETA IN NORMAL AND LATERAL HYPOTHALAMIC-DAMAGED RATS. Marc De Ryck* and Philip Teitelbaum. Dept. of Psychology, University of Illinois, Champaign, 61820.

It has been pointed out that in normal rats hippocampal rhythmic slow activity (RSA or theta) accompanies voluntary actions such as walking, running, rearing, etc. Large irregular amplitude activity (LIA) goes along with immobility and automatisms such as grooming, chewing, and licking (Vanderwolf, Psych. Rev., 1971, 78:83). We have repeated and verified Vanderwolf's observations. Lateral hypothalamic (LH) damage, sufficient to produce aphagia, abolishes voluntary actions (walking, rearing, orienting) leaving intact automatisms such as grooming, chewing, and sneezing (Levitt & Teitelbaum, PNAS, 1975, in press). Nevertheless, like Robinson & Whishaw (JCPP, 1974, 86:768), we find slow theta present in LH animals. Such theta appears in grooming, chewing or even reflex automatisms such as head-shake to tactile stimulation of the auditory meatus. Re-examination of automatisms such as grooming in normal awake rats revealed LIA in face grooming (when head is held immobile but paws move) whereas theta occurs in grooming when the head and neck move. Indeed, theta seems to occur whenever head and neck are moved, regardless of whether the pattern is automatic or voluntary. More detailed description of behavior patterns may be necessary for a better understanding of hippocampal theta.

840 EFFECTS OF LESIONS IN DIFFERENT REGIONS OF HIPPOCAMPUS UPON COLOR MATCHING BY RHESUS MONKEYS. William J. Jackson. Dept. of Physiology. Medical College of Georgia, Augusta 30902

Although previous reports have indicated hippocampal lesions do not impair acquisition of matching when errors are corrected, present findings indicate that monkeys with certain types of hippocampal damage are impaired in ability to match if training is conducted without error correction. Monkeys with anterior (uncal), middle, or posterior hippocampal lesions were compared to appropriate controls in ability to match colored symbols. The sample stimulus was presented simultaneously with 2 choice stimuli.

Findings showed that while all groups initially formed position habits, only middle and posterior hippocampal lesion groups were unable to break position habits and match at above-chance levels within a limit of 3,500 trials. Although monkeys with anterior hippocampal damage ceased position habits at the normal rate, they began to show a peculiar "short-session effect" just when correct matching began to reach above-chance levels. The short-session effect was that affected monkeys rejected the food pellet reward and ceased working after completing an average of 25 of the usual 200 trials per daily session. Usually correct matching was above the 90% level for those 25 trials, but the monkeys would not eat the food pellets. Animals with middle or posterior hippocampal lesions were also able to cease position responding when given benefit of appropriate hand-shaping, but they also manifested the short-session effect when performance just began to reach above-chance levels. The short-session effect continued for months in several instances, but was alleviated permanently by 5mg./kg. Oxazepan administered daily for one week.

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841 REVERSAL LEARNING AND SEPTAL LESIONS. Thomas Chin*, Roger Sikorszky*, and Peter J. Donovick. Dept. Psychol., State Univ. N.Y., Binghamton, N.Y., 13901.

We previously reported (Chin, T., Burright, R. & Donovick, P., presented at Psychonomic Soc., 1974) that septal lesions disrupt the reversal of a brightness discrimination. However, the magnitude of the reversal deficit was found to be related to experience with the discrimination cues and the response measure employed.

The present two experiments were designed to examine (1) whether a reversal deficit would appear if original learning occurred prior to production of septal lesions; (2) changes in response strategies over repeated reversals; and (3) if repeated reversal experience on a brightness discrimination would differentially alter subsequent septal vs control groups acquisition and reversal of a spatial discrimination.

Even when original learning took place prior to surgery, rats with septal lesions were originally deficient on the reversal of a brightness discrimination. As noted in our previous reports the difference between lesioned and control animals was most apparent in the repeated error measure. By the third reversal, however, both groups performed comparibly.

In the second experiment naive animals were trained on a brightness discrimination and given four reversals on that problem prior to being trained on a spatial discrimination and four reversals in a T-maze. Once again the septal deficit as reflected in the repeated error measure on the brightness discrimination was only seen in the first two reversals. Rats with septal lesions were slower to reverse in the spatial discrimination situation. There was no indication that prior brightness discrimination and reversal training improved performance of lesioned animals relative to that of control rats.

842 COMPLEXITY AND REVERSIBILITY OF THE SEPTAL PASSIVE AVOIDANCE DEFICIT. Wail A. Bengelloun*, and Richard G. Burright* (Sponsor: J. L. Fuller) Dept. Psychol., State Univ. N.Y., Binghamton, N.Y., 13901.

Male rats were tested on the acquisition of a water passive avoidance task with electric footshock as the aversive stimulus. Rats with septal lesions performed as well as control animals (both in terms of days to criterion and number of shocks to criterion) when limited to one punished response per day, but exhibited the classic septal deficit when permitted to receive as many shocks as they initiated during daily 5-min sessions. Whereas a presurgical history of sodium depletion did not affect the behavior of controls, it eliminated the septal lesion-induced passive avoidance deficit. In a second experiment subjects were cued (offset of houselight) if they approached the water spout. Here it was found that old rats (300-400 days) required more shocks to meet criterion in both control and septal lesion conditions, when compared to younger (100 day) animals. Moreover, the availability of the cue did not diminish the lesion-induced deficit in older animals. These results further indicate the importance of both experiential and situational variables in determining the effects of septal lesions.

843 EVALUATION OF THE ROLE OF THE MAMMILLARY BODIES, ANTEROVENTRAL THALAMUS, ANTEROMEDIAL THALAMUS, AND DORSOMEDIAL THALAMUS IN SHORT-TERM MEMORY. Jennings N. Naranjo* and Ernest G. Greene. Dept. of Psychol. University of Southern California, Los Angeles, Ca., 90007.

A number of clinical studies have suggested that the hippocampus and mammillary bodies are essential in the storage of memory, but this has received only limited support from work using animals. This research compared rats with lesions of mammillary bodies, anteroventral thalamus, anteromedial thalamus and dorsomedial thalamus in their ability to perform in a task which is sensitive to short-term memory deficits (delayed-alternation). Placement of lesions was verified using Nissl sections, and by tracing the pattern of projections using a silver degeneration stain. The results indicate that mammillary body lesions impair delayed-alternation memory, but there was no impairment following damage to the other structures. These results are consistent with the position that the hippocampus does play a role in the storage of memory. Postcommissural output to the anterior thalamic groups is not needed for the performance of this memory function, but the projections to the mammillary bodies are essential.

844 SYSTEMATIC ISOLATION OF THE PREOPTIC AREA WITH A MICROKNIFE: A NEUROANATOMICAL AND BEHAVIORAL ANALYSIS OF THE DISRUPTION OF SEXUAL BEHAVIOR IN MALE RATS. Henry Szechtman*, Anthony R. Caggiula* and David Wulkan*. (SPON: C. W. Malsbury), Psychobiology Program, Dept. Psych., Univ. of Pittsburgh, Pittsburgh, Pa. 15260.

The medial preoptic-anterior hypothalamus (MPOAH) is crucial for sexual behavior in a number of species. In an attempt to identify the fibers which communicate with the MPOAH in mediating sexual behavior in the male rat, a specially designed 1.7mm long microknife was used to systematically sever MPOAH innervation in 4 planes: anterior, posterior, parasagittal and dorsal to MPOAH. Only cuts parasagittal to the MPOAH lowered the likelihood of copulation: 34 out of 71 males did not copulate on 2 or 3 of the 3 half-hour weekly tests. Cuts most effective in producing this impairment fell bilaterally within .2mm of the junction between the MPOAH and the lateral preoptic area; even if one of the cuts was more than .2mm lateral or medial of the MPOAH-lateral preoptic border, the sexual deficit was less severe. Furthermore, the most effective cuts were also coextensive with the whole MPOAH continuum although cuts coextensive with the medial preoptic nucleus were more effective than cuts coextensive with the anterior hypothalamic nucleus. The lowered probability of copulation was partly a reflection of the difficulty these males had in starting to mate since, 1) if a male intromitted he invariably reached an ejaculation without any difficulty; 2) on tests on which copulation was observed, the latencies to the initial mount and intromission were dramatically extended; and 3) some males mated sporadically. Since similar behavioral changes are found after MPOAH or medial forebrain bundle lesions, it was suggested that parasagittal knife cuts interrupted vital medial-lateral connections between the 2 structures.

A neurological examination revealed that males with parasagittal knife cuts were still responsive to visual, olfactory and somesthetic sensory input and that their genital reflexes appeared unaltered. Furthermore, they still preferred the odour of a receptive compared to a nonreceptive female. However, males which recovered the ability to copulate, were very dependent on the female to initiate copulation, since, more than controls, they did not copulate with females which displayed lordosis but not lure behavior. Moreover, the lack of evidence for a disruption of the pituitary-gonadal system juxtaposed with several behavioral parallels to castrated and immature males, led to the hypothesis that the parasagittal knife cuts interrupted the influence of hormones on sexual behavior.

Although only 1 of the 18 males with cuts dorsal to the MPOAH did not copulate on 2 of the 3 postoperative tests, nevertheless, the pattern of copulatory performance was altered dramatically by these cuts. Males with cuts which interrupted dorsal innervation to the MPOAH required more time to reach an ejaculation, more intromissions and more time between each intromission response. However they were quicker to resume mating after an ejaculation. In contrast, males with transverse cuts anterior to the medial preoptic nucleus or posterior to the anterior hypothalamic nucleus showed no change in copulatory performance.

845 A DIRECT AMYGDALO-PREFRONTAL CONNECTION IN THE RHESUS MONKEY. Stanley Jacobson* and Elliot M. Marcus. Depts. Anat. and Neurol., Tufts Univ. Sch. Med., Boston, MA 02111.

Several afferent systems converge on the prefrontal granular cortex. These systems originate in associational areas of the visual, auditory and somatosensory cortices. Recently with tritited amino acids in the rat and cat amygdaloid projections have been traced to the homologue of primate prefrontal cortex in the rat and cat. In view of these findings we have studied the prefrontal granular cortex of the rhesus monkey to determine whether a similar connection exists in the cerebrum of this primate.

In adolescent rhesus monkeys horseradish peroxidase (HRP) was injected throughout the convexity and medial surface of the prefrontal zone. HRP positive cells were seen in the amygdala following injections confined to the dorsal half of the convexity and medial surface of the hemisphere in areas 46, 10, 9 and 8b. The cells were confined to the lateral division of the basal nucleus.

This finding of a direct amygdaloid input to prefrontal granular cortex suggests that this region in the prefrontal zone may be the cortical area which is superimposed on the limbic cortical and subcortical regions just as the motor cortex is superimposed on the other cortical motor and subcortical motor regions. These observations on amygdaloid-prefrontal connections will be compared to connections seen following injections of tritiated amino acids and HRP into the amygdala and other frontal and temporal cortical areas.

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846 SOME OBSERVATIONS ON THE HIPPOCAMPAL-NUCLEUS ACCUMBENS RELATIONSHIP. DeFrance, J. F. and Yoshihara, H. Morin Memorial Laboratory, Wayne State University, School of Medicine.

The nucleus accumbens septi (nAcS) has been grouped, on the basis or morphological appearance, with the caudate nucleus of the basal ganglia. However, through its input-output pathways it establishes a close relationship with limbic and paralimbic structures. As a first step in an electrophysiological analysis of the nAcS, the distribution and nature of the hippocampal input was examined in acutely prepared cats.

The field response following hippocampal or fimbria stimulation, is dominated by a large negative wave. The negativity was found to be an action current envelope of unitary discharges. Power and paired-stimulus testing indicated that the field extracellular unitary response represented the monosynaptic activation. Intracellular power studies of EPSPs confirmed the extracellular unitary analyses. Some units which responded to fimbria stimulation in a monosynaptic manner also responded to medial forebrain bundle stimulation.

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847 EFFECTS OF CRYOGENIC BLOCKADE OF DORSAL FORNIX AND FIMBRIA UPON HIPPOCAMPAL THETA RHYTHM AND SEPTAL CELLULAR ACTIVITY. Charles L. Wilson, Brad C. Motter*, and Donald B. Lindsley. Depts. Psychol., Physiol., Psychiat., and Brain Research Inst., UCLA, Los Angeles, 90024.

Because septal cells display rhythmic patterns of discharge in phase with hippocampal theta rhythm, and theta rhythm cannot be recorded in hippocampus after septal lesions, these cells are generally considered to act as "pacemakers" for hippocampal theta activity. Anatomical and electrophysiological studies have emphasized the prominent reciprocal pathways connecting these two structures and the present study was undertaken in order to determine whether feedback from the hippocampus is necessary for the maintenance of the rhythmic activity of septal "pacemaker" cells. Bilateral cryoprobes were placed in dorsal fornix-fimbria and recording electrodes in hippocampus and septum of cats under halothane anesthesia. Recordings were obtained during artificial respiration with oxygen and nitrous oxide after immobilization with gallamine triethiodide and infiltration of wound margins and pressure points with lidocaine. Amplitude, frequency and regularity of theta activity was markedly attenuated in dorsal hippocampus during local, reversible cooling (5° to 15°C) of the dorsal fornix-fimbria pathways. After cooling was terminated and brain temperature returned to normal, hippocampal theta rhythm reappeared. Septal cellular activity simultaneously recorded showed rhythmic bursting before, during and after cooling. Septal rhythmic bursting was also recorded after lesions of dorsal fornix-fimbria which abolished theta rhythm in hippocampus. The results of this study indicate that septal cellular rhythmicity can occur independently of hippocampal input. (Supported by USPHS grants NS 8552 and GM 22552 to D. B. Lindsley)

848 HIPPOCAMPAL UNIT ACTIVITY DURING CONDITIONING IN THE RAT. Michael R. Best* and Phillip J. Best. Department of Psychology, University of Virginia, Charlottesville, Virginia 22901.

Previous research on hippocampal single cell responses during conditioning of tones to footshock or during habituation have found decreases in hippocampal activity during tone presentations. These results suggest an important role of the hippocampus in orienting behavior, in conditioning and in decisions about the importance of external stimuli. However, Mays and Best (Exptl. Neurol. 1975, 47, 268-279) showed that during habituation, tones presented to the awake animal do not reliably affect hippocampal unit activity, but the same tones cause reliable inhibition when they arouse the animal from sleep.

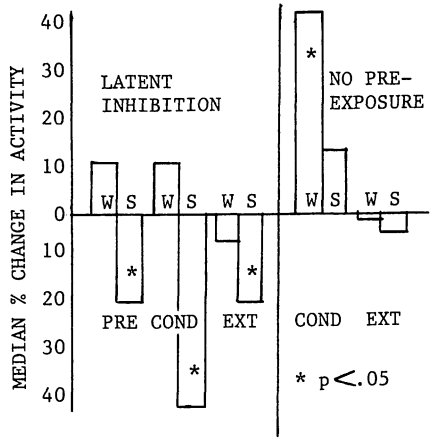
This study asks if the previously found inhibitory response of hippocampal units to conditioned tones is also due to arousal from sleep.

Frequently in conditioning studies the stimulus is presented to the animal in the absence of the reinforcer prior to conditioning. Such stimulus preexposure reduces the salience of the stimulus and retards subsequent conditioning. It has been termed "latent inhibition". The present study also examines the effect of latent inhibition on hippocampal unit responses to conditioned tonal stimuli.

Single cell activity was recorded from 27 hippocampal neurons in 15 male Sprague-Dawley rats (250-300g). The latent inhibition group (LI) received 15-20 preexposures of the tone (5.4 KHz, 75 db, 20 sec), followed by 15-20 (conditioning) tone presentations terminated by footshock (.6 ma, 1 sec pulsed D.C.), followed by 15 non-reinforced tone presentations (extinction). The non-preexposed group (NP) was just given conditioning and extinction. The behavior of the animals and the neocortical and hippocampal EEG were continually monitored. Half of the tones were presented when the animal was awake and half during slow wave sleep.

The figure shows the median percent change in activity caused by tone presentation. Group LI showed significant reduction in activity to tones which aroused the animals from sleep (S) during preexposure, conditioning and extinction. However no significant changes were seen in awake (W) animals. Group NP showed significant increase in activity to tones in the awake animals during conditioning and no other significant changes.

The changes in activity during preexposure agree with the results of Mays and Best. Further, the marked reduction in activity during conditioning to the preexposed sleeping animals is similar to the results from previous conditioning studies. The absence of reliable changes during conditioning in the preexposed awake animals indicates that in previous studies the conditioned stimuli must have been arousing the animals from sleep. Unfortunately neither the animal's behavior nor neocortical EEG were observed in the previous studies. Since the only reliable response to the tones during conditioning in the awake animals was an increase in activity seen in the NP animals one must use extreme caution in drawing conclusions about inhibitory functions of hippocampus in conditioning on the basis of previous inhibitory results.



The results indicate that the hippocampus is responsive to changes in arousal level and is differentially sensitive to manipulations of stimulus salience.

849 AFFERENT CONNECTIONS OF THE HABENULAR NUCLEI IN THE RAT. Miles A. Herkenham* (SPON: W.J.H.Nauta). Psychology Department, Massachusetts Institute of Technology, Cambridge, Mass. 02139.

The patterns of the retrograde cell-labeling resulting from variously localized microelectrophoretic injections of horseradish-peroxidase (HRP) in the habenular complex indicate that the major afferent connections of the medial and lateral habenular nucleus, respectively, have markedly disparate origins. The major source of afferents to the lateral habenular nucleus appears to be the entopeduncular nucleus: HRP confined to the lateral nucleus labeled a multitude of cells within this pallidal region embedded in the cerebral peduncle, and additional cells in lateral preoptic and hypothalamic regions, the nucleus of the diagonal band, the substantia innominata, the nucleus centralis tegmenti superior, the dorsal and median raphe nuclei, and the locus coeruleus. By contrast, HRP injections involving only the medial habenular nucleus caused labeling of numerous cells of the nuclei triangularis and septofimbrialis of the supracommissural septum; only sporadic labeled cells appeared in such cases in the nucleus of the diagonal band and the nucleus basalis of the substantia innominata. Labeling of large, deep-lying cells of the olfactory tubercle appeared to be a consequence of involvement of the subjacent mediodorsal nucleus.

These findings suggest that major afferents of the habenular complex originate from components of both the corpus striatum and limbic system. Although this identifies the complex as a convergence point of striatal and limbic circuits, the evidence that the two categories of afferents remain segregated in the habenula suggests that their actual convergence may occur only in more caudal brain regions receiving habenular projections.

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850 LOCALIZATION AND ANATOMICAL IDENTIFICATION OF THETA AND COMPLEX SPIKE CELLS IN DORSAL HIPPOCAMPAL FORMATION OF RATS. S. E. Fox* and J. B. Ranck, Jr. Dept. of Physiology, Downstate Med. Ctr., SUNY, Brooklyn, N.Y. 11203.

Micro-electrodes were passed through the dorsal hippocampal formation of unrestrained rats, recording for at least five minutes each 35.3 um. At each site the amplitude and duration of action potential (A.P.) spikes, frequency of firing, relation to slow wave theta rhythm, and presence of complex spike (C.S.) or theta cells was recorded. As reported previously by Ranck (EXP. NEUROL. 41: 461, 1973), C.S. cells and theta cells differ in pattern and frequency of firing, duration of extracellularly recorded negativity, presence of complex spikes, relation to slow wave theta rhythm and behavioral correlates. 1,014 neurons were recorded from. (When recording from many neurons simultaneously the "number" of neurons were "counted" in an arbitrary and approximate way.) Of 949 non-theta cells greater than 80 uV in amplitude, only one was not in the hilus of fascia dentata or in a layer of cells which overlapped stratum pyramidale and stratum granulosum. These are the locations of the cell bodies of projection cells (pyramidal cells and granule cells). This layer is, however up to 400 um thicker than stratum pyramidale. Theta cells were seen in sites of cell bodies of projection cells and also in stratum oriens of CA1, suprapyramidal layers of CA3, and the dorsal part of the hilus of fascia dentata. The frequency of occurence in these locations corresponded to the distribution of cell bodies of interneurons. We conclude that the class of projection cells and the class of non-theta cells have a very large overlap and that the class of interneurons and the class of theta cells have a very large overlap. (Supported by: NIMH (MH 12979), NSF (GB 26184) and NIH (NS-10970).

851 AXONAL TRANSPORT IN THE HABENULAR-INTERPEDUNCULAR CHOLINERGIC TRACT - BIOCHEMICAL AND AUTORADIOGRAPHIC STUDIES. P. L. McGeer, T. Hattori and E. G. McGeer. Kinsmen Laboratory of Neurological Research, Dept. Psychiatry, University of British Columbia, Vancouver, Canada.

Autoradiographic studies of rat brain 2 days after injections of tritiated leucine into the habenular nucleus indicated very heavy specific labelling of the interpeduncular nucleus with some specific labelling of the ventral tegmental area of Tsai. Some of the grains in the ventral tegmental area of Tsai appeared adjacent to dopaminergic cell bodies which had been labelled by horse radish peroxidase transported in retrograde fashion from nerve endings in the nucleus accumbens. Electron microscopic autoradiography was used to define the morphology of the radioactively labelled nerve endings. Lesions of the habenular nucleus caused major decreases in choline acetylase activity in the interpeduncular nucleus and minor decreases in the same enzyme in the ventral tegmental area of Tsai. Biochemical studies on the amount of labelled protein accumulated in the interpeduncular nucleus as a function of time after habenular injections suggested more rapid transport and/or turnover of labelled protein in the habenular-interpeduncular tract than in the nigro-striatal tract.

(Supported by a grant from the Medical Research Council of Canada)

852 THE HIPPOCAMPAL ELECTROENCEPHALOGRAM IN THE PERMANENTLY ISOLATED FOREBRAIN OF THE CAT. Charles E. Olmstead and Jaime R. Villablanca. Dept. Psychiat., Mental Retardation Research Ctr., UCLA, Los Angeles, 90024.

It has been postulated that lower brain stem influences are necessary for the spontaneous appearance of hippocampal theta (θ). It is known that the chronic "cerveau isolé" shows alternating periods of electrocortical desynchrony (ECoD) and synchrony (ECoS), (Villablanca, 1965). In the intact animal these EEG patterns are accompanied by θ and hippocampal synchrony (HCS) respectively. We have examined the EEG of the dorsal hippocampus, neocortex and pontine reticular formation in 5 chronically maintained (8 to 138 days) cats with high mesencephalic transections. In chronicity, protracted periods of θ were observed. This θ exhibited similar waveform and frequency characteristics to that seen in the intact cat. Compared to preoperative values there was a 40 to 140 percent increase in the overall amount of spontaneous θ. In the absence of θ the dominant EEG activity of the hippocampus was HCS. The relationships between ECoD and θ and between ECoS and HCS were as noted above for the intact cat. An exception was observed during the onset of spontaneous or evoked (olfactory) EEG arousal of the isolated forebrain when the hippocampal θ led the ECoD by up to one second. To further define the characteristics of θ in the isolated forebrain, Eserine (.1 mg/kg i.p.) and Atropine SO_4 (1 mg/kg i.p.) were used. As in intact cats, Eserine produced uninterrupted θ coupled with ECoD for periods of 2 to 3 hours. Atropine produced ECoS and HCS for a similar amount of time. From this study we conclude that: 1) the caudal brain stem is not essential for the spontaneous appearance of θ; confirming the notion that the neuronal circuitry for its production resides in the forebrain; and, 2) in the permanently isolated forebrain the EEG of the hippocampus and the neocortex continue to co-vary the same as in the intact animal. (Supported by USPHS Grants HD-05958, MH-07097 and HD-04612).

853 AFFERENT CONTROL OF HIPPOCAMPAL THETA RHYTHMS. Lauren K. Gerbrandt*, Theodore G. Weyand*, and R. Michael Snider*. (SPON: Claude F. Baxter)
Dept. Psych.,Calif. State Univ., Northridge, Ca, 91324.

We previously interpreted the phase shifts of hippocampal theta rhythms in the curarized rat as evidence for multiple origins of the hippocampal theta rhythm. This study extends these findings in several ways. Microelectrodes are used to sample the area of phase shifting in finer detail and over a greater range of depth than we previously reported. Here all electrode penetrations pass through the CA 1 region into the dentate granule cell layer. Experimental control over amplitude and phase profiles was attempted by lesioning the contralateral hippocampus or entorhinal cortex unilaterally by suction ablation.

Results from the unlesioned group indicate a prominent, abrupt phase shift (approx. 120^0)occurs in the CA 1 region near where inactivation type unit activities disappear (about 200 microns below the pyramidal cell layer). This region of abrupt phase shifting occurs at the trough of the amplitude profile and is bracketed by a dorsal amplitude peak (35% of max. amplitude) in deep stratum oriens and a ventral amplitude peak in stratum lacunosum-moleculare. The data are largely fit by a 180^0 step function; exceptions are a slight phase lag which develops just above the amplitude trough and a phase shift in excess of 180^0 which develops after the ventral amplitude peak. The slight phase lag is exaggerated by entorhinal ablation, and the greater than-180^0 phase shifts are reduced by contalateral hippocampal ablation. We interpret these results as evidence for multiple origins of hippocampal theta rhythms in the CA 1 region, the dominant source being a 180^0 phase shift, and a secondary source indicated by phase lags exaggerated after entorhinal ablation. It may be necessary to postulate a third origin of theta rhythms in order to explain the greater than 180^0 phase shifts seen in the dentate gyrus.

854 LIMBIC RESPONSES TO INTRAARTERIAL BRADYKININ. C. McGuinness* and G. Krauthamer. Department of Anatomy, CMDNJ - Rutgers Medical School, Piscataway, New Jersey 08854.

Portions of the limbic system were explored in order to determine their responsiveness to noxious stimulation by bradykinin (BK), an endogenous polypeptide known to activate central pain mechanisms. Flaxedyl paralyzed cats were maintained under light to moderate barbiturate anesthesia and branch arteries supplying somatic limb muscles and visceral organs were cannulated for the administration of 20 to 100 μg. doses of BK. Bipolar concentric steel electrodes were used to record the spontaneous activity in the amygdala and hippocampus. Changes in activity during and following BK administration were most consistantly observed in anterior and lateral areas of the amygdala. The random frequency, random amplitude preinjection pattern became rhythmic, often in the theta range. During the period of rhythmic activity amplitudes were stabilized. The specific BK induced changes in frequency and amplitude observed in the amygdala varied over time and in different preparations; they are possibly related to the level of anesthesia and state of preinjection activity. Onset and duration of these changes coincided with previously reported alterations of neocortical activity. Changes in hippocampal activity were rarely observed, except in some preparations where the administration of BK repeatedly led to a high amplitude, seizure like discharge which lasted for several minutes. These observations suggest that even under barbiturate anesthesia the response to BK is not limited to pain pathways but extends also to some limbic structures.
(Supported by NIH grant NS 10922).

855 BEHAVIORAL CORRELATES OF HUMAN LIMBIC NEURONAL FIRING. Eric Halgren, Thomas L. Babb, and Paul H. Crandall.* Brain Research Institute, UCLA, Los Angeles, 90024.

The behavioral effects of mesial temporal lobe lesions in man, and the results of studies of the limbic system in animals, have suggested possible roles of the hippocampus in memory or spatial orientation, and of the amygdala in emotion. We have attempted to test these hypothesized roles by recording from human mesial temporal lobe neurons during an interview including repeated periods of behaviors involving verbal and non-verbal immediate, short-, intermediate-, and long-term memory, spatial orientation, eating, drinking, 'emotional' memories, hyperventilation, and movements differing in the amount of skill and effort required for their execution, or in the degree to which they have been learned. The neurons were recorded from fine wires chronically implanted in temporal lobe epileptics as a part of evaluation for surgery (Babb et al, Electroenceph. Clin. Neurophysiol. 34: 247, 1973). We have analyzed neuronal activity from 37 hippocampal gyrus, 21 hippocampal and 22 amygdala electrodes in 7 patients. Of these, 8 electrodes showed clear-cut behavioral correlates: changes in firing rate that were specific, strong, and repeatable over the entire interview (up to 6 hours). Four of these electrodes, all in the hippocampal gyrus, were strongly correlated with the response period of an intermediate-term memory task (e.g., paired associates). The other 4 electrodes, all in the hippocampus proper, were correlated with certain movements (e.g., forceful chewing). Other electrodes also showed behavioral correlates, but with less specificity, strength, and/or repeatability. These other electrodes support the conclusion that some hippocampal gyrus neurons change firing during memory recall, and some hippocampal neurons change firing during specific movements. They also suggest that a minority of amygdala neurons may change firing during the recall of emotional memories.

856 EFFECT OF NEONATAL HYPOTHYROIDISM ON DEVELOPMENT OF SYNAPTOSOMAL FRACTION MITOCHONDRIA ISOLATED FROM RAT CEREBRAL CORTEX. C. Battie* and M.A. Verity*. (SPON: R.L. Smith). Depts. Anat., and Path., UCLA, Los Angeles, Ca. 90024.

Dendritic and axonal hypoplasia is a feature of neonatal hypothyroidism. In this study biochemical correlates of hypothyroid modulated synaptogenesis were studied. Neonatal hypothyroidism was produced in rats by the addition of propylthiouracil to lactating mothers' food and water. Synaptosomal fractions were prepared from the cerebral cortex of rats aged 6,8, 15 and 22 days (J.Neurochem. 19:1305, 1972). EM studies of the fractions showed some variation in fraction purity, size and organelle content as a function of maturation. Activities of two mitochondrial inner membrane (IM) enzymes, Cytochrome C Oxidase and Succinate cytochrome C reductase, two mitochondrial outer membrane (OM) enzymes, MAO and rotenone-insensitive NADH cytochrome C reductase, and lactate dehydrogenase were assayed. The mitochondrial enzymes showed similar distribution profiles after hyposmotic shock and discontinuous gradient centrifugation. Ratios of the IM markers (MAO/NADH Cyt.) increased with age. All enzyme activities increased with age. No significant difference was found in the OM or IM ratios between control and hypothyroid groups at any age. IM enzyme activity, therefore, increased in synchrony while OM enzyme activity changed independently; hypothyroidism did not appear to change this relationship. In both groups the ratio of MAO/IM enzymes decreased with age. At 15 and 22 days, OM/IM ratios in the hypothyroid animals were significantly higher than age matched controls. Since the total synaptosomal fraction activity of MAO and NADH Cyt. was similar in control and hypothyroid animals at 15 and 22 days, the observed increase in OM/IM ratio in hypothyroid animals presupposes a selective defect in development or organization of inner membrane enzymes.

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857 INTRINSIC CONNECTIONS OF SNAKE CEREBRAL CORTEX. Philip S. Ulinski. Dept. Anat., Univ. Chicago, Chicago, Illinois, 60637.

Snake cerebral cortex consists of four rostrocaudally aligned areas which are named, according to their position, medial (M), dorsomedial (DM), dorsal (D), and lateral (L) cortex. Each area contains three distinct layers numbered 1, 2, and 3 with layer 1 being most superficial. Connections between the areas were studied by making small cortical lesions in water (Natrix sipedon) or garter (Thamnophis sirtalis) snakes and processing the brains by the Fink-Heimer II procedure after survival times of 7-28 days. Lateral cortex projects ipsilaterally to the outer third of layer M1. Dorsal cortex projects ipsilaterally to the middle third of layer M1. Dorsomedial cortex projects bilaterally to the inner third of layer M1 and to M3 and also to a terminal field in the contralateral layer DM1. This field is situated adjacent to layer DM2 medially and slants dorsally toward the area's lateral edge. Medial cortex projects ipsilaterally to the full depths of the dorsomedial and lateral areas and to layer D1. The experiments demonstrate two general organizational features. First, medial cortex occupies a nodal point in cortical organization in that it receives projections from each of the other three areas and gives rise to feedback projections to each of the areas. Secondly, there is a laminar organization of afferents in each of the cortical areas in which each system of afferents terminates at a specific depth in the cortex. (Supported by PHS Grant NS 10137 and the Block Fund of the University of Chicago).

858 CHANGES IN NEURON AND GLIA PACKING DENSITY AND LIPOFUSCIN ACCUMULATION IN THE CEREBRAL CORTEX WITH AGE IN A NONHUMAN PRIMATE. J. Mark Ordy and Kenneth R. Brizzee. Delta Regional Primate Research Center, Covington, Louisiana 70433.

Two of the more important problems in neurobiology and aging are the quantitatively unresolved loss of neurons and the possible involvement of lipofuscin or "age" pigment in the presumed loss of cells with age in the mammalian nervous system. The aims of this study were: 1) examine with quantitative histological procedures, neuron and glia packing density and intraneuronal lipofuscin content in somatosensory areas 1, 3 and motor area 4 of the left cerebral cortex in 6 young and 5 old rhesus monkeys; 2) determine the correlations between age differences in neuron packing density and intraneuronal lipofuscin accumulation content in the laminae of somatosensory area 3.

There was a significant decrease in neuron and a significant increase in glia packing densities between young and old subjects. Neuron packing density and lipofuscin content were correlated significantly and negatively or inversely with age in lamina IV. Lipofuscin correlations with age in the 5 laminae were all positive and significant and the highest correlations with age was again in lamina IV. From the quantitative histological and statistical evaluations it was concluded that loss of neurons and lipofuscin accumulation were correlated significantly in the cerebral cortex. Supported by NIH Grant RR00164-14.

859 POSTNATAL GAMMA IRRADIATION EFFECTS ON BEHAVIOR, CHEMICAL COMPOSITION, CYTOARCHITECTURAL DIFFERENTIATION OF THE VISUAL AND SOMATO-SENSORY CORTEX, AND GROWTH OF THE BRAIN, PITUITARY AND ADRENALS IN THE RAT.

Bernice Kaack* and L. Horrocks Delta Regional Primate Research Center, Covington, La. 70433 and Tulane University, New Orleans, Louisiana 70118.

Sprague-Dawley 3-day old rats were exposed to 300 or 600R whole-body gamma irradiation to determine the effects on postnatal development of behavior, the chemical composition of the brain, the cytoarchitectural differentiation of visual and somatosensory regions of the neocortex, and growth of the pituitary and adrenals. Radiated and sham-control rat offspring were compared on maturation of neuromuscular reflexes and open-field exploration on postnatal days 13, 27 and 60. Following testing, they were sacrificed for chemical and morphological evaluations. There was a significant dose-dependent increase in mortality of the irradiated rat offspring. Eye opening, grooming and autonomic reactivity were significantly delayed by irradiation. There was also a dose-dependent decrease in open-field exploration and increase in open-field latency to emerge from the starting compartment. Total brain and regional DNA, RNA, protein, cholesterol, water and electrolytes were significantly altered by radiation. Body, brain, pituitary and adrenal weights were significantly reduced by irradiation. Previous studies of the effects of "differential experience" in the neonatal rat on dendritic spine counts and neuronal connectivity have remained inconclusive. In this study, preliminary quantitative ultrastructural observations in lamina I and II of the visual and somatosensory cortex indicated significant decreases in proportion of cortical tissue occupied by synaptic end bulbs following whole-body gamma irradiation. Supported by NIH RR 00164-13.

860 TWO-DIMENSIONAL DISTRIBUTION OF NADH FLUORESCENCE IN RAT CEREBRAL CORTEX AS DETERMINED BY FLUORESCENCE PHOTOGRAPHY. Sungchul Ji*, Britton Chance and Robert Nathan*(SPON: M. Reivich). Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pa. 19174 and Jet Propulsion Laboratory, California Institute of Technology, Pasadena, Cal. 91103.

In the 4th Annual Meeting of this Society, Chance, Mayevsky and Stuart (1) reported preliminary results of two-dimensional recording of NADH fluorescence from rat brain cortex by using a fluorescence photographic technique. We have now extended their work and confirmed the observations (1,2) that the distribution of NADH fluorescence intensities as well as of fluorescence changes over the cortical surface are regional and heterogeneous. The technical details of NADH fluorescence photography were previously described(3). The fluorescence and reflectance photographic negatives were densitized by a Joyce-Loebl double-beam recording microdensitometer. The densitometric tracings (about 20 tracings per negative) were then digitized using a computer-assisted TV digitizer (100 points per tracing), and the resulting digital data were reassembled into 3-dimensional surface plots by a PDP 8 computer. The most striking feature of the 3-D surface plots is the presence of deep valleys and undulating ridges in both the fluorescence and reflectance surfaces, with the fluorescence surface showing approximately 50% greater fluctuations in optical densities than the reflectance surface. In general, the valleys were identifiable with major blood vessels (200 to 400 in diameter) and the ridges with cortical regions relatively free of such vessels. In the fluorescence difference plot (anoxic minus normoxic), the valleys due to blood vessels were still visible, but all of the deep valleys were almost obliterated in the reflectance difference plot. Similar results were obtained from another set of photographic negatives which were analyzed at the Jet Propulsion Laboratory employing a 20-square-micron scanner. The computer-reconstructed fluorescence difference photograph showed regions of varying fluorescence changes, whereas the corresponding reflectance difference photograph revealed little regional differences.

The above results may be attributable to one or more of the following several factors: (1) heterogeneous distribution of mitochondrial populations in the tissue, (2) regional differences in the rates of energy metabolism, (3) inhomogeneity in tissue oxygen delivery, and perhaps (4) regional variations in tissue blood volume.

(1) B. Chance, A. Mayevsky and B. Stuart, Neuroscience Abstr., 1974.

(2) B. Stuart and B. Chance, Brain Research, 76, 473(1974).

(3) S. Ji, B. Chance, F. Welsh and B. Quistorff, Biophys. Abstr. 319a (1975).

861 *REDUNDANT INFORMATION IN AUDITORY AND VISUAL MODALITIES: INFERRING DECISION-RELATED PROCESSES FROM THE P300 COMPONENT.* *Emanuel Donchin, Nancy K. Squires* and Kenneth C. Squires*, Dept. Psych., Univ. of Ill., Champaign, Ill. 61820 and Steven Grossberg*, Dept. Math, MIT, Cambridge, Ma. 02139.*

When subjects are presented with a train of Bernoulli trials with one of the two outcomes considerably less probable than the other, an event-related brain potential (ERP) characterized by a large P300 component will be elicited by the rare events, particularly when the subjects are instructed to count the number of occurrences of the rare events. We report two studies of ERPs elicited by compound, bimodal, stimuli designed to determine if the relationship between event probability and P300 holds when subjects are presented with two conjoint series of Bernoulli trials. We were interested in the degree to which it is possible to infer from the P300 component if either of two modalities overshadows the other and the circumstances in which such overshadowing may occur.

Six subjects were presented with series of events; in each series events consisted of the occurrence of one of two auditory stimuli (1000 Hz or 1500 Hz) or the illumination of one of two figures (a right-pointing arrow or a left-pointing arrow) or one of two audio-visual compounds (e.g., the 1000 Hz tone presented simultaneously with a right-pointing arrow). Stimuli in any series were either all auditory, all visual, or all audio-visual. The subjects counted the number of occurrences of one of the two stimuli in each sequence. The probability of this target stimulus was 0.10. In the ERPs associated with unimodal series the P300 components were elicited by the target stimuli. The latency of P300 elicited by visual and auditory rare events differed by 100-150 msec, the visual stimuli eliciting a longer latency P300. When a Bernoulli series in one modality was presented simultaneously with an unvarying stimulus in the other modality the P300 elicited by the rare events was that characteristic of the relevant modality. The waveforms associated with compound stimuli carrying redundant information (that is, when a rare visual event was presented simultaneously with a rare auditory event) suggest that the auditory stimuli overshadowed the visual stimuli. The waveform of the ERP elicited by such compound stimuli was virtually identical to that elicited by unimodal auditory targets.

The predominance of the auditory stimuli could be attributed either to an intrinsic difference between the two modalities or to the fact that the auditory discrimination was somewhat easier to perform than the visual discrimination. The second experiment was conducted to assess the role which discrimination difficulty may play in determining the predominant modality. Six new subjects were again presented with a series of Bernoulli events and instructed to count the number of occurrences of rare events. In each modality we used two different series, one presenting an easy and the other a difficult discrimination. The compound stimulus series were constructed from all possible combinations of easy and difficult auditory and visual stimuli. The scalp distribution of the P300 components elicited by all stimuli were identical, but the latency of the P300 component associated with a difficult discrimination was always longer than that associated with an easy discrimination. When subjects were presented with a compound stimulus, the P300 elicited by the compound, if any, will be that associated with the element of the compound which elicits a P300 with the shorter latency. In other words, when given the choice subjects, at least to the extent reflected by their ERPs, will prefer an easy route.

These data suggest that the P300 components which are often reported to vary in latency over a range of more than 250 msec are all manifestations of the same endogenous cortical process whose timing is determined by the temporal characteristics of the cognitive process with which it is associated. *(Supported by the Advanced Research Projects Agency of the Department of Defense under Contract No. DAHC 15 73 C 0318 and the US San Diego State University Foundation under ONR Contract No. N00014 70 C 0350.)*

862 THE ORBITAL FRONTAL CENTER AND MONKEY SOCIAL BEHAVIOR. F. R. Ervin, M. Raleigh* and H. D. Steklis*. Dept. of Psych., Sch. Med., UCLA, Los Angeles, California 90024

In order to assess the effects of orbital frontal lesions on social behavior, a colony of vervet monkeys (Cercopithecus aethiops) was observed for 10 months. Preoperatively the patterns of social interaction was determined by noting the frequency, duration and intensity of both affiliative and agonistic behaviors. Following bilateral or bital-frontal lesions in selected members of the group there were profound alterations in the social behavior of the operated animals. Patterns of grooming, huddling, playing, sexual behavior, aggression, maternal behavior and spatial relations changed dramatically. While all lesioned animals exhibited changes in behavior, there were significant differences between them related to the age and sex, the peroperative role in the social group, and the behavior directed toward the lesioned animals by the normal ones.

(It is noted that) it would be exceedingly difficult to predict the effects of lesions on complex and important social functions on the basis of results of formal cognitive testing of individual animals or on the basis of their cage behavior in the laboratory.

The effects of orbital frontal lesion, amygdalectomy temporal pole and inferior temporal pole lesion on social behavior are compared. Emphasis is placed on the conceptual problems posed by this class of experiments.

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863 AN INTEGRATIVE CENTER FOR THE DETECTION OF THE MATING CALL IN ANURANS?
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Anurans (frogs and toads) are well suited for investigations of the neural substrate of acoustic behavior. Their vocal repetoire is limited and highly stereotyped. Of the various types of vocalizations, the mating call is the most common and serves as a species identification signal to enable reproductive isolation. Previous behavioral studies in several ranid and hylid species have shown that optimal detection of the mating call is dependent upon the presence of energy in disjoint low- and high-frequency regions which correspond to the two spectral peaks in the call. Related electrophysiological studies have identified auditory units in the VIIIth nerve and in central nuclei of the medulla and midbrain which respond to one or the other or both of the two spectral peaks in the particular species' mating call. However cells have not yet been found that respond exclusively to the simultaneous presence of energy in both frequency regions. The existence of such units could be considered the basis for a "mating call detector" in the anuran's central auditory system.

Neary (Anat. Rec. 178: 425, 1974) recently reported an auditory projection from the torus semicircularis to the posterocentral nucleus of the diencephalon in bullfrogs (Rana catesbeiana). This is the highest known auditory region in the anuran brain. We therefore studied the responses of populations of auditory units in the dorsal thalamus of leopard frogs (Rana pipiens) using evoked potentials and multiunit recording techniques. Neural responses in this area have latencies of 30-40 msec and adapt very rapidly to stimulus presentation rates of once every 10 seconds,or greater. Evoked potentials were recorded in response to either low (250-350 Hz) or high (1.4-1.8 kHz) frequency tone bursts in the range of 60-80 dbSPL. The amplitude of the evoked response was maximum in a circumscribed region of the dorsal thalamus adjacent to the posterior commissure. In this region simultaneous presentation of low and high frequency tones produces a large response which cannot be explained by simple summation of the responses to the two tones presented separately. We have not found this selective response in lower auditory centers. The dorsal thalamus may therefore be the first auditory center involved in integrating the stimulus features necessary for the behavioral detection of the mating call in anurans. Supported by NIH grant NS-09244.

864 CENTRAL CONTROL OF SONG IN THE CANARY, SERINUS CANARIA. Fernando Nottebohm, Tegner M. Stokes* and Christiana M. Leonard. The Rockefeller University, New York, 10021, and Dept. Anat., Mount Sinai Sch. Med. of the City University of New York, 10029.

We have traced central nervous pathways controlling bird song in the canary using a combination of behavioral and anatomical techniques. Unilateral electrolytic brain lesions were made in adult male canaries whose song had been previously recorded and analysed on a sound spectrograph. After several days of postoperative recording, the birds were sacrificed and their brains processed histologically for degeneration staining with the Fink-Heimer technique. Although large lesions in the neostriatum and rostral hyerstriatum had no effect on song, severe song deficits followed damage to a discrete large-celled area in the caudal hyperstriatum ventrale (HVc). Degenerating fibers were traced from this region to two other discrete nuclei in the forebrain: one in the parolfactory lobe (area X, a teardrop-shaped small celled nucleus); and a round large-celled nucleus in the archistriatum (RA). Unilateral lesions of X had no effect on song; lesions of RA, however, caused severe song deficits. Degenerating fibers from RA joined the occipitomesencephalic tract and had widespread ipsilateral projections to the thalamus, nucleus intercollicularis of the midbrain, reticular formation, and medulla. It is of particular interest that direct connections were found onto the cells of the motor nucleus innervating the syrinx, the organ of song production. Unilateral lesions of n. intercollicularis (previously implicated in the control of vocal behavior) had little effect on song.

One bilateral lesion of HVc resulted in permanent (9 months) and complete elimination of the audible components of song, although the bird assumed the posture and movements typical of song. Preliminary data suggest that lesions of the left hemisphere result in greater deficits than lesions of the right one. This finding is consistent with earlier reports that the left syrinx controls the majority of song components. Results reported here suggest a localization of vocal control in the canary brain with an overlying left hemispheric dominance.

865 EFFECT OF METHALLIBURE ON SOCIAL BEHAVIOR AND FERTILITY IN PARADISE FISH. Roger E. Davis. Mental Health Res. Inst., UM, Ann Arbor, MI 48104

Methallibure (I.C.I. 33828), or dithiocarbamylhydrazine has antigonadotropic effects in fishes (Breton, et al, J. Endocr., 59:415, 1973). The behavioral effects of methallibure (MB) have been investigated in few fishes (Kramer, Experientia, 28:1195, 1972). In paradise fish (Macropodus opercularis), oral MB results in decreased frequency of social display, fertility, and gonadosomatic index (G.S.I.). Adult males in individual tanks were fed 20 to 40 mg daily of control diet or one of 3 diets: (A) 0.1 mg, (B) 1 mg, (C) 10 mg MB/g of Tetramin fish food for 5 weeks. The 4 groups showed equivalent mean frequencies of lateral displays (Davis, et al, Behav. Biol., 11, 497, 1974), in a 10 min trial with a live male conspecific, following 3 weeks but not 5 weeks of treatment. At 5 weeks, the controls showed an increase in display frequency, compared to the 3-week level, the A- and B-diet fish showed no change, and the C-diet fish showed a decrease in display frequency. The controls, and the A- and B-diet fish showed similar G.S.I. values, while the C-diet fish showed a significantly lower G.S.I. Spawning success was tested in a replicate control and C-diet group following 5 weeks of treatment: 5 out of 6 controls spawned in 5 days, with high fertility, one did not spawn; one C-diet fish spawned with high fertility, two spawned but produced few or no fertile eggs, and one did not spawn. Chronic administrations of MB in male paradise fish thus reduce intermale social behavior, testes weight, and fertility but not spawning behavior.

866 VISUAL ORIENTING BEHAVIOR IN PRAYING MANTIDS. James Y. Lea* and Conrad G. Mueller* (SPON: W. D. Neff). Dept. of Psychology and Center for Neural Sciences, Indiana University, Bloomington, Ind. 47401

Praying mantids orient toward objects in their environment by means of rapid, saccadic movements of the head. Responses were elicited with a small spot of light, subtending 30' of arc, which could be accurately positioned around a circular arena. Orienting responses were elicited by flashing a light in a particular position, flashing a light in successive positions, or by moving a continuous light. High speed photography was employed to obtain values for the latency of responding and to investigate the dynamic properties of the saccade. Maximum angular velocities of 500 deg./sec. were observed in the Carolina mantis, Stagmomantis carolina. Lower values (350-400 deg./sec.) were obtained from the Chinese mantis, Tenodera aridifolia sinensis. In both species the duration of the saccade was 150 msec. or less. The orientation of the head following a saccade appeared to be attained without visual feedback from the target, since the orientation was found to be precise even when the target disappeared before the head began to move. The stimulus parameters involved in the maintenance of the orienting behavior were studied by a quantitative analysis of visual tracking behavior as well as analysis of initial orienting saccades. The reliability with which a mantis followed successive positions of a stimulus depended on the stimulus intensity, length of time the stimulus was on, and the rate at which the positions were changed. Several species differences were observed. Results of these experiments suggest possible neural mechanisms underlying the behavior.

867 THE SHEET WEB AS A TRANSDUCER, MODIFYING VIBRATION SIGNALS IN SOCIAL SPIDER COLONIES OF MALLOS GREGALIS. J. W. Burgess* (SPON: P. N. WITT). North Carolina Mental Health Division, Raleigh, N. C. 27611.

The sheet web of Mallos gregalis tailors vibrations to fit the colony's behavior patterns. In a social context, it is important to locate acceptable prey on the web and differentiate it from other colony members. B. Krafft has identified close-range chemotactic signals inhibiting predation in social spiders (Agelena consociata). Mallos gregalis on three-dimensional sheet-webs in nature and the laboratory orient to struggling prey at distances over 10 cm, but do not orient to web vibrations caused by colony members. Electronically generated vibration transmitted through the web to a magnetic pick-up shows that transmission is limited to a band between 50-500Hz (measured on an oscilloscope), and sine-wave vibration is amplified at peaks within that band. Pure tones introduced on the web over a range of 10-100,000Hz elicit predation behavior (orientation and directional movement) only within the 50-500Hz band, proportional to the web-response curve. Measured on the web, vibration of a trapped housefly is prominent within the web-response band, recorded on a Brüel and Kjaer audio analyzer, while this vibration measured off web is spread over a wide frequency range, from 50-5000Hz. The fly's vibration is modified by the web into a signal to which spiders readily respond, but other spiders moving on the web do not generate measurable vibrations within this band. Since intra-colony predation is not seen, it is suggested that the filter/amplification characteristics of the web act to stimulate fly predation, while inhibiting inter-spider predation. (Supported by NSF Grant GB25274 to P. N. Witt.)

868 EFFECTS OF CATECHOLAMINES ON THE PREDATORY BEHAVIOR OF GRASSHOPPER MICE, ONYCHOMYS TORRIDUS. Richard McCarty* (Spon: B. Talamo). Dept. Pathobiology, The Johns Hopkins Univ., Baltimore, Md. 21205.

The grasshopper mouse, Onychomys torridus, is a cricetid rodent that inhabits the semi-arid scrub deserts of the southwestern United States and northern Mexico. The majority (up to 90%) of the natural diet of this species consists of arthropods and small vertebrates that are stalked, captured, killed, and consumed. The predatory behavior of grasshopper mice is easily studied under controlled laboratory conditions and is not dependent on prior field or laboratory experience with prey species. A variety of drugs that alter catecholaminergic functioning was injected into adult male and female O. torridus to assess effects on predatory behavior toward house crickets (Acheta domesticus). Predation tests were conducted in a large plastic enclosure that contained 5 large crickets and 5 food biscuits. Attack latency, number of attacks, total predation time, and number of crickets incapacitated or eaten were recorded for each 10-min bout. L-DOPA, with or without a peripheral decarboxylase inhibitor, produced a dose-dependent decrease in all measures of predatory behavior as well as a decrease in motor activity. The effects of l-tyrosine were biphasic; a lower dose (25 mg/kg) was more effective than higher doses (50 and 100 mg/kg) in inhibiting predatory behaviors. Both stereoisomers of amphetamine (1 or 10 mg/kg) produced dose-dependent decreases in predation with little evidence of enhanced motor activity or compulsive gnawing. A time-dependent increase in predatory behavior was observed in grasshopper mice pretreated with αmethyltyrosine, an inhibitor of tyrosine hydroxylase. These results suggest that the putative neurotransmitters, dopamine and norepinephrine, are involved in the expression of predatory behavior in the grasshopper mouse, Onychomys torridus.

869 RESPONSES TO SPECIES-SPECIFIC VOCALIZATIONS OF SINGLE UNITS IN THE SQUIRREL MONKEY FRONTAL LOBE. John D. Newman and David F. Lindsley. NIH, Bethesda, Md. 20014 and Dept. of Physiology, Sch. Med., USC, Los Angeles, 90033.

A possible role in auditory processing by the primate frontal lobe has been suggested both by demonstrable pathways to this region from auditory association cortex (superior temporal gyrus), and by deficits resulting from some frontal ablations. However, the responsiveness of single neurons in the frontal lobe to a variety of acoustic stimuli has not been tested. This investigation attempted to close this gap by testing single neurons in the frontal lobe of awake squirrel monkeys with clicks, steady tone bursts over the range, 0.5-18 kHz, and tape recorded species-specific vocalizations. About 15% of the tested units clearly responded to at least one auditory stimulus. Many of these responsive units responded both to a broad frequency range of tones and to several vocalizations. Our results suggest that acoustically responsive units in the frontal lobe represent a more homogeneous population than those found in the auditory cortex.

870 RESPIRATORY ACTIVITY IN LARVAL LAMPREYS. Carl M. Rovainen, Shinji Homma* and Marc Schieber*. Department of Physiology & Biophysics, Washington University Medical School, St. Louis, Missouri 63110.

The neural control of respiration was investigated behaviorally in intact larval sea lampreys and electrophysiologically in isolated preparations.

Unidirectional ventilation was measured with the larva in a glass tube whose tapered end formed a seal with the mouth. Resting ventilatory volumes were 0.6 ± 0.2 ml/min/gm and breathing rates 1.3 ± 0.1/sec at 20°. Hypoxia, obstruction of breathing, and prior exercise stimulated ventilation several fold both through increased stroke volume and through higher frequency. Evidently, both motoneurons and pacemakers were more active. Pressure opposing ventilation reduced stroke volume and frequency of breathing.

Periodic breathing movements continued in isolated brain-velum-gill preparations. The activities of respiratory neurons were recorded extracellularly during the ventilatory cycle. Motoneurons to velar and branchial muscles were identified by visible movements following intracellular stimulation and by antidromic stimulation. Velar motoneurons were located in the V motor nucleus and were apparently driven by nearby pacemakers.

871 CONNECTIONS OF THE THALAMUS IN THE MONITOR LIZARD. Hansjürgen Distel* and Sven O. E. Ebbesson. Dept. Neurosurgery, University of Virginia School of Medicine, Charlottesville, Va. 22901 USA.

Hodological studies in Varanus benegalensis involved first the delineation of thalamic afferents from retina, tectum and spinal cord. These studies revealed nothing new, except a direct spinal projection to the ventrolateral area of the thalamus suggesting that this region is homologous to the ventrobasal complex of mammals. Thalamic targets of these fiber systems were then lesioned selectively employing stereotaxic techniques. The consequent degeneration of axons and axon terminals were studied with the Nauta and Fink-Heimer techniques. The projections of the major thalamic nuclei have been determined and will be reviewed.

Preliminary observations suggest no overlap of these systems in the telencephalon, e.g. the auditory projections from the nucleus reuniens involve the most medial corner of DVR (dorsal ventricular ridge) whereas the visual rotundo-telencephalic projections terminate in the ventrolateral portion of DVR, anterior to the auditory area. The principal source of afferents to general cortex appear to be nucleus dorsolateralis anterior. Possible evolutionary implications of these findings will be discussed.

872 EEG FREQUENCY BAND ANALYSIS OF THE DIURNAL LIZARD.
Xavier Lozoya and Ranulfo Romo-Trujillo*. Sci. Res. Dept., Natl. Med Ctr., I.M.S.S., México, D. F.

Present day, reported evidence suggests that the essential anatomical structures for active and quiet sleep are present in lower vertebrates like reptiles. It could be expected that at least some of these animals would show a primordial kind of active, fast sleep as well as slow wave sleep. Although the visual inspection of the EEG recording has produced contradictory facts about the existence of these two types of mammalian sleep in reptiles, we decided that a power-spectral analysis of the EEG might reveal subtle differentiations such as the beginnings of the evolution of synchronizing and desynchronizing processes. In the present paper a study of the EEG frequency band analysis in a diurnal lizard (Phrinosoma regali) is reported. After two months of behavioral study, electrodes were chronically implanted in the surface of the brain. EEG activity was channelled to a frequency band analyzer-integrator equiped with different band-filters. A one minute integration interval was used for long lasting records (8-12 and 24 hrs). Power spectra was obtained from 30 sec epochs during different circumstances. Results suggest the existence of active and quiet sleep with different temporal and electrical characteristics with respect to mammalian EEG. It is concluded that both types of sleep are part of the same phenomena as a whole, and phylogenetically present in lower vertebrates with primitive manifestations that can be established with the used quantitative approaches.

873 EFFECTS OF TELENCEPHALIC LESIONS ON THE SOCIAL BEHAVIOR OF THE WESTERN FENCE LIZARD, Sceloporus occidentalis. Robert S. Tarr, Dept. of Physiol., Univ. of Ill., Urbana 61801, and Dept. of Physiol., Chicago Coll. of Osteopath. Med., Chicago 60015.
(SPON: I. M. Korr, Ph.D.)

Western Fence Lizards were enclosed in seminatural environments (100 sq. ft.) with controlled light and temperature conditions. These animals have a complicated social structure based on several fixed action pattern displays. Social behavior was recorded: territorial and challenge displays; location and postures within the territory; response(s) to displays by others; and courtship and feeding patterns. Animals were selectively removed and telencephalic lesions made electrolytically. When returned, the changes in above behaviors were recorded. After all observations were completed, lesion sites were verified histologically. Ten lizards with bilateral lesions involving the "amygdala" (n. sphericus, n. ventromedialis and posterior hyperstriatum) all showed substantial changes in aggressive behaviors, characterized by diminished spontaneous behavior, lack of response to aggressive behavior in others and changes in location preference and posture that reflected these losses. There were only occasional slight changes in feeding and courtship. Ten lizards had bilateral lesions that were outside the amygdala; two had changes in aggressive behavior. Twenty animals were removed and anesthetized, but not lesioned. One of these controls showed a small change in aggressive behavior.
(This work was supported by USPH 1 T01 10715-05 BLS, and NSF-GB-13797.)

874 A SURVEY OF THE PERIPHERAL TRIGEMINAL SYSTEM IN THE COMMON BOA CONSTRICTOR. Judith D. Bodnar*, Dennis A. Poulos and Daniel N. Tapper. Depts. of Physiol. and Neurosurg., Albany Med. Coll., Albany, N.Y. 12208.

Electrophysiological recordings were obtained from single afferent fibers in snakes anesthetized with pentobarbital. Sensory fibers composing the mandibular and maxillary divisions were found to be organized in a general somatotopic manner. An overall functional segregation of fibers subserving specific modalities was not seen. Evidence was obtained to support existence of functional microlocalization in that small nerve filaments often contained identical afferent types subserving similar peripheral receptive fields. Rapidly adapting, less rapidly adapting, and slowly adapting mechanoreceptive neurons were found to innervate precise receptive fields in both intraoral tissues and scales. Proprioceptive afferents were found in motor and not sensory branches of the trigeminus. The region of labial scales was found to be densely innervated by neurons that increased their rate of firing to cutaneous warming. These heat detectors were exquisitely sensitive and capable of appreciating small temperature changes occurring several centimeters away from their peripheral receptive fields. Heat detectors could be silenced by either cooling or intense warming. Unlike mammalian warm receptors, many of the heat detectors displayed a burst of activity ("off response") following removal of stimuli sufficiently intense to inhibit activity.

It would appear that: 1) the anatomical organization of the boa trigeminus does not differ appreciably from that of mammalian forms; 2) the boa trigeminus contains the principal mechanoreceptive neuronal types found in mammalian forms with the exception of receptors associated with hair; and, 3) a difference exists between reptilian heat detectors and mammalian warm receptors in that the latter do not display "off" responses.

Work supported by NIH grant NS 11384.

875 COMPARISONS BETWEEN ELECTRICAL BRAIN STIMULATION ELICITED AND NATURALLY OCCURRING VOCALIZATIONS OF THE TURKEY. S. Anschel. Dept. Zool., Univ. of Md., College Pk., Md. 20742.

Brain sites for eliciting vocalizations were explored with electrical brain stimulation in the free-moving, socially isolated turkey. All call types except those normally associated with dominance threat behaviors were observed. Anomalous vocalizations were limited to stimulation of a single site in the hyperstriatum ventrale. These calls were frequently extended in duration or segmented into rapid pulses. Counterparts for all other vocalizations could be found in the natural repertoire of the turkey. Vocalizations were also elicited that closely resembled contact and alarm calls in frequency, duration and transitions similar to those associated with changes in behavioral state. Charge (μcoul) was the most critical stimulus parameter affecting these transitions. However, mechanical sounding calls, unaffected by changes in stimulus parameters, were elicited with stimulation of the central neostriatum caudale and paleostriatum. The nature of these calls was comparable to those reported by Phillips et al. (1972) for stimulation of the torus externus of the chicken. Vocalizations that are normally emitted in response to sensory stimulation were also elicited. Singing, normally evoked by the sight of a predator, was shorter in duration with mesencephalic grey stimulation than either when naturally occurring or in response to telencephalic and optic tectal stimulation. Gobbling was similar to natural or tone evoked calls except for the relative frequency of occurrence of certain syllables and rapid habituation to brain stimulation.

876 MAPPING POSITIVE AND NEGATIVE REINFORCEMENT SITES BY ELECTRICAL STIMULATION OF THE BRAIN IN MACACA MULATTA. Douglas M. Bowden. Dept. of Psychiatry and Behavioral Sciences; Regional Primate Research Center, University of Washington, Seattle, WA 98195.

Rates of intracranial self-stimulation (ICSS) were determined at 2,900 sites in 178 cerebral structures of 8 rhesus monkeys. To determine whether the reinforcement value of stimulation at a given site was positive, negative, or neutral, the rate of bar-pressing for stimulation at that site was compared with bar-press rates during trials of equal duration when no stimulation was administered. Exploration sites ranged from the medulla to the frontal poles and from midline to the temporal lobe; 31% were positive, 17% negative, and 52% neutral. All animals performed ICSS and there was no indication that the proportions of positive and negative sites changed during the course of extensive exploration in individual animals. Positive sites were found in all of the structures reported to support ICSS in the rat (German and Bowden, Brain Res. 73:381-419, 1974). In addition, numerous positive sites were found in globus pallidus (pars interna), capsula externa, several thalamic nuclei (dorsalis medialis, ventralis anterior, ventralis lateralis, pulvinar) and in the region of the cerebellar nuclei. Negative sites were located primarily along the classical sensory and motor pathways, reticular formation of the medulla, pons, and dorsal tegmentum, portions of central gray, globus pallidus (pars externa), putamen, and several cortical areas, particularly gyrus rectus and the hippocampal, uncinate and inferior temporal gyri. The proportions of positive, negative and neutral sites, and their anatomical distributions, confirmed and extended findings of other investigators who have mapped many of the same areas in the monkey using quite different measures of the reinforcing value of electrical brain stimulation (Plotnik et al., Int. J. Psychobiol., 2:1-21, 1972).

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877 OLFACTION AND FOOD-SEEKING BEHAVIOR IN PROCELLARIIFORMES. Lyle J. Rausch, Robert J. Shallenberger*, and Bernice M. Wenzel. Dept. Physiol., UCLA Sch. Med., Los Angeles, CA 90024 & Ahuimanu Productions, Kailua, HA 96734.

Evidence for reliance on olfactory cues in food-seeking behavior of procellariiform birds was collected in field studies conducted during two cruises aboard the R/V Alpha Helix. On the first cruise (Hawaii-San Diego, 4-18 Dec 73), the dominant procellariiform was the albatross. Experiments included 1) creation of surface slicks of variable odorous and visual characteristics (fish oil, griddle fats, motor oil, dyes); 2) addition of odorized air to an airstream emanating from the moving ship; and 3) launching of inner-tube floats with or without fish oil odors. The use of olfaction was supported by one observation of greatly increased density around slicks of animal fats and by the dispersal of albatrosses during airborne presentation of fish odors, possibly stimulating aerial search patterns. Otherwise, results suggested that visual cues are predominant in eliciting investigative behavior in the albatross. The petrel was the dominant procellariiform on the second cruise (Seattle-San Diego, 22-29 Sep 74), followed in frequency by shearwaters and albatrosses. Both the behavior and increased density of petrels near tuna oil slicks floated on the water strongly suggested olfactory control. The petrels landed and appeared to feed on the slicks after fluttering in characteristic fashion over the oily surface; seagulls attracted to the area neither landed nor fed on the slicks. Control experiments with floating puffed rice or mineral oil slicks as visual cues were ineffective in attracting petrels. Present data from various sources agree in suggesting that olfaction is important in behavioral regulation of petrels. (Supported by NSF grants GD 41402, BMS 7307068, GD 41493, GD 28838, GD 34462, and GB 39268 to Scripps Institution of Oceanography for the Alpha Helix Research Program, and USPHS grant NS 10353 to B.M. Wenzel.)

878 ULTRASONIC CLICKS WITH POSSIBLE BAT REPELLENT PROPERTIES PRODUCED BY THE PEACOCK BUTTERFLY (Inachis io). Lee A.Miller. Biologisk Institut, Odense Universitet, Odense, Denmark. and Bertel Møhl[+]. Zoologisk Laboratorium A, Aarhus Universitet, Aarhus, Denmark.

Hibernating peacock butterflies produce a pair of intense ultrasonic clicks during a wing display, which consists of a rapid forward and downward movement of the wings following tactile or visual stimulation. The clicks have intensitities of from 100 to 110 dB SPL (at 10 cm), and durations of from 30 to 60 μsec. The maximum energy lies between 30 and 60 kHz, with an effective bandwidth of 45 kHz. Clicks are produced in a 1 to 2 square mm area of wing membrane located between the costal and subcostal wing veins at the base of each forewing. This area is mechanically bi-stable, producing an intense click as it buckles during a wing display.

Hibernating butterflies display when captive vespertillionid bats (one Plecotus auritus, and one Pipistrellus pipistrellus), maintained in small cages, crawled to within a few cm. The bats responded by retreating, with head jerks, with pinna movements, or by emitting audible chirps. Preliminary results indicate that the bats respond to the intense ultrasonic clicks and not to other components of the wing display. In addition, the energy spectrum of a click matches nicely with the auditory sensitivity of the vespertillionid bat, Myotis lucifugus, (Dalland, Science 150: 1185, 1965). (These studies were supported in part by the Danish Research Council.)

879 ORGANIZATION OF CRUSTACEAN NEUROPIL: IDENTIFIED SYNAPTIC PROCESSES IN STOMATOGASTRIC GANGLION. David G. King. Dept. of Neurosciences, UCSD, La Jolla, CA 92037.

The structure and synaptic contacts of several of the motor neurons in the stomatogastric ganglion of a spiny lobster (Panulirus interruptus) were examined in detail by serial-section electron microscopy. Neurons were identified electrophysiologically prior to fixation and an accurate soma map was prepared. Correct identification was confirmed in a few cases by tracing the neuron's axon to the appropriate motor root. Tracing and reconstruction of neuropil processes were accomplished by matching profiles in neighboring sections. No intracellular markers were used. This procedure imposed a practical limit on the extent and resolution of the reconstructions; few processes smaller than two microns in diameter were followed for any distance.

All neurons appear to share several common features of organization of their dendritic branches: 1. The major processes of each neuron are heavily ensheathed by glia and devoid of synaptic contacts (Figure 1). 2. Distal to this sheathed region, nearly all dendrites form elaborate swellings containing presynaptic vesicles. The vesicles are clustered at synaptic contacts characterized by pre- and post-synaptic membrane specialization (Figure 2). 3. Such synapses occur typically at corners and involve two post-synaptic processes (Figure 2, arrows). 4. Post-synaptic processes are often short narrow branches arising at or near a pre-synaptic enlargement (Figure 2, asterisks).

Each presynaptic swelling can be classified according to type of vesicles into one of the following groups: Class A contains mostly clear flattened or irregular vesicles; class B contains mostly clear smoothly rounded vesicles; class C contains in addition to clear irregular vesicles a large population of larger dense-core vesicles. Class A includes PD, VD, and LPG neurons. Class B includes LP, PY, AM, DG, and EX neurons. No intrinsic neurons have been found in class C; it is believed from this and the work of E. Maynard and B. Friend (U. of Oregon) that such profiles containing accumulations of dense-core vesicles may represent terminals of axons entering the ganglion through the stomatogastric nerve.

Figure 1 is a stick-figure reconstruction of a PD neuron; heavy line shows glial sheath; asterisks show synaptic regions.

Figure 2 shows a reconstructed synaptic complex. A PD neuron is presynaptic to an LP neuron and an unidentified process (shaded). Both the LP and the unidentified neurons are themselves presynaptic to many other processes (not shown).

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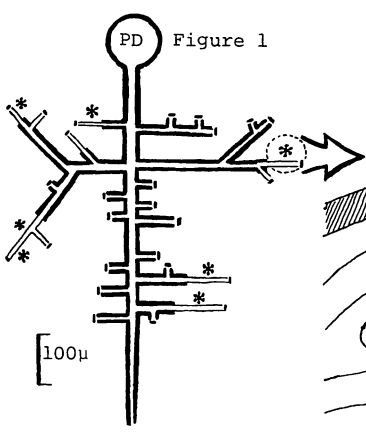

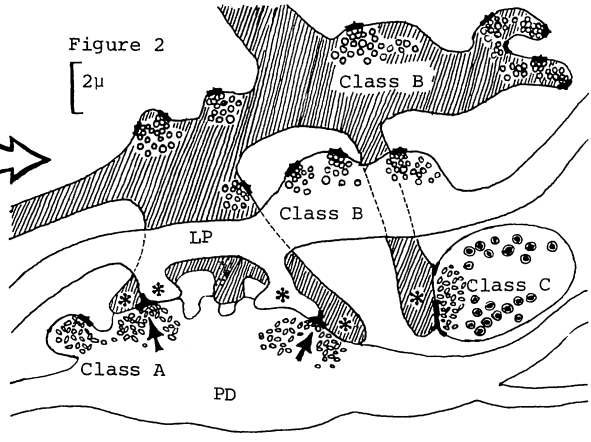

880 EFFECT OF DELTA-AMINOLEVULINIC ACID ON THE CRAYFISH STRETCH RECEPTOR. H.N. Dichter, L. Taddeini, and G. F. Ayala, Dept. of Neurol., Univ. of Minnesota Mpls. Mn. 55455 and Dept. of Medicine, Ramsey Hospital, St. Paul, Mn.55101

Acute Intermittent Porphyria (AIP) is an inborn error of prophyrin metabolism which results in accumulation, in the body fluids, of the Porphyrin precursors Porphobilinogen (PBG) and delta-Aminolevulinic Acid (ALA). The clinical manifestations of AIP are characterized by major symptoms of nervous system dysfunction. The pathogensis of these symptoms remains yet to be explained. We have studied the effect of ALA on an isolated neuronal preparation, the crayfish stretch receptor (CSR). The most striking results obtained upon introduction of ALA into the perfusion chamber was a rapid and large decrease of membrane resistance. This effect was rapidly reversed upon removal of ALA from the chamber. Perfusion with ALA produced modest changes in resting membrane potential (RMP) of the receptor and the amplitude of the antidromically elicited action potential was decreased slightly. We investigated the effect of ALA on the CSR with crayfish saline containing different concentrations of K+ and Cl- ions. The membrane conductance change due to the application of ALA, decreases linearly with the decrease in the external concentration of chloride. Modest changes of conductance were observed with different external concentrations of K+. GABA, the known inhibitory neurotransmitter of the CSR, is similar in structure to ALA. Both compounds, GABA and ALA, appear to increase Cl- conductance in this preparation. Addition of ALA to the bath either decreased or abolished the IPSP and the RMP shifted to the value of the apparent reversal potential of the IPSP. Picrotoxin antagonizes the effect of ALA on membrane resistance. Our data indicate that most of the effects of ALA on the neuronal membrane are similar to those produced by GABA. (Supported by NIH Grant NS09784 and St. Paul Ramsey Medical Foundation.)

881 THE IDENTIFICATION OF A NEW LEECH INHIBITORY MOTOR NEURON AND AN ANALYSIS OF ITS JUNCTIONAL POTENTIALS. Masashi Sawada* and Richard E. Coggeshall. Marine Biomedical Institute and Dept. of Anatomy, University of Texas Medical Branch, Galveston, Texas 77550.

Three pairs of inhibitory cells have been identified in each segmental ganglion of the leech. We have found a 4th pair of cells along the lateral margin of the ganglion in the position of cell 119 in the nomenclature of Ort, et al. (J. Comp. Physiol., 94: 121-154, 1974). The right and left cells are electrotonically connected. Upon stimulation, these cells give rise to inhibitory junctional potentials (IJP's) in the leech body wall muscle cells. The mechanism of the IJP appears to be that a Cl^- gate is opened in the muscle cell. Since 5-hydroxytryptamine (5-HT) is known to hyperpolarize leech muscle cells by opening a Cl^- gate (Sawada and Coggeshall, submitted), a search for 5-HT in the cell bodies of these inhibitory cells is beginning. In addition we are attempting to identify the specific terminals of these cells by labelling the cell body with radioactive material. (Supported by grant NS 10161 from the USPHS.)

882 RECEPTORS FOR AMINO ACID NEUROTRANSMITTERS ON APLYSIA NEURONS. Paul J. Yarowsky* and David O. Carpenter, (SPON William A. Alter, III), Neurobiology Dept., Armed Forces Radiobiology Research Inst., Bethesda, Md. 20014 and Dept. Physiol., the George Washington University, Washington, D.C. 20037

Using 5 barrel iontophoretic electrodes we have examined the sensitivity of Aplysia neurons to the amino acids γ-aminobutyric acid (GABA), L-glutamic acid (GLUT), and L-aspartic acid (ASP). There are separate and distinct receptors for each of the substances, causing changes in conductance to various ions. A majority, but not all cells respond to GABA. The most common response to GABA is a hyperpolarizing Cl- conductance increase, although Na^+ and K^+ conductance increases are occasionally found. Contrary to previous reports, not all cells responding to GABA respond to acetylcholine (ACH) in the opposite manner. GLUT and ASP receptors, unlike receptors to GABA or ACH, are located predominately in the neuropile. GLUT and ASP receptors cause either a depolarizing Na^+ conductance or a hyperpolarizing Cl^- conductance increase. Occasional cells had GLUT receptors causing a K^+ conductance increase. No potentiation between the effects of glutamate and aspartate were found. All receptors showed desensitization. Some cells show a biphasic response to GABA, either depolarizing-hyperpolarizing or hyperpolarizing-depolarizing. The late depolarization appears to be due to a slow Na^+ conductance increase similar to that reported by Gerschenfeld and Paupardin-Tritsch (J. Physiol. 243: 427, 1974) for serotonin. The depolarizing response to GABA and the hyperpolarizing responses to both GABA and GLUT are curare sensitive. Traditional GABA antagonists, picrotoxin and bicuculline, block all types of GABA receptors, but also depress responses to ACH.

883 INNERVATION AND PHYSIOLOGICAL PROPERTIES OF PENIS RETRACTOR MUSCLE OF APLYSIA. J. Hill* and J.E. Blankenship. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550.

The innervation, electrophysiological properties and morphology of the penis retractor muscles of Aplysia have been examined using intracellular, sucrose gap and tension recordings and electron microscopy. The muscle fibers are of the smooth type and have diameters of from 3-5μ. They have a high resting conductance to K^+ and Cl^- ions as determined by ion substitution experiments. The fibers may be weakly electrically coupled. Individual fibers appear not to sustain overshooting action potentials, but each receives at least one spontaneous excitatory junctional potential (ejp) and one inhibitory junctional potential (ijp). Stimulation of the major nerve trunk (from right pedal ganglion) to this muscle elicits ejp's, but no obvious hyperpolarizing ijp. Stimulus induced ejp's show frequency-dependent facilitation and depression and posttetanic potentiation, which can be correlated with changes in tension. Serotonin (5HT, 10^{-6}-10^{-5}M) produces depolarization and contraction of the fibers. Dopamine (DA, 10^{-4}-10^{-3}) produces excitation and contraction. Lower concentrations of DA (10^{-6}-10^{-5}) and acetylcholine (ACh) prevent 5HT-induced contractions and produce relaxation of muscle already contracted by 5HT or stretch. ACh and DA also increase membrane conductance based on shunting of nerve stimulus induced ejp's. While ACh does not produce an obvious hyperpolarization of the muscle in normal sea water, in Cl^- free medium it causes depolarization and contraction. ACh effects are partially blocked by curare, but not by atropine or hexamethonium. No clear antagonist to 5HT has been found, but dehydroergotamine (10^{-6}-10^{-5}M) will block the DA relaxation effect. It is tentatively concluded that 5HT may be an excitatory transmitter and ACh and dopamine inhibitory transmitters in this system. (Supported by NIH Grants NS 11255 and NS 70613.)

884 IDENTIFICATION OF GAMMA-AMINOBUTYRIC ACID AS A NEUROTRANSMITTER IN THE BARNACLE SHADOW REFLEX PATHWAY. R. Clark Lantz* and Ronald J. Millecchia. Dept. of Physiol., West Virginia University, Morgantown, WV 26506.

When a shadow is presented to a barnacle that is actively feeding, the animal will withdraw into its shell. This is known as the shadow withdrawal reflex. The identification of gamma-aminobutyric (GABA) as the transmitter involved at the first synapse of this reflex pathway is described. Two criteria were used in identifying the transmitter. 1) Does the proposed transmitter mimic the effects of the naturally occuring transmitter on the postsynaptic cell? and 2) Is the proposed transmitter found in large quantities in the presynaptic cell? Microelectrode recordings from the postsynaptic cell showed that the cell hyperpolarized during illumination of the photoreceptor. Upon shadowing the photoreceptor, the postsynaptic cell depolarized causing the initiation of action potentials in the postsynpatic cell. The response to shadowing seen in the postsynaptic cell could be abolished by application of GABA or its noncompetitive inhibitor, picrotoxin, to the bathing medium. These effects are consistant with the idea that GABA is the naturally occuring transmitter. Using a specific enzymatic assay, large amounts of GABA (35000 μg/g dry wt. of tissue) were found in the presynaptic cell. These values are comparable to values found by Kravitz (J. Neurophysiol. 126: 729, 1963) in inhibitory fibers of lobster and crab where GABA has been identified as the transmitter. From these observations we feel that GABA is the transmitter involved at the first synapse in the barnacle shadow reflex pathway.

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885 MODULATION OF INTRINSIC RHYTHMICITY OF INSECT MUSCLE BY (OCTOPAMINERGIC) DUM NEURONS. G. Hoyle, Biology Department, University of Oregon, Eugene, OR 97403.

The locust or grasshopper extensor and flexor tibiae show an intrinsic rhythm (IR) of tonic contraction. This rhythm is modulated by acceleratory and inhibitory thoracic neurons. The inhibitors are the dorsal, unpaired, median (DUM)neurons which terminate in and on selected muscle fibers that also receive the fast axon only. Terminals are filled with large dense-core vesicles. The rhythm persists and may be enhanced following excision of the leg, but it is reduced or abolished by perfusion with insect salines. Contractions average 4 s. duration and recur at an average frequency of 3.5/min., with maximum amplitude of 2 g. A burst of 10 or more impulses in the DUM neuron innervating the extensor tibiae, DUMETi, at a frequency above about 4 Hz. results in complete cessation of the rhythm for about 3 minutes. Weaker bursts attenuate the contractions and slow the rhythm. Stronger ones lead to longer periods of inhibition. The common inhibitor also inhibits IR, but the rhythm starts immediately upon cessation of stimulation. Single slow or fast axon impulses can trigger an IR contraction prematurely. All three axons can lead to early termination of an IR contraction. The rhythm appears to be confined to proximally-located fibers. Slow depolarizing waves of up to 15 mV maximum amplitude accompany the rhythm in some of these. The inhibitory effects are mimicked by noradrenaline, dopamine and octopamine. Sensitivity to the latter is about 200x greater. The DUMETi axon swells above a ligature and dense-core vesicles accumulate but they do not fluoresce after Falck treatment. The transmitter may thus be octopamine. It is suggested that the function of the rhythm is to ensure exercise of fast-innervated muscle fibers.

This research was supported by NSF Grant #BMS75-00463.

886 SEROTONIN MEDIATED INCREASE IN CYCLIC AMP: A POSSIBLE BIOCHEMICAL STEP IN THE INCREASED TRANSMITTER OUTPUT ACCOMPANYING BEHAVIORAL SENSITIZATION IN APLYSIA. M. Brunelli,* V. Castellucci, E.R. Kandel, Div. Neurobiol. & Behavior, Columbia University, P & S.

A strong stimulus to the head of Aplysia produces behavioral sensitization of both an habituated and a non-habituated gill-withdrawal reflex. Sensitization involves a facilitation in synaptic efficacy at the excitatory synapses made by an identified cluster of sensory neurons on the gill motor cells and can be produced in the isolated ganglion by stimulation of the connectives from the head ganglia. This facilitation can be simulated by perfusing the ganglion with 10^{-4}M serotonin (see also Shimahara and Tauc, 1972), but not by dopamine or octopamine. The facilitation produced by stimulating the connectives is reversibly blocked by cinanserin (10^{-4}M). Since the cyclic AMP in the ganglion is increased both by electrical stimulation of the connectives and by perfusion with serotonin (Cedar et al., 1973a,b), we examined whether serotonin might mediate sensitization via cAMP. In some experiments we perfused the ganglion with dibutyryl cyclic AMP and theophylline; in others we intracellularly injected cyclic AMP into the sensory neurons. Both procedures simulated the action of serotonin and enhanced synaptic transmission within minutes. Intracellular injection of cyclic GMP was without effect.

Although the nerve cells that mediate sensitization have not yet been identified, these data suggest that they may be serotonergic and may mediate their actions by increasing cAMP in the sensory neuron terminals. We speculate that cAMP may in turn enhance transmitter release by increasing the level of free calcium.

887 AXONAL TRANSPORT OF VESICLES: AUTORADIOGRAPHIC LOCALIZATION OF ^{3}H-GLYCOPROTEINS IN IDENTIFIED APLYSIA AXONS AFTER INTRASOMATIC INJECTION OF ^{3}H-FUCOSE. E.B. Thompson, E.R. Kandel, and J.H. Schwartz, Columbia University College of Physicians and Surgeons, N.Y., N.Y. 10032.

To study the organellar localization of rapidly transported ^{3}H-glycoproteins within single neurons of Aplysia we have analysed autoradiographs of the cholinergic interneuron L10 after intrasomatic pressure injection of ^{3}H-fucose. Silver grains were localized to intracytoplasmic membranes in both cell body and axon 3 h after injection. Grains also appear at this time over those areas of synapses which contain vesicles. In the cell body the Golgi apparatus was labeled, as were vesicles, multivesicular bodies, pigment granules, smooth endoplasmic reticulum, mitochondria and peroxisomes. After 16 and 24 h label became increasingly associated with cell membrane and axolemma, which had not been labeled at 3 h. Intraaxonal vesicles were the most intensely labeled organelle in the neuron, having a relative specific activity of 40.4 (% silver grains/% area), an intensity 10 times that of similar appearing somatic vesicles, and 4.5-10 times that of other organelles (multivesicular bodies, mitochondria, smooth endoplasmic reticulum) in the axon. The preponderant labeling of vesicles in the axon parallels the rapid and preferential transport of glycoprotein components described by Ambron et al. (J. Cell Biol., 61:649-664 and 665-675, 1974) and may indicate that specific glycoprotein molecules can be identified as components of these vesicles. Within the cell body the most intensely labeled organelle is the Golgi (rel. sp. act.= 9.5). Over 50% of the silver grains are associated with the Golgi and with somatic vesicles, and at least 32% of the axonal silver grains are associated with vesicles. It appears that the biosynthetic machinery of these neurons is heavily involved in the production of vesicle membrane destined for transport along axons and to terminals.

888 TRANSPORT AT AN AXON BIFURCATION IN AN IDENTIFIED SEROTONERGIC NEURON OF APLYSIA. D.J. Goldberg, J.E. Goldman,* and J.H. Schwartz, Dept. Physiol., Div. Neurobiol. & Behav., Columbia U., Coll. of Phys. & Surg., N.Y. 10032.

^{3}H-serotonin (5HT), injected into the metacerebral neuron in the cerebral ganglion, is incorporated into particulates, presumably serotonergic vesicles, which are transported along axons. The axon of this neuron bifurcates near the cell body to send branches of approximately equal diameter into the lip nerve and cerebro-buccal connective. Normally, 6 h after intrasomatic injection, the lip nerve contains 11.5%, and the connective 19.5% of the ^{3}H-5HT in the neuron. We wished to determine the effect of a major alteration in the configuration of the axon tree on parameters of transport. When the connective was cut close to the bifurcation prior to injection, more than twice the normal amount of ^{3}H-5HT was transported into the lip nerve. We analyzed the distribution of radioactivity along the lip nerve of neurons in which the connective had been cut 0.5 h or 3 h prior to injection. The increase in transport was accompanied by a dramatic increase in the average displacement of ^{3}H-5HT along the axon. The patterns of radioactivity along the axon also strongly suggested that this incremental material was diverted, with a delay, from the cut stump. Diversion of transmitter indicates that serotonergic vesicles from the connective can be transported along the lip nerve, and suggests that vesicles in one branch of the axon are identical to those in the other. Another important implication of these observations is that the rate of axonal transport may depend upon the local concentration of the transported organelle. This suggests a novel hypothesis for the mechanism of transport wherein organelles are translocated, perhaps in association with microtubules, at an unalterable maximum rate. The rate-limiting step in transport would be the reversible binding of the organelle to the translocation mechanism.

889 DENSE-CORE VESICLES IN THE AXON OF THE METACEREBRAL CELL, AN IDENTIFIED SEROTONERGIC NEURON OF APLYSIA, ARE LABELED AFTER INTRASOMATIC INJECTION OF ^{3}H-SEROTONIN. J.E. Goldman* and J.H. Schwartz, Dept. Physiol., Div. Neurobiol. & Behav., Columbia U., Coll. of Phys. & Surg., N.Y. 10032.

Axonal transport of serotonin is a process which consists of at least two steps: export from the cell body and translocation along axons. Biochemical and pharmacological experiments (Goldman and Schwartz, J. Physiol., 242: 61, 1974) suggested that both steps involve movement of the transmitter within vesicles which are specific to serotonergic neurons. Loading of these vesicles is selective: ^{3}H-serotonin was exported from the cell body soon after it was injected into the metacerebral neuron in the cerebral ganglion and moved along the cell's axons at a fast rate; neither its precursor, ^{3}H-5 hydroxytrytophan nor its metabolite, a serotonin glycuronide, was packaged in the cell body or transported by a fast mechanism. Up to about 4 pmoles, the amount of ^{3}H-serotonin appearing in the axons depended on the amount of transmitter injected into the cell body; greater amounts were saturating. Half-saturation occurred when the total (labeled and endogenous) amount of transmitter in the neuron was about 5 pmoles, a value in the range Weinreich, McCaman, McCaman, and Vaughn, (J. Neurochem., 20, 969, 1973) have found normally present in this cell. Using autoradiography with both the light and electron microscopes, we saw that ^{3}H-serotonin was restricted to the axon of the injected metacerebral neuron, which also contained characteristic, dense-core vesicles. These ellipsoid (66 x 79 nm) profiles, similar to aminergic granules in other animals, were absent from cholinergic neurons. In the metacerebral neuron, they were the only membranous organelles significantly labeled.

890 INTERACTIONS BETWEEN BINDING OF ^{125}I-LABELLED α-BUNGAROTOXIN (αBT) AND CHOLINERGIC ANTAGONISTS TO IDENTIFIED NEURONS OF APLYSIA. W. Shain, L. A. Greene* and D. O. Carpenter, AFRRI, Bethesda, Md. 20014 and Harvard Medical School, Boston, Massachusetts 02115.

Different identified neurons of Aplysia will respond to acetylcholine (ACh) by conductance increases to either Na^+, Cl^-, or K^+. The pharmacologic sensitivities of the responses differ in that the Na^+ response is specifically sensitive to hexamethonium (HEX), the Na^+ and Cl^- responses are sensitive to d-tubocurarine (d-TC) and the K^+ response is blocked by tetraethylammonium (TEA). Kehoe (J. Physiol. 225:115, 1972) has proposed that these 3 responses are associated with different ACh receptors. ^{125}I-αBT-binding studies of whole ganglia homogenates have demonstrated only a single toxin binding site (Shain et al., Br. Res. 72:225, 1974). This observation suggests that a single receptor may be associated with the 3 different responses. We have studied the binding of ^{125}I-αBT to homogenates of pooled identified neurons with known but different responses to ACh. The apparent K_m for toxin binding to 6 different neurons was 1.8 ± 1.3 nM, and showed no apparent differences among neurons with the three different ACh responses. The mean toxin bound per cell was 10.8 ± 5.5 fM with a range of 3.8 ± 16.9 fM. Interactions between ^{125}I-αBT and antagonists were studied on 9 different cells. The I_{50} values for HEX, TEA, and d-TC were 1.2 ± 0.4 mM, 0.22 ± 0.07 mM and 5.4 ±2.4 μM, respectively and showed no apparent differences among the cells. These values are not significantly different from those for a whole ganglion homogenate (0.8 mM, 0.22 mM and 7.6 μM, respectively). These results are consistent with the hypothesis that there is a single ACh receptor complex in Aplysia with binding sites for HEX, TEA and d-TC and with the potential for 3 different responses.

891 PHARYNGEAL SENSORY NEURONS AND FEEDING BEHAVIOR IN THE OPISTHOBRANCH MOLLUSC NAVANAX. D. C. Spray & M. V. L. Bennett, Dept. of Neuroscience, Albert Einstein Col. of Med., N. Y., N. Y. 10461.

Feeding in this carnivore involves 1) pharyngeal protraction that, on making contact with prey, is followed by 2) rapid pharyngeal expansion leading to ingestion and 3) peristaltic swallowing with movement of prey from pharyngeal cavity to esophagus. Electrotonically coupled motoneurons in the buccal ganglion mediate pharyngeal expansion. Swallowing is accompanied by IPSPs in these neurons that decrease coupling and allow asynchronous firing, presumably contributing to swallowing. The motoneurons are controlled at least in part by two groups of small primary sensory neurons on the dorsal surface of the buccal ganglion. One group occupies the caudal 2/3 of the surface and forms inhibitory synapses on the motoneurons. Each neuron in this group has a discrete receptive field covering about 5% of the pharyngeal wall. Touch in this field elicits abruptly rising axonal impulses. Touch in a much larger surround causes EPSPs, and repetitive stimulation of one cell can recruit neighbors; thus the neurons are mutually excitatory. Activity of a few cells may spread to others and cause prolonged inhibition of the motoneurons and decoupling. The other group of sensory neurons lies in a band just caudal to the expansion motoneurons on which they form excitatory synapses. These neurons innervate the lip and anterior 1/3 of the pharyngeal wall. They have smaller receptive fields, and are also mutually excitatory. Activation of these neurons alone may elicit synchronous firing and pharyngeal expansion. Activation with inhibitory sensory neurons may lead to the asynchronous activation of expansion motoneurons during swallowing.

892 CONTROL OF FEEDING BEHAVIOR BY THE METACEREBRAL GIANT NEURON OF PLEUROBRANCHAEA. Rhanor Gillette* and William J. Davis. Div. Nat. Sci., UCSC, Santa Cruz, Calif., 95064.

The metacerebral giant (MCG) neuron of Pleurobranchaea has been analyzed by means of intracellular dye injection and intracellular stimulation/recording in whole animals, semi-intact preparations and isolated nervous systems. The soma is located in the brain. The neuron sends an axon out the mouth nerve and a descending axon through the cerebrobuccal connective to the buccal ganglion. Graded extracellular stimulation of brain nerves causes large (2 - 15 mv) compound excitatory postsynaptic potentials and action potentials in the MCG, presumably by activation of sensory paths from the periphery. In whole-animal preparations food stimuli on the oral veil excite the MCG and initiate feeding behavior. In isolated nervous systems intracellular depolarization of the MCG soma can initiate cyclic motor output of the kind that normally underlies feeding behavior. In spontaneously active semi-intact preparations MCG stimulation accelerates the ongoing feeding rhythm. The MCG makes mono- and polysynaptic connections with identified feeding interneurons and motorneurons in the brain and buccal ganglion, and in turn receives synaptic inputs from identified feeding neurons. During cyclic feeding output the membrane potential of the MCG shows large (5 - 10 mv), cyclic fluctuations in phase with the feeding rhythm caused by synaptic excitation and inhibition from the above sources. Bursts of action potentials are generated during the depolarization. Endogenous membrane properties of the MCG also contribute to its cyclic activity.

The collective data imply that the MCG is one of several neurons that normally act in concert to initiate and sustain feeding behavior. The MCG receives extensive feedback from the feeding network that it drives, and apparently exercises its command function by virtue of access to the sensory influences that normally excite feeding behavior.

893 A SELF-INHIBITORY SYNAPTIC POTENTIAL (SISP) IN APLYSIA BUCCAL GANGLIA. Daniel Gardner. Cornell Univ. Medical Coll., New York, N.Y. 10021.

The buccal ganglia of Aplysia contain four identified cholinergic interneurons, each of which mediates a variety of synaptic actions. I now report that each interneuron also appears to monosynaptically inhibit itself. A self-inhibitory synaptic potential (SISP) is recorded in an interneuron following each action potential and superimposed upon the nonsynaptically mediated component of the spike afterpotential. The SISP is blocked by curare and enhanced by raising external Ca^{++}. One-for-one following and persistence in threshold-raising high Ca^{++} solutions suggest that the SISP is monosynaptic. Iontophoretic ACh responses and the decrease in SISP amplitude at high rates of firing imply that the interneuron possesses the same rapidly-decrementing hyperpolarizing cholinergic receptor seen on other buccal ganglia cells. Under voltage clamp, the cell was depolarized >50 mv for 3 msec to produce an action potential, then examined at one of several holding potentials for 300 msec to locate the currents responsible for the SISP. In order to isolate cholinergic synaptic currents from late currents due to conventional voltage-dependent conductances, the ganglia were bathed in curare. Currents obtained after curarization were subtracted from corresponding currents in control solutions, revealing the curare-sensitive currents presumably underlying the SISP. Similar currents were obtained when records from ganglia bathed in high Mg^{++} were subtracted from records obtained in high Ca^{++}; this dependence upon divalent ions suggests transmitter release. The postsynaptic currents rise to a peak and decay exponentially with a time constant of 50 msec. The peak conductance of 0.2 μmho in high Ca^{++} does not appear to vary with membrane potential over a 70 mv range. A curare- and Ca^{++}/Mg^{++}-sensitive voltage-independent conductance change thus underlies the SISP. Supported by USPHS grants NS11555 and RCDA NS00003 from NIH-NINCDS.

894 THE NONUNITARY NATURE OF AN ALL-OR-NOTHING EPSP IN APLYSIA NEURON R15.
Peter Harley, Department of Psychology and Marine Sciences Research Laboratory, Memorial University of Newfoundland, St. John's, Newfoundland.

Stimulating the right pleuro-visceral connective evokes, in cell R15, an all-or-nothing EPSP which has been studied by several different laboratories, and always described as being unitary and monosynaptic. This EPSP is definately not unitary, and it may not be monosynaptic. If the connective is stimulated at many different sites, it is usually possible to find a point at which a small, excitatory response appears below threshold for the large one, and with an identical latency. Alternatively, another means of observing this small, component response is to stimulate through the seawater at high intensities, with a colinear arrangement of anode and cathode. This causes an anodal block on either side of the cathode, and often, the large EPSP will fall out and leave the small response occurring with the same latency. Stimulating the connective at two sites with pulse pairs temporally as close as the absolute refractoriness in the pathway will allow, it is also possible to demonstrate partial failure of the EPSP due to the failure of some action potentials at the stimulating electrode more remote from the ganglion. Finally, if the ganglion is soaked in collagenase and hyaluronidase, the amplitude of the EPSP eventually comes under the control of the right connective stimulus intensity. In the most successful preparations, it is apparent that there are at least 7-10 presynaptic elements contributing to the response. In these experiments, prior to the development of stimulus control over the EPSP amplitude, there is a stage characterized by dramatic augmentation of the evoked 1-hz response. This is suggestive of recruitment.

895 MONOSYNAPTIC CONNECTIONS IN THE CEREBRAL GANGLION OF APLYSIA. Steven M. Fredman and Behrus Jahan-Parwar, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The neurons of the cerebral ganglion are organized into bilaterally symmetrical pairs of clusters (Fig. 1A). We have found extensive excitatory synaptic connections between neurons in two pairs of clusters located in the caudal portion of the ganglion. Neurons in the A and B clusters receive some common synaptic input, but of opposite signs. Tactile stimulation of the tentacles produces IPSPs in the A neurons and EPSPs and spikes in the B neurons. Intracellular current injection into A neurons reveals that they make excitatory monosynaptic connections with both ipsilateral and contralateral B neurons (Fig. 1B) While it is impossible to test all the permutations of possible connections among these neurons, by impaling 3 B cells and then sequentially penetrating and driving a minimum of 10 cells in each A cluster with a fourth electrode one can demonstrate that these connections are very extensive. In a similar fashion, it is possible to show that B neurons make reciprocal excitatory monosynaptic connections with both ipsilateral and contralateral B neurons. It appears that the majority of A neurons synapse on the majority of B neurons, and that the B neurons synapse on each other (Fig. 1C). Failure to demonstrate these connections was usually associated with either poor resting potentials or noisy electrodes in the follower neurons. No synaptic connections were found between either ipsilateral or contralateral A neurons. In addition to the direct connections, there also appears to be some polysynaptic excitatory feedback from the B neurons to the A neurons (Fig.1D). Although the function of this circuit is not known, there is some indirect evidence that it may be involved in a defensive withdrawal reflex. Experiments are now in progress to test this hypothesis. Supported by PHS NS 11452 to B.J.P.

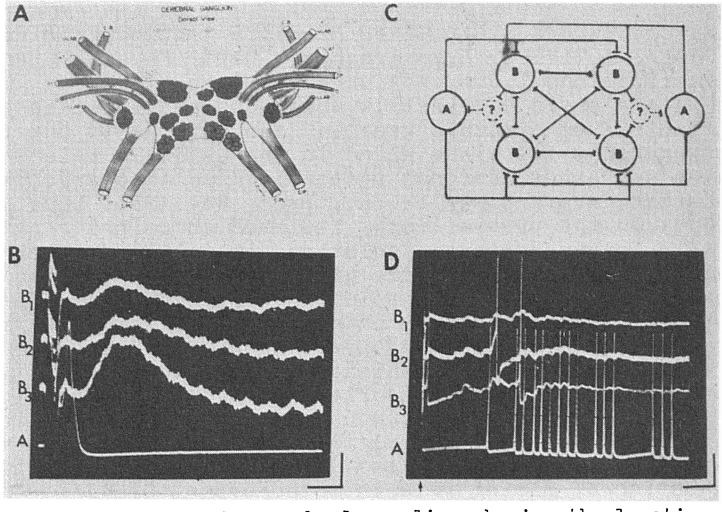

Fig. 1. A. Diagram of the cerebral ganglion showing the location of the clusters. B. Spike in A cell produces EPSPs in 3 B cells. Gain: 2mV, 20mV; Time: 20mSec. C. Schematic representation of the synaptic connections between A and B neurons. For simplicity only one A neuron and two B neurons on each side have been shown. Dashed line is feedback pathway involving an unknown number of neurons. D. Spike in A (arrow) produces EPSPs in B_1 and B_2 and a spike in B_3. Subsequent EPSPs in B cells are probably due to synaptic connections between B cells. The excitation produced is of sufficient magnitude to cause A to fire and re-excite B_2 and B_3. Gain: 10mV, 20mV; Time: 200mSec.

896 ACTION POTENTIALS AND PREPOTENTIALS FROM NEUROSECRETORY CELLS OF APLYSIA ABDOMINAL GANGLION. F.E. Dudek and J.E. Blankenship. Marine Biomedical Institute, Univ. Texas Med. Br., Galveston, Texas 77550.

Neurosecretory cells (bag cells, BCs) in the abdominal ganglion of Aplysia release a hormone(s) that causes egg-laying behavior. We utilized simultaneous intra- and extracellular recording to determine (1) the cellular locus of BC spike initiation during prolonged repetitive firing, (2) the ionic basis of the BC action potential, and (3) the properties of the somatic prepotentials while the BCs are refractory.

When stimulating distally on either connective (beyond the BC processes), the temporal occurrence of ipsilateral extracellular BC action potentials indicated that they were initiated in the processes on the connective and propagated toward the cell bodies. During a prolonged after-discharge, nearly all BC spikes were still initiated in the ipsilateral peripheral processes and only rarely were they conducted from the cell bodies out the processes of the ipsilateral connective. However, the BCs could also be activated into a prolonged after-discharge by direct extracellular stimulation of the BC bodies. Preliminary evidence indicates that the long duration action potentials of the BCs are Na+ dependent and can occur in Ca++-free sea water.

After the BCs completed an episode of prolonged firing, they became refractory and stimulation caused small prepotentials that summated and potentiated. All somatic prepotentials were preceded by extracellular spikes in the BC processes of the ipsilateral connective. Our results indicate that the prepotentials represent passive invasion of the soma by spikes that are blocked in the BC processes. Potentiation of the somatic prepotentials was directly correlated to increased amplitude of the extracellular BC spikes. (Supported by NIH Grants NS 11255 and NS 70613.)

897 THE CRUSTACEAN SINUS GLAND RELEASES A HORMONE DEPRESSING NEURONAL ACTIVITY. Hugo Aréchiga. Dept. Physiol. Centro de Investigación, I.P.N. México 14, D.F.

On the basis of eyestalk ablations and injections of extracts from eyestalk ganglia, the hypothesis has been put forward that the sinus gland in the eyestalk releases a peptide which depresses neuronal responsiveness (see Aréchiga, H., Huberman, A., and Naylor, E., Proc. Roy. Soc. Lond. (B) 187-229 1974). In the present work, isolated abdominal ganglia were placed in van Harreveld solution, in one compartment of a two-cell chamber, while in the other compartment, isolated eyestalk were placed in the same solution. Both sections of the chamber were interconnected by a channel and one-way flow from the eyestalk section was secured by means of a valve and a negative pressure system. The spontaneous electrical activity of motoneurons (mostly the tonic flexor neurons to superficial abdominal muscles) was continuously recorded with suction electrodes. The electrical stimulation of the sinus gland with repetitive shocks (1-10/sec.) during 1-5 min. resulted in a reduction of firing rate in motoneurons. Similar results were obtained when eyestalk were incubated in high K^+ solutions (10-80 mM). The magnitude of the depression was proportional to K^+. The effect started within 1 min. of incubations and 50% depletion was attained in 30 min. Electrical stimulation or incubation of other parts of the eyestalk apart from the sinus gland failed to produce any effect on motoneurons activity. Incubation of the solution containing the released product with proteolytic enzymes abolished the effect. These results support the notion of a peptidic hormone depressing neuronal activity.

898 PEROXIDASE LABELING OF FLIGHT MOTONEURONS ON NORMAL AND MUTANT DROSOPHILA MELANOGASTER. John C. Coggshall* (SPON: R. W. Wyman). Dept. Biol., Yale University, New Haven, Conn. 06520

Horseradish peroxidase is injected into individual flight muscle fibers. The peroxidase is transported up the axon to the soma and dendrites of the single motoneuron innervating the injected muscle fiber. After histochemistry, the neuron and its branches are darkly stained and visible in the light and electron microscope. The motoneurons innervating the six fibers of the dorsal longitudinal muscle have been extensively investigated. Each muscle fiber is apparently innervated by a different motoneuron. The cell bodies for the two most dorsal units lie contralaterally and dorsally in the mesothoracic area of the ganglion. The cell bodies for the four ventral units lie on the ipsilateral side, ventral, and more anteriorly. All the dendritic trees ramify throughout the dorsal mesothoracic neuropil. The trees extend more or less equally in the contralateral and ipsilateral neuropil.

The same technique has been applied to flight neuromotor mutants in order to correlate anatomical changes with their behavior and electrophysiology. A number of flightless mutants have been isolated, all having normal wing morphology. Some have abnormal muscle morphology and some apparently have defects only in the nervous system. Peroxidase labeling of motoneurons in these mutants has not indicated any gross changes in the dendrite morphology at the light microscope level.

899 AUTOACTIVE SALIVARY NEURONS IN A TERRESTRIAL MOLLUSK: REFLEX FUNCTION AND SYNAPTIC MODULATION. David J. Prior and Alan Gelperin. School Biol. Sci. Univ. Kentucky, Lexington, Ky. 40506 and Dept. Biology, Princeton Univ., Princeton, N. J. 08540.

The giant garden slug, Limax maximus, possesses a pair of discrete salivary glands and associated contractile salivary ducts. Rhythmic contractions of these ducts serve to expel salivary secretions into the anterior esophagus.

Each salivary duct is innervated by a nerve from the ipsilateral buccal ganglion. En passant recordings from the salivary nerves reveal the presence of a single unit in each nerve that fires bursts of spikes in a regular array (about 1/5 sec at 20°C). Each burst by these salivary nerve bursters (SNB) initiates a peristaltic contraction of the ipsilateral salivary duct.

The somata of the two SNBs are symetrically positioned, one in each buccal ganglion. Each SNB has a single axon in the ipsilateral salivary nerve. Intracellular recordings from the SNB reveal a slow oscillation in resting potential, each depolarization resulting in a burst of spikes. The ongoing burst pattern of the SNBs is very regular. However, when the motor output characteristic of "feeding activity" is generated by the CNS the activity pattern of the SNB is considerably altered. The frequency of spikes within a burst is increased and the bursts are of longer duration, becoming closely synchronized with bursts of motor activity.

The endogenous nature of the basic bursting pattern is indicated by the persistance of the general burst pattern while the isolated preparation is bathed in reduced Ca^{++}, increased Mg^{++} saline. Furthermore, the phase of the SNB burst pattern can be altered by the insertion (by current injection) of an extra burst into the ongoing pattern.

Simultaneous recordings from pairs of identified buccal or cerebral ganglion cells and SNBs have indicated both direct electrical and chemical synaptic inputs capable of altering the bursting pattern of the SNB. Injection of subthreshold depolarizing current into buccal cell B7 can elicit an extra burst in the ipsilateral SNB. Hyperpolarizing current injected into B7 can block burst production by the SNB. Activation of the metacerebral giant cell transiently suppresses bursting in both SNBs.

Therefore, the basic burst pattern of the SNBs appears to be endogenous, with a significant potential for modulation by way of identified synaptic inputs.

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900 SPIKE RATES OF IDENTIFIED NEURONS DETERMINE THEIR ROLE IN INITIATION OF RESPIRATORY RHYTHMICITY IN APLYSIA. Nelson Sinback* and Bert Peretz (SPON:R.A. Morantz). Armed Forces Radiobiology Research Institute, Bethesda, MD 20014 and U. of Kentucky Medical Center, Lexington, KY 40506.

Rhythmic activity endogenous to the parieto-visceral ganglion (PVG) initiates pinnule filling movements, PF movements, which pump blood through the gill. Each PF movement is composed of two constituent contractions: a flare of the gill pinnules away from the efferent vessel and twitch contractions of the efferent vessel. PF movements are stereotyped behaviors with invariant constituent contractions, sequence of contractions, and periodicity (1/90 sec). Suppression of spiking of PVG neurons established that motoneuron LD1 elicited pinnule flares and motoneuron Leff elicited efferent vessel contractions during PF movements. In addition, non-motoneurons L10 and L11 and motoneuron L7 each spiked in fixed pattern during PF movements. L7 spike rates above 2sps elicited gill contractions. During PF movements, L7 spiked at 1.5 sps and hence did not elicit a gill contraction. Increased spiking induced by intracellular stimulation of Leff, L7, L11, or L10 caused a five fold prolongation of the interval between PF movements. The fact that simultaneous spike suppression of 2 neurons shortened intervals suggests that Leff, L7, L11 and L10 are part of a network initiating PF rhythmicity. L7 caused maximum prolongation of PF movement intervals at 1-2sps, Leff caused maximum prolongation at 3-4sps, and L11 and L10 caused maximum prolongation at 3 sps. In each neuron higher or lower spike rates caused less prolongation. Thus, changes in a neuron's spike rate could alter its role in initiation of PF rhythmicity. For motoneuron L7, the spike rate which caused maximum prolongation(1-2sps) was less than the spike rate which elicited gill contraction (>2sps). Hence, L7 can perform either of two roles - initiate PF rhythmicity or elicit gill contractions - depending on its spike rate. This research was supported by MH 18611 to B.P.

901 UNIT ACTIVITY OF THE VISCERAL GANGLION OF THE MUSSEL, MYTILUS EDULIS AND THE CONTROL OF CILICARY MOVEMENT. Martin D. Hamburg and Anthony A. Paparo* (SPON: B. Kaplan). Dept. Anat., Cornell U. Medical College, New York, New York 10021 and School of Medicine, Southern Illinois University, Carbondale, Ill. 62901.

The relative simplicity of the molluscan nervous system holds promise for the combined histological, histochemical and electrophysiological investigation of the same, repeatably identifiable elements. At the same time, even in lower invertebrates, the degree of organization and differentiation of the nervous system is sufficiently high that the combined patterns of anatomical connection, electrical activity and transmitter characteristics in the control of behavior might serve as a useful model valid for higher species. This investigation considers the effect of pharmacological modification of unit activity of the visceral ganglion of the mussel, Mytilus edulis and the control of ciliary beating.

Unit activity was recorded from the cells of the visceral ganglion in intact animals. Lateral ciliary activity was also studied in isolated gill-ganglion preparations. Direct application of dopamine (DA) to the visceral ganglion produced a long-term inhibition of electrical activity from cells of the visceral ganglion. Application of low concentrations (10^{-7} M) of acetylcholine (ACh) increased unit activity of the visceral ganglion while high concentrations (10^{-3} M) of ACh inhibited visceral ganglion activity. Both effects were blocked by prior application of hexamethonium (Hex). The inhibition of unit activity produced both by DA application and high concentration ACh was prevented by prior treatment with phenoxybenzamine (PBZ). Application of norepinepherine (NE) failed to influence neuronal activity of the visceral ganglion.

DA applied either to the isolated gill or isolated ganglion prevented cilio-excitation due to electrical stimulation of the branchial nerve. Serotonin (5-HT) excited ciliary beating when applied to the isolated gill, but when applied to the isolated ganglion affected neither ciliary beating nor neuronal activity of the visceral ganglion. Applications of Hex to the isolated gill prevented the excitation of ciliary activity produced by electrical stimulation of the cerebro-visceral connective and produced a long lasting cilio-inhibition that followed stimulus offset.

These data suggest that (a) the release of ACh in the visceral ganglion stimulates cilio-excitatory neurons and also small DA releasing interneurons that inhibit these same neurons; (b) the effect of this system is to provide for ACh mediated self-regulation of ciliary activity within the visceral ganglion; and (c) besides DA releasing interneurons present within the visceral ganglion, DA releasing axons that terminate directly at the gill originate outside the visceral ganglion and can be found in the cerebrovisceral connective.

902 COLOR DISCRIMINATION CAPABILITY IN THE CRAYFISH OPTOKINETIC SYSTEM.
Richard F. Olivo, Yoko Hiraga* and Karen Koumjian*. Dept. Biol. Sci., Smith College, Northampton MA 01060.

Crayfish have been shown to possess yellow-sensitive (560 nm) and blue-sensitive (450 nm) photoreceptors, and thus could have color vision. But because of the sparsity of blue receptors (10-30% of the total) and the theoretical possibility that the blue receptors' function is to extend the spectral sensitivity range rather than to permit color discrimination, we have examined color discrimination behaviorally, utilizing the optokinetic response. Restrained crayfish were placed at the center of a drum made of red (665 nm) and blue (440 nm) translucent stripes. Illumination from separate red and blue sources, adjusted with neutral density filters, permitted the relative luminance of the red and blue stripes to be varied independently. The drum was oscillated through 36° at approximately 0.1 Hz and the animals' eye movements were recorded as they tracked the drum. Color discrimination was tested by holding the blue stripes at constant luminance and varying the red stripes from very bright to very dark; the amplitude of the tracking response was initially high, decreased to a null, and increased again as the red brightness was changed. The null presumably represents the minimum brightness contrast between red and blue stripes; the tracking amplitude at the null was typically 20% (and sometimes as great as 60%) of the maximum. The response of the same animals to a control stimulus (a drum made entirely of red stripes) was unmeasurably small. Thus, these experiments indicate that the crayfish can detect the pattern of red and blue stripes regardless of the brightness contrast between the stripes. The implication is that the crayfish optokinetic system is capable of color discrimination.

903 NEUROPIL RECORDING IN THE LOBSTER STOMATOGASTRIC GANGLION.
John P. Miller. Dept. of Biology, UCSD, La Jolla, CA. 92037

A new technique has been developed for marking the recording sites in neuropil penetrations of cells in the stomatogastric ganglion of the lobster Panulirus interruptus. Fiber-filled glass capillary microelectrodes are back filled with 4% (w/v) Procion Black H-GSA (PB). Cells are marked at the end of an experiment by passing 500 msec. 25-50 nanoamp hyperpolarizing pulses at 1 hz. for about 15 min. Unlike other Procion dyes currently in use, PB does not diffuse throughout the cell cytoplasm, but remains localized at the point of injection within a radius of 10-15μ. PB electrodes of up to 350MΩ have been used to record from and mark small(~10μ) neuropilar processes.

Experiments were done with a PB electrode in the neuropil and a Procion Yellow (PY) electrode in the soma of the same cell. The cell could thus be marked at the neuropilar site as well as filled with PY for fluorescence microscopic visualization and reconstruction. Ganglia were fixed in Bouins solution, dehydrated in an alcohol series, and cleared in methyl benzoate.

Dual neuropil-soma recordings, with neuropil electrodes 300-500 microns from the somata, show that spikes are decremented as much as 50% in passing from neuropil to soma. However, very little(<5%) decrement of PSP's occurs from 10μ neuropil processes to soma. The decrement of activity from neuropil to soma is therefore largely due to capacative filtering rather than to resistive decrement, and PSP activity monitored in the soma is apparently a good measure of sub-threshold activity going on in the integrative region.

Preliminary experiments show that marking and filling might be done with a single electrode filled with a mixture of PB plus either PY or Procion Rubine (PR). PB, as well as PR, contains a heavy metal complex, and should be easily located in electron micrographs. This method might prove valuable in studies involving blind penetrations through vertebrate CNS.

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904 CONVULSIVE BURSTING IN NEURONS IN THE ABSENCE OF NEGATIVE CONDUCTANCE.
L. Donald Partridge* (SPON: A. O. D. Willows). Friday Harbor Labs, Friday Harbor, Washington, 98250.

The membrane potential instability produced by a region of negative conductance in the steady state I-V curve for a neuron has been proposed to underlie bursting activity. The study reported here demonstrates the existance of a negative conductance characteristic in a neuron which does not show bursting behavior, furthermore this characteristic is eliminated when bursting is induced in the neuron.

Steady state I-V curves for a non-bursting neuron, LP16, in the pleural ganglion of Tritonia were generated continuously by applying triangular wave command signals to a voltage clamp. The typical I-V curve for this neuron in sea water showed a decreasing slope conductance from resting potential in the depolarizing direction and then a negative slope conductance from about 10 mV depolarized until a spike current was produced in an uncontrolled region of the neuron. When 80 mM pentylenetetrazol was applied to these neurons, typical bursting activity developed within about 5 minutes. In each case the negative slope conductance region in the steady state I-V curve was found to be considerably reduced and usually abolished altogether. It is suggested that the inherent instability of the negative slope conductance region of the steady state I-V characteristic is not a necessary mechanism to model pentylenetetrazol induced bursting activity.

905 BEHAVIOR OF THE FOREGUT OF THE SPINY LOBSTER.
William J. Tighe, Jr. Dept. of Biology UCSD, La Jolla, CA 92037

The stomatogastric system in the spiny lobster, Panulirus interruptus, is one of the most completely described small motor nervous systems. However, the transformation of its motor patterns into behavior of the foregut has not been studied.

In semi-intact behaving lobsters, a system for the sensitive monitoring of multiple positions in two dimensions in time has been prepared for attachment to the stomach.

Low inertia arms, generating D.C. signals based on miniature Hall-Effect devices act as position transducers. With these transducers, fine wire electrodes are inserted into muscles for the recording of E.M.G.s simultaneous with the motions.

Preliminary work has clearly demonstrated that gastric mill muscles GM 6b close the lateral teeth and GM 3c opens the lateral teeth. The basic gastric mill rhythm seen in these semi-intact preparations is clearly identical with that in isolated preparations. Additionally longer term modulations of these rhythms have been seen and are in the process of being fully described. Continued work will describe the dynamic and temporal range of the stomatogastric system.

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906 INNERVATION OF PUTATIVE MECHANORECEPTOR NEURONS IN A SQUID SUCKER.
P. A. Santi and P. P. C. Graziadei. Department of Biological Science, Florida State University, Tallahassee, Florida, 32306.

Presumed mechanoreceptor neurons have been described in a squid sucker on both light and electron microscopical levels. The neurons are distributed around the oral surface of the sucker in a narrow basal zone of an epithelium which is crucial in the formation of the hermetic seal of the sucker. The neurons have a distinctly multipolar shape with numerous processes or dendrites branching along a single lateral plane. Their axon is thick, unbranched, and was traced to at least the level of the sucker ganglion's neuropil. The most striking observation was that some if not all of the neurons are innervated by thin accessory nerve fibers. Synaptic contacts have been observed from the accessory nerve fibers onto the receptor neuron's soma, axon, and dendrites. Both the occurrence of intraepithelial multipolar neurons and their peripheral innervation is unusual. The position, distribution, and shape of these neurons suggest that they may function as mechanoreceptors; however, it is not clear whether they are sensitive to stimuli impinging upon the epithelium or stimuli from the muscles upon which the epithelium rests. Previous behavioral experiments indicate that cephalopod suckers possess a high degree of chemotactile sensitivity. Nevertheless, the function of these neurons and their innervation awaits further electrophysiological or behavioral experiments.

907 SUSTAINED OSCILLATIONS AND TEMPORAL FILTERING IN THE CRAYFISH OPTIC NERVE.
Raymon M. Glantz and Harvey B. Nudelman, Dept. of Biology, Rice University, and Dept. of Psychiatry U.T. Medical School, Houston, Texas.

The steady state discharge of the sustaining fibers (SF.) of the crayfish optic nerve exhibit marked regularity. Interspike interval histograms reveal a multimodal structure with a natural period of about 100ms and all other peaks at integral multiples of the natural period. Autocorrelograms reveal a periodic structure with peaks at the natural period extending for at least 10 cycles after any arbitrary spike. These data suggest that constant illumination results in a sustained oscillation of a neural process intermediate between the retinula and the SF. A possible physical basis of this oscillator is the lateral inhibitory network subserving the convergent lamina ganglionaris cells presynaptic to the SF. If this is the case the periodic activity of the SF would arise from the synchronization of its convergent inputs and the natural period should reflect the time course of the group inhibition. Such a network would be expected to resonate if driven at its natural frequency. This hypothesis was tested with sinusoidally modulated illumination. If the SF mean discharge rate is examined with respect to the stimulus modulation frequency the plot exhibits a broad peak with a high frequency cut-off at or near the reciprocal of the natural period. Examination of the SF firing time with respect to the stimulus phase reveals that the stimulus modulation frequencies associated with enhanced SF mean discharge rate result in distinct phase locked firing. These results are interpreted in terms of the entrainment of an endogenous oscillator.

908 SYNCHRONIZED OSCILLATIONS OF CRAYFISH SUSTAINING FIBERS: EVIDENCE FOR A NETWORK ORIGIN. HB Nudelman and R.Glantz. Dept Psychiatry Univ Tex Med Schl, Dept Biology Rice Univ. Houston Texas.

Evidence has been presented in the companion abstract that the crayfish sustaining fiber(SF) under high flux large field conditions exhibits oscillating type behavior. This "oscillator" could reside either in the individual SF's or in some common neural network. If the latter is true one would expect to find synchronized oscillations among pairs of SF's. This hypothesis is easily tested by recording simultaneously from 2 SF's in the same lead and electronically separating them for computer analysis. Joint PST scatter diagrams were plotted online to look for correlations between SF's under broad field high flux conditions. These correlations would be reflected by diagonal structures in the joint PST scatter diagrams(Gerstein & Perkel Biophys J 12,5 1972). Diagonal densities along the major diagonal and parallel to it were observed in a variety of SF pairs. The existence of these diagonal densities was intensity dependent.

Detailed examination of one pair,038 & 056, showed their interval histograms both having a peak at 100 ms. 056 exhibited peaks at multiples of 100 ms. The joint PST scatter diagrams showed diagonal densities whose periodicity was 100 ms. When a PST of 056 spikes is made triggered from an 038 spike it is clear that 056 spikes are phase locked to the occurrence of the 038 spike. The peaks of this histogram are separated by 100 ms. These results suggest that these SF's are entrained by a common oscillating network. A lateral inhibition based model is suggested. NSF BMS72-02010 A02

909 NEURAL COUPLING MECHANISMS FOR REFLEXIVE AND CIRCADIAN MOVEMENTS. William H. Gordon* and James L. Larimer. Dept. of Zoo., University of Texas at Austin, Texas, 78712.

Surgical lesions in the circumesophageal connectives (CEC's) of crayfish were used to locate interneurons responsible for coupling two locomotor behaviors to their respective driving systems in the brain. Page and Larimer (J. Comp Physiol 78; 107 (1972): 97; 80 (1975)) have shown that one of these behaviors, the "lights-on," is a reflexive response, requiring a retinal input. The other, occuring at "lights-off," is a circadian behavior that can be entrained via an extraretinal pathway. Both behaviors are lost after complete sectioning of the CECs. Animals with one quadrant of a single CEC remaining were continuously monitored for locomotor or for leg movement activity. Animals having any one of the quadrants except the dorso-medial (DM) retained both behaviors. Furthermore, in approximately 90% of the animals with the DM quadrant, both behaviors were lost concurrently. These data imply that 1) there are multiple coupling interneurons mediating these behaviors, and 2) the coupling neurons for the reflexive and circadian behaviors are either co-distributed in the cord quadrants, or are in fact the same CEC elements. These alternatives are now being investigated using chronically implanted microelectrodes in selected cord quadrants to better characterize the responsible interneurons.

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910 CONDUCTION BLOCK AT BRANCH POINTS OF SENSORY NEURONS IN THE LEECH CNS. King-Wai Yau* (SPON: J. G. Nicholls). Dept. of Neurobiology, Harvard Med. Sch., Boston, Mass. 02115 and Fleischmann Labs, Stanford Univ. Med. Center, Stanford, Ca. 94305

When cutaneous sensory neurons in the leech CNS that respond to touch fire repetitively they become hyperpolarized through the action of an electrogenic Na pump. As a result impulses can fail to propagate through certain processes. In the present experiments a correlation has been made between the geometry of a cell's processes and the sites where conduction block occurs. The technique was to inject horseradish peroxidase intracellularly; this provided a detailed picture of the arborizations and revealed sensory axons which had not been observed in earlier studies. These newly found processes innervated the skin through nerve roots of the adjacent anterior ganglion in contrast to the major processes which passed through the nerve roots of the ganglion containing the cell body. These 'extra' sensory axons were susceptible to conduction block when firing for brief periods at low frequencies produced by moderate mechanical stimulation of the skin. Their morphology, together with experiments in which artificial block was produced by passing hyperpolarizing current into cell bodies, suggested that conduction block occurred at branch points of cell processes. The tendency of a branch point to fail depended on the relative diameters of the fibers. Intracellular recordings from motor cells which were postsynaptic to pressure sensory neurons indicated that synaptic transmission was modified when conduction block occurred in the presynaptic sensory cells. Other experiments suggested that branch points with particularly low safety margins of conduction could operate as low-pass filters, so that action potentials separated in time by short intervals were unable to get through.

911 EFFECTS OF NERVE AND CONNECTIVE LESIONS ON ENTRAINMENT OF THE OCULAR OSCILLATOR OF APLYSIA BY RED LIGHTCYCLES. Gene D. Block and David J. Hudson*. Dept. of Psychology, University of Oregon, Eugene, Oregon 97403.

The eye of Aplysia contains a circadian oscillator. When the eye with its nerve is isolated in darkness compound action potentials (CAPs) occur autorhythmically and their frequency expresses a circadian rhythm. The peak CAP frequency occurs near dawn of the previously applied lightcycles (Jacklet, 1969). Ocular photoreceptors are sufficient for entraining the eye rhythm since isolated eyes can be entrained by white lightcycles in vitro (Eskin, 1971). This is not the case, however, when red lightcycles are employed as the zeitgeber. With red light entrainment only occurs if the optic nerve remains attached to the rest of the central nervous system (Block et al., 1974). Since the eye is relatively insensitive to red light (Waser, 1968) and efferent fibers have been demonstrated in the optic nerve (Eskin, 1971), it seems likely that entrainment of the ocular oscillator by red lightcycles occurs by means of extraocular photoreceptors communicating with the eye via efferents in the optic nerve.

The present study investigated which of the various extraocular photoreceptors might be responsible for ocular entrainment by red lightcycles. Extraocular photoreceptors have already been described in the abdominal ganglion (Arvanataki & Chalazonitis, 1961) and in the cerebral ganglion (Eskin, 1971; Block & Smith, 1973). In addition we have found red sensitive photoreceptors in the tentacles whose response can be recorded in the optic nerve. After cutting all cerebral nerves and connectives except for one optic nerve, red lightcycles continued to entrain the eye whose nerve was intact. Red lightcycles had no effect or only a weak effect on the eye whose optic nerve had been cut. Barring possible hormonal factors which might "jump over" the lesions, it seems likely that the cerebral ganglion contains extraocular photoreceptors responsible for entrainment of the eye by red lightcycles.

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912 MODULATION OF SYNAPTIC TRANSMISSION AND BURSTER NEURON ACTIVITY AFTER RELEASE OF A NEUROHORMONE IN APLYSIA. Earl Mayeri and Sandra Simon*. Dept. Physiol., University of California, San Francisco, CA 94143.

In the marine mollusk Aplysia californica a hormone released by electrical stimulation of neurosecretory bag cells (BCs) induces egg laying when the medium bathing the BCs is injected into another animal (Kupfermann, JN 33: 877, 1970). The BCs are located in the abdominal ganglion and send extensive granule-filled neural processes into the ganglion sheath, ending in presumed neurohaemal sinuses near other types of ganglion neurons, but not innervating them (Coggeshall, JN 30: 1263, 1967; Frazier et al, JN 30: 1288, 1967). Since egg laying involves the release of a stereotyped behavior, we began to investigate the possibility that a hormone released from the BCs can influence behavior by a direct action on the central nervous system.

Recording simultaneously from one of the BCs and from other identified neurons in the isolated ganglion with intracellular microelectrodes, a BC cluster was extracellularly stimulated with a short train of pulses (5 msec, $\leq$10 mA pulses at 5 Hz for 1-5 sec) to elicit a burst of BC spike activity lasting 3-10 min. The BC burst was followed by two types of responses. First, there was an increase in the size of excitatory synaptic potentials in some identified cells produced directly by the identified interneuron L10. In cells of the RB cluster the increase develops to a maximum in 20-40 min and returns to baseline level usually within 1-2 hr. With strong responses there is a five fold or more facilitation of the synaptic potentials. Synaptic facilitation, monitored during spontaneous L10 spike activity, is often accompanied by a transition from tonic L10 discharge to periodic burst discharges. As a result of these changes, the synaptic connections from L10 to RB cells temporarily become highly effective ones, L10 strongly driving the postsynaptic cells sometimes for an hour or more. Second, in the parabolic burster neuron R15 there was an increase in the amplitude of the slow potential that underlies the endogenous periodic burst discharge of this neuron, resulting in an increase in the spike rate and number of spikes during the burst, and an increase in average spike rate. Potentiation of the slow potential begins in a few minutes, peaks within 20-60 min and lasts for 2 hr or more.

Neither of the two responses has been observed in the absence of a BC discharge after ganglion nerves and connectives were stimulated. Both responses were observed on occasions when the BCs burst spontaneously. There was no sign of synaptically mediated interactions between BCs and other ganglion cells, including L10, RBs, and R15, during BC activity. These results, and our preliminary studies of the effects of an extract of BCs applied to the ganglion, provide evidence that a neurohormone released under nearly physiological conditions induces long term changes in synaptic transmission and endogenous burst activity. Previous studies indicate that the neurohormone(s) released from the BCs is a low molecular weight polypeptide, and that vertebrate neurohypophyseal peptide hormones produce a response in R15 similar to the one reported here. Since L10 is a command interneuron mediating cardioacceleration via excitation of one of the RB cells (Koester et al, JN 37: 476, 1974), it should be possible to determine if these neural changes result in behavioral ones, and to analyze the underlying cellular mechanisms in considerable detail.

913 HABITUATION AND ITS MODIFICATION BY EXPERIENCE IN CRICKET GIANT INTERNEURONS. R.K. Murphey, S.G. Matsumoto*, R.B. Levine*. Dept. Biology, S.U.N.Y., Albany, New York 12222.

The process of habituation to tone pulses has been examined in the medial giant interneuron (MGI) of Acheta domesticus using intracellular recording methods. This neuron responds phasically with a short burst of 5-10 spikes at the onset of a tone pulse. Depression of the response occurs for tone repetition rates above 0.25 Hz. Increasing the tone repetition rate causes a more rapid depression of the response. Increasing the tone duration also causes more rapid depression.

The sensory receptors activating MGI consist of a group of mechanoreceptive hairs located on the cerci (more than 300 hairs/cercus). Single mechanoreceptive hairs were left free to move while all other hairs on both cerci were covered with vaseline. The rate of depression of the excitatory post synaptic potential from a single hair is very similar to the rate at which the overall response of MGI declines. Thus the characteristics of the receptor and its synapse with MGI are sufficient to account for habituation of the MGI response.

The habituation rate to tones is modified by exposing the specimen to tones for various periods. Short term (10 mins.- 3 hrs.) exposure of a specimen to an habituating stimulus produces depression of the response. Recovery from this depression occurs in 5-10 mins. Exposure of the specimen to an habituating stimulus from hatching to adulthood (60-90 days) produces a specimen which exhibits less depression than control specimens to a test stimulus. This effect is stable for at least 2 hours. We conclude that the synaptic connection is more "stable" in experienced than it is in naive specimens. Supported by NIH Grant # 1 R01 NS 10825-01A1 PHY. Work done at the University of Iowa and at the University of Oregon.

914 HABITUATION AT THE TERMINALS OF NEURON L7 IN THE GILL OF APLYSIA. Jon W. Jacklet and Jasper Rine*. Dept. Biology, SUNYA, Albany, 12222.

Tactile stimulation (5 gms) of the siphon of Aplysia evoked withdrawal of the gill and concomitant spiking in central neuron, L7. The neuron fired at rates comparable to those observed by others (Kupfermann et al., 1970). With repeated tactile stimulation at 30 sec intervals the gill withdrawal amplitude habituated to 10% of the initial value in 8-10 trials but the number of spikes evoked in L7 was relatively unchanged. If the rate of firing of L7 produced by tactile stimulation of the siphon was mimiced by selective intracellular depolarization of L7, withdrawal of the gill was never produced. This result suggests that L7 contributes very little or nothing to gill withdrawal during repeated tactile stimulation at 5 gms. However, withdrawal of the gill, comparable in amplitude to that produced by tactile stimulation of the siphon, was produced by selective intracellular depolarization of L7 if the evoked rates of firing were 15/sec for 2 sec. L7 never fires at such sustained rates during 5 gm siphon stimulation. When selective intracellular stimulation of L7, sufficient to evoke 15 spikes/sec for 2 sec, was repeated every 30 sec (habituation paradigm), the amplitude of gill withdrawal progressively decreased with a time course comparable to habituation evoked by tactile siphon stimulation. The gill response to L7 stimulation could be dishabituated by tactile stimulation of the siphon or mantle without changing the subsequent evoked spike rate in L7 by more than a few per cent. These results show that, during weak or moderate (up to 5 gm) stimulation of the siphon, L7 contributes very little directly to habituation of the gill withdrawal. The results also show that the terminals of L7 in the gill are a site of habituation and dishabituation. This site could be at neuromuscular junctions or at peripheral gill neurons. Supported by NIH grant 08443 to J.W.J.

915 THE INTERACTION OF CENTRAL AND PERIPHERAL PATHWAYS MEDIATING GILL WITHDRAWAL REFLEX HABITUATION IN APLYSIA, K. Lukowiak* and B. Peretz
University of Kentucky Medical Center, Lexington, Kentucky 40506

Central and peripheral pathways mediate habituation of the gill withdrawal reflex to tactile stimulation of the siphon or gill (Peretz, 1970; Jacklet et al, 1975). The central (CNS) and peripheral nervous (PNS) systems are proposed to be independent (Kupfermann et al, 1974), but other work suggests an interaction (Lukowiak, 1973; Peretz and Howieson, 1973). An interaction would be shown if activity in one pathway affected the response mediated by the other pathway. We did four experiments to test for the interaction. (1) Repeated electrical stimulation of the cut ends of the branchial or ctendial nerves (efferent pathways to the gill) results in habituation of the gill response. Tactile stimulation of the gill or siphon dishabituates the nerve-elicited response. The dishabituatory effect of tactile stimulation shows that activity evoked in the PNS affects the response elicited by the central pathways and that the interaction occurs peripherally. (2) Repeated depolarization of L7, a gill motor neuron in the CNS, results in habituation of the elicited gill response. The interposition of a tactile stimulus to the gill dishabituates the elicited response. Tactile stimulation of the siphon, even with the siphon nerve severed, also dishabituates the elicited response. (3) Repeated tactile stimulation of the gill, which causes gill movement that does not involve L7, changes the efficacy of L7's ability to elicit gill movement. (4) The depolarization of L7 interposed during habituation (to tactile stimulation of the gill) results in dishabituation of the response. The results show that activity of a single central neuron, L7, affects the response mediated by the PNS and that activity in the PNS affects the response elicited by a single central neuron. Thus, the CNS and PNS involved in the gill withdrawal reflex interact.

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916 TWO FUNCTIONAL CONSEQUENCES OF DECREASED CONDUCTANCE EPSPs: INCREASED ELECTROTONIC COUPLING AND AMPLIFICATION. Thomas J. Carew and Eric R. Kandel, Div. Neurobiol. & Behavior, Coll. Physicians & Surgeons, Columbia Univ., New York, N.Y. 10032

In an attempt to relate various forms of synaptic actions in Aplysia californica to behavior, we have examined the functional consequences of both increased and decreased conductance PSPs in a common effector system: the inking motor system. Inking is triggered by a noxious stimulus applied to head or siphon. The response is mediated by three electrically coupled identified motor cells ($L14_A$, $L14_B$ and $L14_C$) located in the abdominal ganglion. Electrical stimulation of peripheral nerves or connectives produces identical synaptic responses in all three cells: a fast EPSP (0.1-0.3 sec) due to an increased ionic conductance, followed by a slow EPSP (10 sec - 3 min) due to a decreased ionic conductance. During the fast EPSP, electrotonic coupling between the ink motor cells is reduced (by up to 40%); however during the slow EPSP, coupling is enhanced (by up to 30%). Thus the degree of electrotonic coupling between the ink motor neurons can be increased or decreased by nearby chemical synapses (Fig. 1).

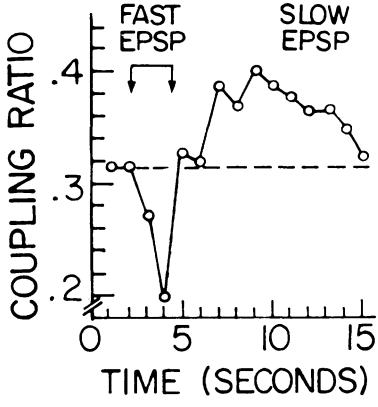

FIG. 1. Increased and decreased electrotonic coupling. Constant current hyperpolarizing pulses were injected (1/sec) into one ink motor cell while recording from all three (coupling between $L14_A$ and $L14_C$ is graphed). Connectives were stimulated (1.5 msec pulses, 6/sec for 2 sec, indicated by arrows) producing a fast (increased conductance) EPSP during the train and a slow (decreased conductance) EPSP following the train. The coupling ratio (0.316, dashed line) is decreased during the fast EPSP (pulses 3-4) and increased during the slow EPSP (pulses 5-15).

In contrast to the increased conductance EPSP, that decreases the amplitude of other synaptic actions because it shunts the membrane resistance, the decreased conductance EPSP can amplify the action of a previously ineffective sensory input (from siphon skin), making it capable of triggering an inking response. The amplification appears attributable to two factors: (1) the synaptic current from the sensory input produces a larger potential change in the ink cells because of their increased input resistance; and (2) the increased coupling between the cells may increase positive feedback among them. The amplification is not simply due to the depolarization from the slow EPSP, since intracellular depolarization comparable to that of the slow EPSP does not amplify the same sensory input. Nor is the amplification simply due to presynaptic facilitation, since intracellular pulses which are just at threshold for spike initiation are also amplified by the slow EPSP.

A suprathreshold noxious stimulus produces inking predominantly by means of the fast EPSP. Electrophysiological and behavioral studies indicate that the slow EPSP provides a second means of triggering inking whereby two subthreshold stimuli delivered sequentially to the same or even to different sites become capable of triggering inking.

917 CENTRAL MECHANORECEPTOR NEURONS IN APLYSIA CONNECT TO PERIPHERAL SIPHON MOTOR NEURONS: A SIMPLE SYSTEM FOR THE MORPHOLOGICAL STUDY OF THE SYNAPTIC MECHANISM UNDERLYING HABITUATION. C. Bailey*, V. Castellucci, J. Koester and E. R. Kandel. Neurobiol. and Behav., Columbia U., P & S.

To study the morphological substrate of plasticity we have developed a preparation that may permit the identification of synapses that mediate habituation. Weak tactile stimulation of the siphon produces habituation of both the gill and siphon withdrawal reflex. Gill withdrawal is centrally mediated; habituation results from a decrease in transmitter quantal output at the synapses made by the central mechanoreceptor neurons on the central gill motor cells (Castellucci and Kandel 1974). Siphon withdrawal has both central (60%) and peripheral (40%) components (Perlman, 1975). The same sensory cells connect to both central siphon and gill motor cells. Since the gill, and the central and peripheral siphon components all have similar kinetics of habituation, we tested the possibility that peripheral siphon motor cells are also innervated by branches of central sensory cells. In the periphery the siphon nerve contains at least two populations of neurons: one type contains large electron dense granules commonly found in neurosecretory cells; the other type contains cells that move the siphon. The putative motor neurons are excited by tactile stimulation of the siphon skin via direct connections from branches of the same central sensory cells that innervate the central motor neurons. The monosynaptic EPSPs from these branches also undergo depression with repeated stimulation. The morphological identification of specific synapses is often hampered by the complexity of the neuropil and requires the use of an intracellular marker for both pre- and postsynaptic cells. The spatial isolation of the peripheral motor neurons suggests that the three-dimensional morphology of sensory to motor synapses may be reconstructed in both the rested and habituated state without labelling the motor cell.

918 THE CONTROL OF WALKING IN THE SCORPION: JOINT RECEPTOR ANALYSIS. R. F. Bowerman. Dept. of Zoology & Physiology, University of Wyoming, Laramie, Wyoming 82071.

Experimentation on the neural mechanisms underlying the control of walking in arthropods has focused primarily on insects and crustaceans. Recently, Bowerman has initiated work on scorpion walking, describing aspects of locomotion in intact animals (Bowerman, J. Comp. Physiol, in press) and in animals with one or several appendages ablated (Bowerman, J. Comp. Physiol, in press), which revealed a contribution of undefined sensory feedback from the appendages to the control of walking. In order to comprehend how proprioceptive input shapes motor output, it is necessary to appreciate the number and discharge characteristics of the total array of proprioceptive elements which monitor the leg joints. The walking legs consist of seven segments; coxa, trochanter, femur, patella, tibia, tarsus (tarsomere 1 and tarsomere 2) and pretarsus (tarsal claws). The C-T joint is quite dextrous, exhibiting movement both in anterior-posterior and dorsal-ventral planes, as well as limited rotation about the long axis. The proprioceptive elements monitoring these movements are correspondingly complex in their sensitivities. More distal joints (T-F, F-P, P-T, T-Tar 1, Tar 1-Tar 2) are planar. Each joint has an array (10-15) of tonic units which code for joint position at either one end of the joint range or the other; e.g., flexion or extension sensitive. An additional complement of phasic units (5-10) provides movement information, reflecting direction and velocity of joint movement. In total, the CNS is provided with extensive proprioceptive information for each joint of the scorpion walking leg.

919 GENERIC DIFFERENCES IN COMMAND FIBER CONTROL OF ABDOMINAL EXTENSION IN CRAYFISH. Charles H. Page. Dept. Physiol., Rutgers Univ., New Brunswick, N.J. 08903.

Coordinated extensions of the abdomen were evoked by stimulating command fibers (CFs) in the circumesophageal connectives (CCs) of Procambarus clarkii. Some extension CFs excited thesuperficial extensor motor neuron (SEMN#4) which innervates the tonic receptor muscle of the muscle receptor organ; others excited one or more of the excitatory SEMNs which innervate only the superficial extensor muscle. The inhibitory SEMN was silent during these extensions. In contrast, CFs which elicited coordinated abdominal extensions were not found in the CCs of Orconectes rusticus and O. virilis. Extensions were never evoked unless two or more CC fibers were stimulated simultaneously.

When P. clarkii are lifted up from the aquarium floor into the water column they usually extend their abdomens. Under identical stimulus conditions O. rusticus and O. virilis usually move their abdomens to an intermediate position. The extension sensitivity exhibited by P. clarkii is correlated with the presence of extension CFs in its CCs while extension insensitivity is associated with the apparent absence of extension CFs in the CCs of O. rusticus and O. virilis. These correlations provide support for the presumption thatabdominal extension of the intact crayfish is elicited by the internal excitation of one or more CF. (Supported by NIH grants #NS 09957 and NS 12262).

920 SEGMENTAL HOMOLOGY AND VARIATION IN FLEXOR MOTONEURONS OF THE CRAYFISH ABDOMEN. Jay E. Mittenthal and Jeffrey J. Wine, Dept. Biol. Sci., Purdue Univ., W. Lafayette, Indiana, 47907, and Dept. Psych., Stanford Univ., Stanford, Ca. 94305.

If a neural subsystem in a segmented animal involves several segments, knowledge of the organization of the subsystem in every segment is essential for understanding its evolution, development, and behavioral function. The subsystems controlling phasic and tonic flexion of the crayfish abdomen have served as paradigms for the organization of motor control. To extend the utility of this preparation we report the location and size of all phasic and tonic flexor motoneurons in the anterior five of the six abdominal ganglia.

Cobalt dye[1] was allowed to diffuse into the cut ends of flexor motoneuron axons by dipping roots containing only those axons into 150mM $CoCl_2$ for 10 - 14 hours. Ganglia were prepared and processed as previously described.[2] From camera lucida drawings the projected soma areas, and position of soma centers with respect to an orthogonal co-ordinate frame (y axis = midline, x axis = line bisecting angle between roots 1 and 2), were measured in more than 100 ganglia.

The somata form three clusters on the basis of position in the ganglia and location of the root in which axons exit. For the phasic flexors, the anterior group shows the greatest departure from serial homology; it has 1 soma in the first abdominal ganglion (G1), 3 in G2 and G3, 2 in G4, and none in G5. The medial group contains the giant motoneuron, the peripheral inhibitor, and two smaller neurons in all five ganglia. The posterior group contains four somata in G1 - G4 and three in G5. The tonic flexor motoneuron somata cluster into the same three groups and are adjacent to phasic flexor somata. The tonic flexor somata are serially homologous in all five ganglia.

Homologous groups, and to some extent homologous neurons, can be recognized in the thoracic ganglia, and in abdominal ganglia of other decapod crustacea. Three groups of motoneurons appear in several orders of insects that are strikingly similar to the groups in decapod crustacea with regard to position of somata in a ganglion, laterality and relative rostrocaudal position of root of axon emergence, putative neurotransmitters, and geometry of neuropilar arborizations. The groups in crustacea and insects may be homologous, and corresponding groups may exist widely within the Arthropoda.

Intersegmental variation in soma size of the two largest motoneurons, which are believed to innervate all phasic flexor muscles within their segments, parallels the variation in volume of deep flexor musculature per segment. It is suggested that the caudal reduction in volume of musculature results in a corresponding reduction in trophic requirements of muscles and in axonal arborization of motoneurons, leading in turn to reduction in the size of the motoneuron somata.

Quantitative differences in maps of soma positions were characterized by a set of affine transformations which map mean positions of either deep or superficial motoneuron somata from each ganglion into the mean positions of homologous somata in G2. The magnitude of distortions required for each transformation are correlated with differences among ganglia in size of homologous somata. However, a transformation does not invariably map the mean image of the ganglionic margin and roots into the margin and roots of G2. Dissimilarities of transformed ganglionic margins can in part be interpreted as a consequence of deviation from serial homology in the anterior group of phasic flexor motoneurons.

[1]Pitman, Tweedle, and Cohen, Science 176:412 (1972)
[2]Wine, Mittenthal, and Kennedy, J. Comp. Physiol. A. 93: 315 (1974)

support: for J. E. M., NIH grant NS-11383; for J. J. W., NSF grant GB-40058.

921 INPUT-OUTPUT CHARACTERISTICS OF THE DEFENSIVE GILL-WITHDRAWAL REFLEX IN APLYSIA CALIFORNICA. John Byrne, Div. Neurobiol. & Behav., Columbia Univ., P & S, and N.Y. State Psychiatric Inst., N.Y. 10032

Reflex withdrawal of the gill of Aplysia produced by tactile stimulation of the siphon skin is mediated by 24 mechanoreceptor neurons, 6 motor neurons and at least three interneurons. To determine to what extent these cells quantitatively account for the behavior, intracellular recordings were made from individual mechanoreceptor and motor neurons while controlled force step indentations of various intensities (.24 to 4.2 gm; 0.5mm probe diameter) were delivered to the skin. The mechanoreceptors gave stable average responses to repetitive stimuli and pooled stimulus-response data could be best fit by a linear relationship. Preliminary experiments suggest that the thresholds for the sensory discharge, the complex PSP in the motor neurons and the resultant gill contractions are nearly identical. The amplitude of the complex PSP and the gill contractions also appear to be linear functions of the intensity of the tactile stimulus over a behaviorally significant range. Thus in addition to the primary sensory mechanoreceptor neurons, the transformations at the central and effector elements may also be linear. Utilizing the techniques of systems analysis, a mathematical model of the mechanoreceptor response dynamics was obtained from step response PST histograms. The model was simulated on a digital computer using the simulation language CSMP and its generality was demonstrated by its ability to predict the response of a mechanoreceptor to a ramp input stimulus. The data indicate the feasibility of specifying the dynamic properties of this reflex behavior based on a knowledge of the input-output characteristics of the individual neural elements.

922 DYNAMIC BEHAVIOR OF THE CRUSTACEAN DORSAL STRETCH RECEPTOR-INHIBITOR SYSTEM. George E. Wolfe* and Boris F. Tolkunov* (Sponsor: D. Junge). Dept. Psychiatry, UCLA, Los Angeles 90024; Sechenov Institute, Leningrad, USSR.

The curve of input vs output rates for the crustacean dorsal stretch receptor (SR) and its inhibitor (I) has been described by Perkel et al. (1964) for a stationary regular input. The system settles into maintained states of locked firing (...,2:1,1:1,1:2,...) in which the SR rate increases in proportion to the I rate, even though the synapse is inhibitory. We examined the input-output rate curve of the system in its dynamic state by driving I with a pulse train modulated triangularly or by white noise. The stabilized states also occur during dynamic modulation, but their nature and degree depend on the frequency of modulation. Rebound, an unimportant system property in static condition, had a significant (and sometimes unexpected) influence on the I-SR rate curve under dynamic conditions. This influence depended on frequency and depth of modulation. The system showed other significant nonlinearities. The main general conclusion was that the system's actual behavior is often quite different from what might be naively predicted for a "simple" inhibitory synapse-pacemaker pair. (Supported by grants USPHS NS 5264 and NS 53453, and NSF 18157).

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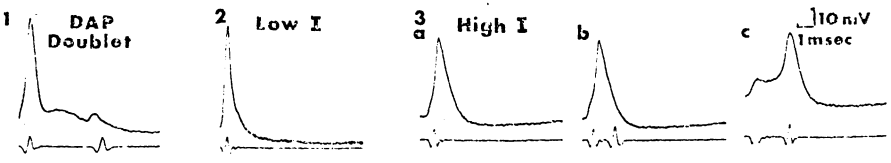
923 EXTRA SPIKES IN LOBSTER STRETCH RECEPTORS: ORIGIN FROM SPATIAL INTERACTION AND CREATION OF METASTABLE STATES. Daniel K. Hartline, Biology Dept., Univ. of California San Diego, La Jolla; and William H. Calvin, Dept. of Neurological Surgery, Univ. of Washington School of Medicine, Seattle.

During rhythmic firing to sustained depolarizations, one is occasionally surprised to see an extra spike perhaps 2 msec after a rhythmic spike. This double spike phenomenon has been seen in many different types of neurons (see Calvin, Sypert abstracts, this volume). Typically, spikes are followed by hump-like depolarizing afterpotentials (DAP) which, when exceptionally large, elicit extra spikes. Particularly in lobster stretch receptor, through the work of Grampp (1966), it is apparent that spatial interactions between soma, dendrites, and the initial segment trigger zone play an important role in the genesis of DAPs and thus extra spikes. The tonic muscle receptor organ of the spiny lobster, Panulirus interruptus, was studied under elevated bath temperatures (e.g., 29°C), which Grampp (1966) found augmented the probability of extra spikes upon antidromic stimulation. Receptors were stimulated by stretch of the muscle or by injection of depolarizing currents through an intrasomatic micropipette; a suction electrode allowed simultaneous recording from the axon. Extra spikes followed the first spike to terminate a long silent period, as first noted by Eyzaguirre and Kuffler (1955); these arose from DAPs (Fig.1). Doublets also occurred during sustained rhythmic firing (Fig.3); they often showed the same very short and fixed interspike intervals (e.g., 2.0 ± 0.2 msec) that have been observed in mammalian neurons. When scanning the dynamic range of the cell by slowly stretching and relaxing the muscle or by injecting a triangular current waveform (e.g., 40 nA peak-to-peak with a 30 sec period) while heating, the first doublets appeared during the relaxation phase of the stretch or the downside of the triangular waveform. They often persisted for a considerable range of declining rates, leading to a striking hysteresis effect in the plot of current vs. total spikes/sec. They could be elicited by downsteps of current: after holding the current at a fixed level, then stepping it to a higher level (the discharge pattern being rhythmic at both firing rates), doublets appeared immediately upon stepping back down to the original current level. This might be called a "metastable" state: the doublet pattern would often revert back to ordinary rhythmic firing after a time, although there were instances where a more stable "latch" was achieved which could be unlatched by a hyperpolarizing pulse. Previous reports of doublets have emphasized the role of DAPs; however, we often saw double spikes in our axonal recording when the intrasomatic recording showed only a broadened single spike (Fig.3b). This was associated with an AB delay, i.e., a slight pause in the rise of the spike which is thought to indicate a slowed retrograde invasion of the soma from the initial segment trigger zone. This offers even clearer evidence for the role of delayed invasion in the genesis of extra spikes: the spike-in-progress in the soma provides a source of depolarizing current at a time when the initial segment trigger zone has recovered its excitability. Extra spikes have been tentatively identified as the source of some of the sterotyped high-frequency bursting firing patterns seen in deafferented and/or epileptogenic regions of mammalian CNS. Perhaps the latent mechanisms for extra spikes have been augmented in such situations. Our results suggest that the extent and the speed of retrograde invasion of the soma and dendrites may control extra spike generation.

Supported by NIH grants NS 09677 and NS 04053 and by NSF grant GJ 43177.



924 SIGNAL MODIFICATION IN AN ISOLATED NERVOUS SYSTEM BY PAIRING OF DISTINCT SENSORY STIMULI. Daniel L. Alkon* (SPON: W. J. Adelman, Jr.) Lab of Biophys., NINCDS, Bethesda, Md. 20014

Each eye of the nudibranch mollusc Hermissenda crassicornis contains two Type A and three Type B photoreceptors. These photoreceptors receive synaptic inhibition from statocyst hair cells (Alkon, 1973). Hair cells, in turn, may be excited, and/or inhibited by photoreceptors (Alkon, 1973, 1975). A stimulus (light) to which the photoreceptors are directly sensitive, and the hair cells indirectly sensitive, is easily delivered and well defined. A comparable physiologic stimulus to which the hair cells are directly sensitive and which permits intracellular recording has heretofore not been available. In the present study such a stimulus is delivered by subjecting the isolated nervous system to centrifugal forces generated by a reinforced Garrard turntable. All of the requirements for intracellular recording, including an amplifier, are mounted on the turntable. Electrical contacts are made via copper-copper slip rings. In response to rotation, hair cells in front of the centrifugal force vector depolarize and increase their firing, whereas hair cells in back of the vector hyperpolarize and decrease their firing. These hair cell responses are independent of the orientation of the nervous system with respect to the center of rotation.

Rotation of the isolated nervous system of Hermissenda with its cephalad end toward the center of rotation causes a synaptic hyperpolarization accompanied by elimination of impulse activity during the steady state phase of the Type A but not Type B photoreceptor's response to light. Rotation of the isolated nervous system with its caudal end toward the center of rotation causes a depolarization accompanied by a substantial increase of impulse activity during the steady state phase of Type A and B photoreceptors' responses to light. Both the hyperpolarizing and depolarizing responses of photoreceptors to rotation can be explained by known synaptic interactions within and between the statocysts and the visual system (cf. Alkon, 1973; Alkon & Bak, 1973; Detwiler & Alkon, 1973). Thus, transformation of responses in specific photoreceptors is dependent not only on appropriate pairing of light and rotation, but also on the orientation of the nervous system (isolated and in the intact animal) with respect to the center of rotation.

It was also found that pairing of an intermittent light stimulus with an intermittent rotation stimulus produces a marked decrement in the number of conducted impulses during subsequent responses of Type A photoreceptors (in the cephalad orientation) to the light stimulus alone. This decrement, which was small or non-existent for Type B photoreceptors, could last at least two hours. Such a decrement did not occur with presentation of either the intermittent light stimulus or the intermittent rotation stimulus alone, nor did it occur when these two stimuli were presented alternately, i.e. in an unpaired regimen.

This prolonged signal modification in Type A photoreceptors produced by stimulus pairing can serve as a model of conditioning on the cellular level. That it is not sensitization is shown by the inability of the unpaired regimen to produce a similar effect. That this modification is specific is shown by the absence of a similar effect for other photoreceptors (Type B) in the same eye. In fact, the data suggest that the design of this neural system provides for modification (cf. Alkon, 1975) of responses in some cells, e.g. the Type A photoreceptors and those hair cells which they excite, while preserving essential visual information in other cells, e.g. the Type B photoreceptors and the second order optic ganglion cells which they inhibit. The prolonged signal modification appears to result from sodium inactivation of the photoreceptor axon membrane. The inactivation, in turn, appears to occur when potassium accumulates near the Type A spike focus as a combined result of the photoreceptor's own impulse activity and synaptic inhibition it receives from hair cells.

925 PHASIC INPUTS TO THE LOBSTER STOMATOGASTRIC GANGLION. David F. Russell* (SPON: A. I. Selverston). Dept. of Biology, UCSD, La Jolla, Ca. 92037.

The stomatogastric ganglion (STG) of Panulirus interruptus produces two motor patterns, gastric and pyloric, which control movements of the lobster foregut during food processing. To study the inputs to these pattern generators, isolated preparations were made which included the bilaterally paired commissural ganglia (CG's), the oesophageal ganglion (OG), and the STG with its motor nerves. Both gastric and pyloric motor rhythms were very active. En passant recordings from the single input nerve to the STG revealed bursts of spikes which participated in four different rhythms. (1) A pair of large input units (E neurons) fired in bursts coordinated with the gastric rhythm; one E neuron originated in each CG. Both E neurons put EPSP's onto the LG, LPG, and GM gastric motorneurons in the STG. (2) Several small input units fired in bursts coordinated with the pyloric rhythm; these inputs to the STG also travelled from the CG's. They put trains of EPSP's onto the AM, DG, and LPG gastric motorneurons. (3) The two CG's generate a rhythmic motor pattern which sets up peristaltic swallowing motions of the oesophagus. Bursts of very small units coordinated with the oesophagus rhythm passed into the STG; several STG cells showed resulting synaptic inputs. The EX cells displayed trains of IPSP's and EPSP's synchronized with the oesophagus rhythm. (4) The OG also produced patterned input to the STG. Two OG units (the IVN fibers), which send axons to the STG, sometimes fired in rhythmic bursts. During a burst the gastric and pyloric rhythms were disrupted. In summary, many inputs to the STG exhibit patterned firing.

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926 NEURAL AND NEUROSECRETORY CONTROL OF A CRUSTACEAN AUXILLIARY HEART. A. Steinacker* (SPON: Elvira Ceccarini), Albert Einstein College of Medicine, Bronx, New York 10461.

Blood pressure in crustaceans is commonly assumed to be a function of the frequency and amplitude of the contraction of the main heart. However, there also exists in many decapods an auxilliary heart (the cor frontale) whose function appears to be the maintenance of blood pressure in the cerebral nervous system. The somata of the four motoneurons which control the heart are located in the supraesophageal ganglion. Each heart muscle fiber is innervated by all four motoneurons. The firing frequency of these motoneurons is inversely proportional to the frequency of contraction of the main heart in the intact crab and to the cerebral perfusion pressure in the in vitro preparation. This coordination is mediated by receptors in the supraesophageal ganglion. In addition, there are excitatory and inhibitory fibers in the circumesophageal connectives and stretch receptors in the muscle tendons that modulate the auxilliary heart. The auxilliary heart appears to be tied into a general regulatory system for maintenance of blood pressure in the animal by the pericardial system. Pericardial peptides, which are known to increase the frequency and duration of bursts of the main cardiac ganglion and to act on the gill motoneurons. These extracts have a similar effect on the motoneurons of the auxilliary heart. In addition, they have a direct effect on the muscle of the auxilliary heart. Both muscle tension and pulse pressure are increased upon application of the pericardial extract. The action of these peptides upon the conductances of the muscle membrane is currently under study.

927 THE STOICHIOMETRY OF THE SODIUM-POTASSIUM PUMP IN THE LOBSTER CARDIAC GANGLION. David R. Livengood. Armed Forces Radiobiology Research Inst., Bethesda, Md. 20014.

The lobster cardiac ganglion is a unit of nine neurons which can maintain their natural beat rhythm for many hours in vitro. An active electrogenic pump has previously been shown to exist in these cells. Four of the nine cells of the cardiac ganglion (the follower cells) are large enough to be penetrated with up to three microelectrodes. If two of the three internal microelectrodes contain sodium salts, passing current between these two electrodes permits the iontophorectic injection of reproducible amounts of sodium ions into the follower cells which in turn activates the electrogenic sodium pump. By knowing the charge used to inject the sodium salts into the follower cells and measuring the time-current area produced by the uncoupled portion of the sodium-potassium pump, one can determine the sodium-potassium coupling ratio. This coupling ratio increases when the external sodium is replaced by choline. This increase in the sodium-potassium coupling ratio therefore produces an increase in the pump electrogenicity. These results suggest that external sodium ions can affect the sodium-potassium coupling ratio, either by inhibiting a non potassium saturated pump from "turning over" or by converting a component of the pump to a sodium-sodium exchange mode. Such a mechanism might have an important function in the maintenance of resting membrane potential under conditions where total pump rate is reduced.

928 UPTAKE OF PARAMETHOXYPHENYLETHYLAMINE (PMPEA) BY ISOLATED SYNAPTOSOMES IN VITRO. C. Vance*, R. Ashkenazi and B. Haber (Spon: J.S. Kittredge). The Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550 and Dept. of Physiology, Hadassah School of Medicine, Jerusalem.

PMPEA, an indirectly acting sympathomimetic amine, in vivo potentiates monosynaptic spinal reflexes, and causes depletion of central serotonin (5HT) and norepinephrine (NE). In vitro, the drug blocks the uptake of 5HT by synaptosomes, and to a lesser degree that of NE and dopamine (DA). Because of the possibility that some of the physiological effects of the amine are due to its storage in and release from presynaptic terminals, we have characterized the transport process for PMPEA in isolated synaptosomes. Transport experiments were performed in Krebs-Ringer pH 7.4, utilizing H^3 PMPEA (custom tritiation) and terminated by rapid filtration through millipore filters. The synaptosomal uptake of PMPEA is saturable, temperature dependent, and the Km (8 micromolar) compares with that observed for the uptake of NE. Unlike NE, the uptake of PMPEA is not sodium dependent, either at 37°C or at 0°, and is not blocked by oubain. The omission of Na+ from the incubation medium stimulates the accumulation of PMPEA, as does the omission of calcium. Metabolic inhibitors such as azide, cyanide, dinitrophenol and iodoacetic acid block the synaptosomal accumulation of PMPEA. Though PMPEA blocks the uptake of 5HT, NE and DA, its own accumulation is not altered by these amines, except at high concentrations. The parent amine, phenylethylamine, as well as the dimethoxy-derivatives (2,3- and 3,4-DMPEA) inhibit the uptake of PMPEA in a noncompetitive manner. These findings suggest that the carrier mechanism for PMPEA in synaptosomes is different from that for 5HT or the catecholamines, and imply that PMPEA may be stored in presynaptic terminals in vivo. (Supported by NIH Grant NS 11255, Welch Grant H-504, and a MDAA grant the Multidisciplinary Mental Health Program, UTMB.)

929 EFFECTS OF VINCA ALKALOIDS ON UPTAKES OF AMINO ACIDS BY RAT BRAIN SYNAPTOSOMES. N.A. Peterson and E. Raghupathy*. Brain-Behavior Research Center, Langley-Porter Neuropsychiatric Institute, UCSF, Sonoma State Hospital, Eldridge, Ca. 95431.

Synaptosomal particles contain transport mechanisms for the rapid accumulation of a number of amino acids. Previous work in this laboratory has dealt with characteristics and developmental changes in these transport systems. In this present investigation we have studied the effects of vinca alkaloids on synaptosomal amino acid uptake in order to ascertain a possible role for a microtubular system in the transport mechanisms. Vinblastine had a substantial inhibitory effect on synaptosomal accumulations of leu, ileu, val, his, tyr, phe, ser, thr and ala. The I_{50} values for vinblastine inhibition of these amino acids were all in the range of 30-50uM. The inhibitory effects of vinblastine on the uptakes of these amino acids were antagonized by 150mM Na^+ and 15mM Ca^{++} or Mg^{++}. At these ionic concentrations the I_{50} values were raised 3-5 fold. In the presence of Ca^{++} or Mg^{++} the vinblastine inhibition was further antagonized by several nucleotides. ATP was the most effective in this respect, causing a 7-10 fold increase in the values for I_{50}. The nucleotides had no effect in the absence of divalent cations.

The ionic factors and nucleotides which modify vinblastine inhibition of synaptosomal amino acid uptakes are known to have a fundamental involvement with the structure and function of tubular protein and microtubular systems. The effects of these factors on vinblastine inhibition strongly suggest that the accumulation of amino acids by synaptosomal particles is mediated by a microtubular system. Additional support for this view stems from the observation that the inhibitory effects of vinblastine on amino acid uptakes were almost abolished when the incubations were carried out at 5° instead of 37°.

930 ^{3}H 2-DEOXY-D-GLUCOSE TRANSPORT IN SYNAPTOSOMES OF ISCHEMIC GERBILS. Maria Spatz, Bogomir B. Mrsulja, Branislava J. Mrsulja* and Igor Klatzo. NIH, Bethesda, Md. 20014.

The effect of ischemia on synaptosomes uptake of ^{3}H 2-deoxy-D-glucose was investigated in Mongolian gerbils subjected to unilateral common carotid artery occlusion for various periods of time. The gerbils were killed by decapitation and the synaptosomes of left and right hemispheres were prepared separately by the method of Diamond and Molfay (J. Neurochem. 19: 1899, 1972). The specific and nonspecific entry of the labeled hexose was measured in duplicate according to Diamond and Fishman (J. Neurochem. 20: 1533, 1973). An altered synaptosomal uptake of ^{3}H 2-deoxy-D-glucose was found in the cerebral hemisphere ipsilateral to the carotid artery occlusion. The saturable ^{3}H 2-deoxy-D-glucose entry in the synaptosomes from the ischemic hemisphere was 32% lower at 30 minutes and 55% lower at 60 minutes than in the contralateral control hemisphere. The carrier mediated glucose transport of synaptosomes was almost completely abolished in the ischemic hemisphere of gerbils subjected to 3 hours of common carotid artery occlusion. The synaptosomal transport activity was not recovered by the addition of various metabolites to synaptosomal suspension. Complete recovery of synaptosomal function was observed 60 minutes after cerebral circulation was reestablished in gerbils subjected to 60 minutes of unilateral carotid artery clipping.

931 Ca^{++} Binding to Synaptosomal Plasma Membranes. Nancy C. Kendrick* and Mordecai P. Blaustein. Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, Missouri, 63110.

A large Ca^{++} concentration gradient must be maintained across the plasma membrane at presynaptic terminals in spite of repeated entry of Ca^{++} ions during synaptic activity. The membrane component responsible for this gradient, thought to be a Na-Ca exchanger or some form of Ca-ATPase must necessarily have a high affinity for Ca^{++}, probably with a K_D of 10^{-6}-10^{-7}M. Accordingly, experiments were designed to characterize synaptosomal plasma membranes with respect to Ca^{++} binding under conditions where low affinity nonspecific Ca binding would be minimized, and where ATP effects or Na inhibition of Ca^{++} binding might be revealed.

A synaptosomal plasma membrane fraction was prepared by a modification of the method of Mahler et al.*(J. of Neurochem.). This fraction was enriched 8-10 fold in Na-K ATPase activity, a plasma membrane marker, and about 1.3 fold in monoamine oxidase, a marker for the outer mitochondrial membrane. Solutions for Ca^{++} binding contained 20 mM imidazole, pH 7.4, 2 mM $MgCl_2$, and 130 mM NaCl, KCl, or choline Cl. Ca concentrations,monitored by atomic absorption spectrophotometry, ranged from 0.5 µM to 1200 µM. Binding of ^{45}Ca to 50-100 µg aliquots of these membranes was measured by incubation of protein with 1 ml of the appropriate ^{45}Ca containing solution, generally for 10 min at 37°, followed by filtration through a Millipore or Nuclepore filter. Blank values were determined for filters without protein and subtracted. Under these conditions, 2 sets of binding sites could clearly be distinguished by means of Scatchard plots. High affinity sites, binding about 0.1-0.5 µMoles per g protein, with a K_D of 3.0-6.0 x 10^{-6} were observed along with low affinity sites binding 6.5-10 µMoles per g, with a K_D of about 2 x 10^{-3}M. The number of sites and affinities did not appear to change significantly when 130 mM KCl or Choline Cl was substituted for NaCl. In contrast, Ca binding to a microsomal fraction was 2-3 times greater in 130 mM KCl or Choline Cl than in 130 mM NaCl. Ca binding to plasma membranes in the presence of 2 mM ATP was found to be enhanced several fold at 30 sec, the shortest interval measured; the ATP induced a Ca^{++} uptake which was linear with time for at least one hour. Ca^{++} uptake was markedly inhibited by 8 mM ATP. Ca^{++} binding to plasma membranes in the absence of ATP remained at a low constant level after about 4 min.

*Mahler et al. (1974) J. Neurochem. 22:281.

932 TYROSINE TRANSPORT BY ISOLATED NEURAL TISSUES. Chung S. Kim*, Lorcan A. O'Tuama, Lester D. Grant and Harriette P. Nichols* Depts. Med. and Peds., and the Biological Sciences Research Ctr. Child Dev. Res. Inst., Univ. N. Carolina Sch. Med., Chapel Hill, 27514.

The neural uptake of L-tyrosine may at times be rate-limiting for catecholamine biosynthesis (e.g., Lloyd and Breakefield: Nature 252: 719. 1974). The present study sought (a) to compare and contrast the patterns of transport of L-tyrosine in C.N.S. regions of differing monoaminergic innervation and in neural barrier tissues; (b) to observe the effects on brain uptake of tyrosine of agents known to affect central turnover of catecholamines.

Cat hypothalamus (HT), caudate nucleus (CN), thalamus (T), choroid plexus (CP), paraventricular tissue (PV) and meninges (M) incubated aerobically in mock CSF showed a concentrative accumulation of ^{3}H-L-tyrosine (20μM) at 5 min. The accumulation was reduced significantly by ouabain (10^{-4}M), anoxia, cold, higher concentrations of ^{3}H-L-TYR (50 to 1000 μM), and by D-tyrosine (20μM). Equimolar amounts of L-phenylalanine, but not of glutamic acid, L-DOPA or GABA, also reduced ^{3}H-L-TYR accumulation. Maximum transport velocity for ^{3}H-L-TYR averaged .500 μ mols/g/min for CP, exceeding by at least twofold the V_{max} for other tissues (HT = CN > M > T > PV). The apparent Km for ^{3}H-L-TYR uptake was lowest (84μM) in PV and highest (375μM) in HT. LiCi (10^{-3}M), chlorpromazine (10^{-4}M), ethanol (10%), prostaglandin E_1 (10^{-6}M), prostaglandin F_2 (10^{-6}M), probenecid (10^{-5}M), acetazolamide (10^{-5}M) and dopamine (5×10^{-3}M) all failed to alter the 5 min tissue-to-medium distribution ratio of ^{3}H-L-TYR. Pb NO_3 (10^{-6}M) variably depressed accumulation of the radiolabel.

The results show several similarities in the accumulation of tyrosine by all tissues studied: the uptake is energy dependent, carrier mediated, specific and stereospecific. Partly distinctive features are shown by the low capacity high affinity uptake systems of T and PV, and by the high capacity uptake of the CP. Systems of the former type may respond rapidly to changes in brain tyrosine levels whereas the latter type may aid in long-term adjustments of tissue levels. This study shows no correlation between the kinetic patterns of tyrosine uptake and the presence or functional state of catecholamine receptors in the isolated brain slice. Further studies of this question employing more homogeneous cell populations are needed.

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933 GOMPERTZIAN (ACTUARIAL) DESCRIPTION OF NERVE ACTION POTENTIAL
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An equation of the form $V = W \exp[-b \exp(kt)]$ (after Gompertz, 1825) is used to describe population death and growth. It is also a fitting description of the collapse and restitution of the membrane potential during a nerve impulse. In one form the Gompertzian shows exponential decay increasing during each moment at a rate depending on the amount of decline yet to occur. In the other it shows exponential growth during each moment at a rate that decreases according to the amount of growth already accomplished. If the action potential is plotted with the spike directed downward, then the leading edge may be fitted by a classical Gompertzian survivorship curve, while the trailing edge is a growth curve obtained when (kt) is negative. For each phase, the "slope" will be fixed by a value of k, while b locates the phases along the time axis. W is set by the amplitude of the action potential. Alterations in action potential form, as for example by changing concentrations of Na^+ and K^+, can be described in terms of changes in parameters of the equation. The Gompertzian growth curve also provides an excellent fit to Hodgkin-Huxley membrane conductance data. (Aided in part by Psychobiology Program, Florida State University).

934 BLOCKING OF THE SQUID AXON POTASSIUM CHANNEL BY EXTERNAL CESIUM IONS. R. J. French and W. J. Adelman, Jr., Laboratory of Biophysics, IR, NINCDS, NIH, Bethesda, Md. 20014.

External Cs produces a voltage dependent block of inward currents through the potassium channel of the squid axon causing the appearance of a region of negative slope in the current voltage relation at sufficiently negative membrane potentials. The negative resistance region appears at voltages closer and closer to zero as the [Cs] increases. A plot of the ratio of current in the presence of Cs and K to the current in the presence of K alone against transmembrane voltage is sigmoid, asymptotically approaching 0 at negative potentials and 1 at the positive end. We use the slope of this curve at the 50% blocking point, where the current ratio is 0.5, as a measure of the voltage dependence of blocking. The data were compared with the predictions of a simple single site model for Cs—K interaction. (A similar model has been successfully used by other workers to describe the blocking of Na currents in the frog node of Ranvier by H, Ca, and procaine.) Although the current voltage relation at each [Cs] had the qualitative form that the model predicts, the following features of the data were inconsistent with the model: (a) blocking is more steeply dependent on [Cs] than predicted, (b) the voltage dependence of the blocking changes with changing [Cs], and (c) at [Cs] of 50 mM or more the voltage dependence of the blocking is beyond the expected range of values. With Cs present on the outside only, one would expect the single site model to describe the data if the Cs ions blocked at the first site of a multisite channel. We conclude that the blocking site is beyond the first ionic binding site reached by an ion entering the outer channel mouth.

935 STUDIES OF VOLATILE ANESTHETICS AND INERT GAS NARCOSIS ON AUTOACTIVE NEURONS OF HELIX ASPERSA. James L. Parmentier and Kenneth M Wilson*
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The application of a series of halogenated hydrocarbons currently used as anesthetics initially increases the firing rates of Helix central neurons but after longer contact suppresses this activity. Both the time distribution of action potentials in the bursts and the minimum quantity of charge (current x time) is altered by exposure to these compounds. The activation threshold is first reduced but as narcosis proceeds larger currents are needed until the point of cell refractoriness is reached. The range of concentrations (10^{-3} M - 10^{-4} M) and the order of potency parallels that found in man. A similar concentration dependent relationship was found for prenarcotic excitability and narcosis induced by the noble gases under hyperbaric conditions. In both cases the data correlate well with known physico-chemical properties such as oil solubility and the square root of the van der Waals "a" constant over a range of partial pressures of five orders of magnitude. Pressure reversal was demonstrable in the case of the weak anesthetic nitrous oxide.

These results support the critical volume hypothesis as developed by Miller et.al. (Mol. Pharmacol. 9:131-143, 1973) and suggest that narcosis involves a continuum of changes in excitability of individual neural membranes which alter triggering and threshold phenomena but not gating mechanisims. Helium at 68-90 ATA produced prenarcotic excitability patterns comparable to those of the other compounds and caused reduced excitability at about 200 ATA. This did not occur under control hydraulic compressions and suggests that helium may have a distinct pharmacology which functions in the range of the"high pressure neurological syndrome" seen in primates. Such an effect may need to be taken into consideration when helium is to be used for experimental or supportive pressurization.

936 EXTRACELLULAR ION SHIFTS RECORDED DURING SPREADING DEPRESSION IN CATFISH CEREBELLUM. R.P. Kraig* & C. Nicholson. Div. of Neurobiology, Dept. of Physiology & Biophysics, University of Iowa, 52242.

Spreading depression (SD) has been reported in the cerebellum of the catfish, Corydoras aneus. Simultaneous measurement of $[K^+]_o$ and $[Cl^-]_o$ with micropipettes containing ion selective resins (Corning 47717 & 47715 respectively) reveals large fluctuations in the extracellular enviroment of the molecular layer. Following KCl injection $[K^+]_o$ rises from 2mM slowly and then rapidly to a peak of 40-60mM. Thirty seconds after the initial change in $[K^+]_o$, $[Cl^-]_o$ begins a rapid decline from 165mM to 70-50mM. The diminished $[Cl^-]_o$ lasts 2-6 minutes before returning to baseline while $[K^+]_o$ returns more slowly. The pronounced magnitude and delay of the $[Cl^-]_o$ change during SD has heretofore been unrecognized. This large shift in $[Cl^-]_o$ almost certainly means there is a large concomitant fall in $[Na^+]_o$. Simultaneous field potential analysis demonstrates that the functional integrity of the neuronal circuitry is restored after these ion shifts. This suggests that the brain is capable of withstanding considerable transient ion imbalances such as occur in SD. (Supported by USPHS research grant NS-09916 from NINDS)

937 A OUABAIN-SENSITIVE CHLORIDE CONDUCTANCE IN AXONS: ITS RELATION TO ELECTROGENIC SODIUM PUMPING. E.M. Lieberman. E. Young* and T.M. Nosek. Dept. Physiol., Bowman Gray Sch. Med., Winston-Salem, N.C. 27103

Isolated crayfish axons were bathed in low chloride and low sodium solutions with/out ouabain. Membrane chord conductance (Gm, mho/cm^2=1/Rm, ohms·cm^2) and membrane potential (Em, mV) were continuously monitored. Gm of axons exposed to 0.5-1mM ouabain or low external sodium (Na) in chloride (Cl) containing solutions doubles relative to control. The same experiments in $0[Cl]_o$ showed that 95-100% of the Gm increase in ouabain can be accounted for by an increased Cl conductance (g_{Cl}). The resting Em in $0[Cl]_o$ was not different from the Em in Cl solutions (Em=-88.1±.7mV). The Gm in $0[Cl]_o$ solution was significantly less ($4.7x10^{-4}$mhos/cm^2 or 0.89± 1.9% of control, $p<.001$). The axons responded similarly to ouabain and $0[Na]_o$ with either sulfate or Isethionate as a Cl substitute. Ouabain, in $0[Cl]_o$ induced a 6.4±.9mV depolarization in 3-5 min accompanied by a 33% increase in Gm due to the depolarization. In the absence of a direct effect of ouabain on Gm (as in Cl solutions) the ouabain induced depolarization represents the inhibition of a 3.0uA/cm^2 hyperpolarizing current. Applied currents of approx. the same value are necessary to increase the Em to the pre-ouabain control. $0[Na]_o$, in Cl solutions caused an increased Gm and no significant Δ Em. In $0[Cl]_o$, $0[Na]_o$ results in a transient 6.3±.5mV hyperpolarization and no Gm change. This is equivalent to 2.7uA/cm^2 hyperpolarizing current. The $0[Na]_o$ hyperpolarization peaked in 3-5 min and decayed to -1.5 to -2mV of the control level in 15-25 min. The Em and Gm are insensitive to ouabain at this time. If ouabain is applied at the peak of the $0[Na]_o$ hyperpolarization an immediate (3-4min) return to control occurs. It is concluded that the ouabain sensitive cation pump controls g_{Cl} and evidence is presented that the pump is electrogenic in the steady-state. Supported by NS08773.

938 EFFECT OF TETRAETHYLAMMONIUM IONS ON THE POSTSYNAPTIC CURRENT IN THE SQUID GIANT SYNAPSE. R.S. Manalis. Dept. Physiol.,Univ. Cinti., Cinti. Ohio, 45267 and Marine Biol. Lab., Woods Hole, Mass. 02543

The influence of the activated nonsynaptic membrane on measurements of the synaptic reversal potential (E_r) for an excitatory synapse has been reduced considerably by the intracellular application of tetraethylammonium (TEA) ions into the postsynaptic cell. TEA has been used with the implicit assumption that it affects only the electrically excitable membrane and not the postsynaptic membrane. Experiments have been performed on the squid (Loligo pealei) giant synapse in order to determine if TEA has any postsyanptic effects. Measurements were made under conditions in which the nonsynaptic membrane remained inactive. Postsynaptic currents (PSCs) were recorded using a voltage-clamp technique in which an axial wire was used for current injection and a microtip for voltage recording. E_r measurements were made by extrapolating from linear regions of PSC-Vm relationships; V_m was varied by the application of hyperpolarizing voltage-steps only. The control E_r was +35.5±1.4 mV (mean ± s.e., n=22). TEA was applied by iontophoresis from micropipettes into the postsynaptic axons for 0.5-5.0 min. In TEA-treated preparations, E_r shifted to a value of +20.3±2.7 mV (n=9) and there was a slight increase in the time-to-peak of the PSC. TEA caused no change in the leak conductance of the nonsynaptic membrane and in E_{Na}. In fact, E_{Na} in TEA-treated preparations was found to be +48.6±1.8 mV (n=10)--a value slightly more positive than the control E_{Na} of +46.1±1.4 mV (n=12). Therefore, the shift in E_r cannot be explained by a fall in E_{Na}. It is concluded that TEA has an effect on the postsynaptic current and that its mechanism of action may be due to one or a combination of the following possibilities: a) TEA substituted for intracellular potassium ions; b) TEA became itself a specific carrier of the postsynaptic current due to the creation of an electrochemical gradient for TEA; c) TEA blocked sodium conductance of the activated postsynaptic membrane.

939 BARIUM DEPOLARIZATION AND RESISTANCE INCREASE IN GYMNOTID ELECTROPLAQUES. T. L. Pencek* and K. Kusano. Dept. of Biology, IIT, Chicago, Ill. 60616.

Barium as low as 0.5 mM produced a depolarization and resistance increase in four species of electric fish which show K-inactivation responses: Eigenmannia sp.; Gymnotus carapo; Electrophorus electricus; Sternopygus sp. Depolarization by Ba was larger in K-free saline than in normal (5 mM K) saline. Resistance increased in K-free saline, as compared to control, and further increased with Ba. E_m vs. log $(K)_o$ curves had an average slope of about 50 mV/10 fold change in $(K)_o$ which was reduced by 2.5 mM Ba to 23 mV in Eigenmannia, 29 mV in Gymnotus and 32 mV in Electrophorus. Eigenmannia had an E_m in 5 mM K of 74.7 mV (avg.) which upon addition of Ba solution (0.5~10 mM) depolarized to about 47 mV; effective resistance went from 4.9 KΩ to 6.3 KΩ. In K-free E_m was 97.3 mV and resistance was 1.3 times the control; addition of Ba depolarized to 48 mV and further increased resistance. Sternopygus electroplaques depolarized from 75.8 mV to 41.6 mV in 2.5 mM Ba with an effective resistance increase from 36.6 KΩ to 46.9 KΩ. Gymnotus had an E_m of 88.2 mV and resistance of 74.8 KΩ. In Ba-media(0.5 ~10 mM) the average E_m was 76 mV and the resistance increased 14 times. In Cl-free saline the cells depolarized from 82.3 mV to 74.6 mV in 20 min. and with 2.5 mM Ba to 58.8 mV. In Na-free choline substituted media the cells depolarize from 89.7 mV to 81.5 mV, addition of 2.5 mM Ba caused a further depolarization to 51.8 mV. In Electrophorus, the Ba depolarization is greatest in K-free saline: control E_m = 91.7 mV; K-free = 125 mV; and in K-free with 0.25 mM Ba = 118 mV, 1.25 mM Ba = 104 mV, 5 mM Ba = 74.3 mV, and 10 mM Ba = 53.7 mV. Decreasing $(K)_o$ increases the Ba effect. In K-free with 10 mM Mg as a control, no depolarization occured over a 90 min. period. The Ba effects are not readily reversible. Depolarization caused by Ba is due to a reduction in the resting K-conductance and the remaining E_m may be due to lowered G_K and by other ions. (Supported by USPHS Grants NS-09618, 12275).

940 CHARACTERIZATION OF RAT BRAIN SUBCELLULAR FRACTIONS BY ELECTROPHORESIS OF SDS EXTRACTS ON SLAB GELS. Anna Korenovsky*, S.P. Mahadik*, M.M. Rapport (SPON: A.S. Perumal) Div. Neuroscience, N.Y. State Psychiatric Institute and Dept. Biochem.Columbia Univ., New York, N.Y. 10032.

Knowledge of the chemical differences between subcellular fractions of brain is still very incomplete, and it is therefore necessary to reexamine these fractions with every new technique. Polyacrylamide gel electrophoresis in SDS permits analysis of the subunit structure of complex proteins. More recently slab gel electrophoresis in SDS has increased the resolution, sensitivity, and reproducibility of this technique. We have modified the method to make it applicable to membranes of rat brain subcellular fractions obtained by conventional gradient centrifugation. After delipidation, membrane fractions gave well-defined, reproducible bands on electrophoretograms. Improved solubility of the membrane proteins resulted in a larger proportion of the polypeptides having lower molecular weights. Electrophoretograms of nuclei, mitochondria, microsomes, and synaptosome membrane fractions contained bands with molecular weights ranging from <10,000 to > 200,000 on slab gels of both 10% acrylamide (over 50 bands) and 9% to 25% gradient acrylamide (over 70 bands). Gradient slab gels showed a sufficient number of qualitative and quantitative differences among the subcellular fractions to provide new criteria of homogeneity for such fractions. To permit presentation of the results, the electrophoretogram has been divided into 5 zones (A to E) representing molecular wt. ranges of < 19,000 (A); 19,000-30,000 (B); 30,000-50,000 (C) 50,000-100,000 (D); and > 100,000 (E). The nuclear fraction (N) has 4 characteristic bands in A (N-A 1to4), 2 in B (N-B5,N-B6), 2 in C (N-C4,N-C5), 1 in D (N-D2), and 2 in E (N-E2,N-E3). Some of the bands in each zone represent subunits of RNA polymerase, and the 4 major bands in A are histones. The mitochondrial fraction has over 17 bands in A, an elaborate pattern that is not seen with nuclear, synaptosomal membrane, and microsomal fractions. Other major bands characteristic of mitochondria are Mt-B1, Mt-B3 Mt-B 5to10, Mt-C4, Mt-C 7to10, Mt-D3, Mt-D5. Of these, Mt-B1, Mt-B3, Mt-C4, Mt-D3, and Mt-D5 correspond to bands indicated in the literature to be characteristic of mitochondria. Although microsomal and synaptic membrane fractions show a high degree of correspondence, zones A and D show discriminating bands. Bands Mc-A 1to4, Mc-C2, Mc-C 7to9, and Mc-D 2to5 are not seen with synaptic membrane fraction. Bands SM-B5, SM-C7, and SM-D2 are not seen with the microsomal fraction. Bands Mc-D6 and SM-D2 are very similar and are not seen in other subcellular fractions. Slab gel electrophoresis appears to be the most powerful method available for characterization and comparison of subcellular fractions prepared by different procedures.

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941 EFFECT OF TEMPERATURE UPON THRESHOLD CHANGES FOLLOWING IMPULSES IN FROG SCIATIC NERVE FIBERS. Michael J. Binder, Stephen A. Raymond. R.L.E., M.I.T., Cambridge, Mass. 02139.

In rested nerve at 18°C, slow variation of temperature ± 8C° does not change threshold appreciably. However, rapid 5C° increases in temperature result in transient increases of threshold that disappear after 15 to 45 min. Cooling by a step of 5C° transiently lowers threshold. Return of threshold to the original level takes longer following a 5C° drop in temperature than it does after a 5C° rise, suggesting that metabolic processes are involved. Thus the rested frog axon behaves as though it controls its threshold to compensate for temperature shifts.

When the nerve is active (3 impulses/sec), impulses are followed by a sequence of threshold oscillations in which the membrane is alternately refractory, superexcitable, depressed. Cooling by 5C° displaces this threshold curve so that the refractory phase is longer, the superexcitable phase is delayed and reduced, and depression is earlier, much stronger and takes longer to recover. Warming has opposite effects. The transitions between the 18°C and the 13°C curves are not monotonic unless the temperature is changed slowly. The threshold transients following sudden temperature steps are in the same direction as for rested nerve, but since active nerve steady-state threshold values at any delay after an impulse depend on the temperature, the transient does not decay to the original threshold value but to a new one. (Supported in part by NIH Grants 5R01 EY01149-02 and 1 T01 EY00090-01 and a Grant from Bell Laboratories, Inc.).

942 EVIDENCE FOR DEFECTIVE INCORPORATION OF PROTEINS INTO QUAKING MOUSE MYELIN. S. Greenfield*, S. W. Brostoff*, E. L. Hogan. Dept. of Neurol., Med. Univ. of S.C., Charleston, S.C. 29401, and P. Morell*, Dept. of Biochem., Univ. of N.C., Chapel Hill N. C. 27514

The Quaking Mouse is a neurological mutant characterized by a deficit in myelin accumulation. Differences in composition of quaking from normal myelin include lowered levels of cholesterol and galactolipid and of basic and proteolipid protein. These findings could be the result of defective protein or lipid biosynthesis, increased turnover of components of the quaking myelin membrane or a defect in the assembly of the membrane itself. As a continuation of our work on quaking myelin as a model for membrane assembly, we have examined the labeling of protein in quaking myelin at several age points.

Quaking and littermate controls received intracranial injections of 150 uCi of ^{3}H-glycine and 25 uCi of ^{14}C-glycine respectively and after two hours their brains were combined and subcellular fractions prepared according to Eichberg, et al. (Biochem. J. 92:91, 1964). The average $^{3}H/^{14}C$ ratio of all subcellular fractions except myelin was 3.0. The $^{3}H/^{14}C$ ratio for the myelin subfraction was 1.88 indicating a decrease of approximately 40% in the incorporation of glycine into myelin relative to the other subfractions.

In other experiments, myelin proteins were separated by discontinuous gel electrophoresis in buffers containing SDS and the $^{3}H/^{14}C$ ratios determined in each gel slice. In contrast to the microsomal subfraction which gave a $^{3}H/^{14}C$ ratio of 3.5 across the gel, the $^{3}H/^{14}C$ ratio of myelin showed large variations with values ranging from 0.67 for proteolipid protein to 2.0 for some of the high molecular weight proteins. Irrespective of age, all proteins were inhibited in incorporation into myelin relative to microsomes, although this was most pronounced for proteolipid protein followed by basic protein and intermediate protein.

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943 PERIPHERAL NERVE GLYCOPROTEINS AND MYELIN FINE STRUCTURE DURING DEVELOPMENT OF RAT SCIATIC NERVE. John G. Wood and Ed Engel*. Dept. Anat., University of Tennesee Center for Health Sciences, Memphis, Tennesee, 38163.

Sodium dodecyl sulfate(SDS) acrylamide electrophoresis and electron microscopy(EM) have been used to correlate the appearance of a major peripheral nervous system(PNS) glycoprotein in rat sciatic nerve. The major protein in PNS myelin is a glycoprotein(Everly et al., J. Neurochem. 21:329-334; Wood and Dawson, J. Neurochem., 21:717-719) which may be easily seen in the gel pattern of whole sciatic nerve. 3 mm sections of nerve were removed at various ages from newborn to adult and immediately delipidated with chloroform-methanol(2:1). SDS electrophoresis of nerves from newborn rats revealed virtually none of the myelin glycoprotein either by protein staining or carbohydrate staining. However there was a considerable amount of a protein which migrated to the same point on the gel as the small myelin basic protein. During the first two postnatal weeks the amount of protein staining of the glycoprotein increased to virtually adult levels, and there was a parallel increase in carbohydrate staining as determined either by PAS staining or concanavalin A staining of the gels. EM was performed on 3mm segments of nerve which were bisected and the pieces embedded and sectioned together so that the EM analysis could be correlated with the gel analysis. The appearance of the major sciatic nerve glycoprotein was shown to correlate well with the overall pattern of myelination in given segments of nerve. In addition the EM analysis revealed the presence of profiles resembling glial growth cones in various stages of contacting non-myelinated axons. Between 4-8 days postnatal identical profiles were seen between internal myelin lamellae and the axolemma. Since the characteristic feature of these profiles is an abundance of membranous material, the possibility exists that during developement some membrane may be added or removed from myelin lamellae internally.

944 STUDIES ON THE NATURE OF THE CHEMICAL BONDS UNDERLYING SYNAPTIC CONNECTIVITY. Paul Kelly, Pamela Kaups*, Gary Lynch and Carl Cotman.
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The bonding mechanisms responsible for the linkage of pre and postsynaptic elements are of central importance in synaptogenesis and synaptic maintenance. In order to examine the molecular basis of synaptic connectivity, intact tissue slices and synaptosomes from brain were incubated in various media known to disrupt certain types of chemical bonds. Quantitative electron microscopic analysis of a relatively homogeneous synaptic field in the rat dentate gyrus was employed in assessing the relative efficacy of various chemical environments to dissociate synaptic junctions.

Synaptic junctions retained near normal morphology with respect to the close apposition of pre and postsynaptic membranes in environments of high ionic strength (2-4M NaCl), ethylenediamine tetraacetic acid, mixtures of carbohydrates, basic pH and reducing agents. Therefore, it is unlikely that these synaptic junctions are held together solely by ionic bonds, coordination complexes, "lectin-like" carbohydrate interactions or interpeptide disulfide linkages. In addition, exposure to urea or guanidine hydrochloride (1-2M), Triton X-100 (1%) or sodium dodecyl sulfate (0.2%) resulted in little synaptic dissociation. Since urea, guanidine and detergents disrupt hydrogen bonding and/or hydrophobic interactions, these results tend to negate a primary role for these bonding mechanisms in maintaining synaptic integrity. The sequential effects of sodium periodate oxidation and sodium borohydride reduction were also examined. Under conditions known to reduce membrane sialic acid content and break internal carbohydrate covalent bonds, no significant synaptic dissociation was observed. The later finding suggests that neither periodate sensitive covalent linkages nor terminal sialic acid mediated coordination bonds are of major importance in synaptic connectivity. In comparison with cytoplasmic organelles and other plasma membranes, the synaptic junction appeared more resistant to detergents as well as other disruptive treatments.

To examine the role of proteins and peptide linkages in cleft integrity, the effect of proteolytic enzymes was studied. Trypsin treatments appeared to dissociate synaptic junctions under conditions where its proteolytic action seemed localized to the outer synaptic membrane surface. Although the specific sites of peptide bond breakage within the cleft remains unclear, these results implicate proteins in an important role in synaptic connectivity in the dentate gyrus.

Our studies show that the synaptic junction is strong and durable and able to survive an extreme set of conditions which would disrupt all weak noncovalent interactions. It is likely that synaptic membranes are joined by peptide bonds or by noncovalent bonds which survive all treatments except those which disrupt or dissolve synaptic membranes.
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945 ACETYLCHOLINE RECEPTOR TURNOVER IN MEMBRANES OF DEVELOPING MUSCLE FIBERS. Peter N. Devreotes* (Spon. D. M. Fambrough). The Johns Hopkins University, Baltimore, Md. 21210

A model for turnover of the acetylcholine receptor in cultured chick embryonic myotubes is described. The model is constructed from observations in which [^{125}I]-mono-iodo-α-bungarotoxin was used as a specific marker for the receptor (Devreotes, P. N. and Fambrough, D. M., 1975 Journal of Cell Biology 65:335). Receptors are incorporated into the surface of myotubes from a "precursor" pool which is distributed on internal membranes and contains about 10% as many receptors as does the surface. Since the rate of incorporation is about 5%/hour, the precursor pool can support about two hours of incorporation in the absence of new protein synthesis. Receptors have a half-life in the surface of about 22 hours and are degraded by an energy-dependent mechanism which probably involves their internalization and subsequent degradation in secondary lysosomes. In addition to the surface and precursor classes of receptors, there is a third class of receptors containing approximately 30% as many receptors as does the surface. These "hidden" receptors in intact myotubes interact only slowly (t 1/2 = 1-2 hours) with extracellular [^{125}I]-mono-iodo-α-bungarotoxin and the significance of this slow interaction is presently under investigation.

946 INTERNODAL POTENTIALS OF MAMMALIAN NERVE FIBERS DERIVED FROM NODE ACTION POTENTIALS PREDICT NODE CURRENTS AND EXTRACELLULAR RECORDS. W.B. Marks and G.E. Loeb. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20014.

The internode of a myelinated nerve fiber can be modelled as an intra-axonal resistance shunted by distributed capacitance and resistance through the myelin to ground potential. We have calculated the time course of the spatial potential profile within the internodal axon that results when node potential waveforms are applied to the ends of this model. For mammalian fibers the node potentials were assumed to have the time course of the unit potentials (scaled to 130 mV) recorded from the cut ends of various fibers of the cat (Paintal, J. Physiol. 184: 79, 1966, for 8 to 100 m/sec). The spatial profile of an action potential was then constructed by joining the spatial potential profiles of successive internodes with appropriate delays.

Node currents were calculated from the gradients of these voltages on each side of a node. For amphibia, the amplitude and waveform agreed with measured values. For mammals, the amplitude of the node currents is related to conduction velocity by

$$i(nA) = 0.062\ c(m/sec) + 0.5\ .$$

About 70% of the node current returns through the myelin capacitance, 5% through the myelin resistance, and 25% through other nodes. The voltage profiles were used to calculate the extracellular signals of fibers captured within tubular electrodes (e.g. chronic recording or filaments in oil). For tubes shorter than 3 internodes waveform depended on node position. For lengths of 2 to 8 internodes, extracellular spike amplitude (mammals) is about 1/3 of the product of node current and tube resistance (center to ends).

947 MEDIATION OF IMPULSE CONDUCTION IN AXONS BY THRESHOLD CHANGES. Stephen A. Raymond and Paul Pangaro.* R.L.E., M.I.T., Cambridge, Mass. 02139.

At regions of low conduction safety in axons, a train of nerve impulses undergoes periods of blockade and periods of conduction. This is called intermittent conduction and has been noted many times. We propose that activity-dependent threshold shifts explain the periodicity of intermittent conduction. Using single sciatic axons of the frog, we measured the after-effects of a variety of conditioning pulse trains. We wrote three exponential functions to predict threshold following arbitrary patterns of impulse activity. Using these relations between activity and threshold, we illustrated on film the following explanation: Impulses arrive at the region of low conduction safety. As each impulse is conducted, the membrane on the distal side of the region becomes superexcitable for a short time. If the next impulse arrives during this time, it is most likely to be conducted. Continuous conduction results as long as successive impulses fall in the superexcitable period. But this high activity leads to a build-up of depression, causing the threshold curve after each impulse to move more and more quickly out of superexcitability. Conduction soon becomes chancy. After the first failure, the threshold climbs still higher, and conduction block ensues. The block persists until recovery from the depression. As soon as the threshold recovers enough, conduction is again possible. The first impulse to conduct generates a transient superexcitability that initiates a new period of continuous conduction. The durations of the intervals of intermittent conduction are related to the frequency of conditioning trains. Threshold changes are sufficient to account for intermittent conduction. This suggests that slow, subthreshold processes have great influence wherever conduction is probabilistic. (Supported in part by NIH Grant 5 R01 EY01149-02 and a grant from Bell Telephone Laboratories, Inc.).

948 OPTICAL MONITORING OF ACTIVITY IN LEECH NEURONS USING CHANGES IN ABSORPTION. L. B. Cohen, B.M. Salzberg* and W. N. Ross*. Dept. of Physiol., Yale U. School of Medicine, New Haven, 06510

The absorption of light by a giant axon stained with a merocyanine dye changes during the action potential (Biophys. J. *14*, 983, 1974). We have now used this optical signal to monitor action potentials in neurons of a central nervous system. Segmental ganglia from the leech, *Hirudo medicinalis*, were incubated with a solution of 'Merocyanine 540' (Eastman Organic Chemicals). A sensory neuron was impaled with a microelectrode and depolarizing current was passed to generate an action potential. An enlarged image of the ganglion was formed with a microscope objective and a pin-hole was inserted in the image plane so that the light reaching a photodiode came mainly from the stimulated neuron. In the best experiment, a single action potential led to an absorption change of only one part in 10^4, but this change was measured with a signal-to-noise ratio of 10:1. We expect that such optical signals can be used in an apparatus that is capable of monitoring activity in many neurons simultaneously.

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949 TEA-INSENSITIVE MUTANT IN Paramecium aurelia. Youko Satow. Lab. Molecular Biology, Univ. of Wisconsin, Madison, Wis. 53706.

The mutant P. aurelia that failed to respond behaviorally to tetraethylammonium (TEA) was examined. In TEA solution (4 mM TEA-Cl, 1 mM $Ca(OH)_2$, 1 mM citric acid, 1.2 mM Tris, pH 7.2), wild type generated series of action potentials but the mutant did not. In this solution, the action potentials (Ca regenerative responses) triggered by applied currents were nearly all-or-none in the wild type. The mutant responses were graded to the strength of current injected. The amplitude and the first derivative of the action potentials were smaller in the mutant than in the normal membrane, although full response could be generated with strong current. After the spikes, the mutant membrane repolarized to levels closer to the resting potentials than the normal membrane. Similar results were obtained in higher concentrations of TEA (up to 16 mM), although the active responses were reduced, apparently due to the loss of resting potentials at this high ionic concentration. Input resistance at 10^{-10} amp outward current was 57.3 $\pm$ 15.3 MΩ in the normal membrane and only 33.5 $\pm$ 16.2 MΩ in the mutant membrane. I-V curves showed that the mutant membrane has a smaller slope resistance at all points measured. This difference was also observed in other solutions containing Ca, K, Na, Mn or Ba instead of TEA. We conclude that this mutant has a conductance, probably a K conductance, enlarged by the mutation. This change affects both the resting properties and the active Ca response by increasing the leakage current. (Supported by PHS,NSF toKung)

950 INTERACTION BETWEEN MANGANESE AND DIVALENT CATION ACTION POTENTIALS IN THE LEECH RETZIUS CELL. Anna L. Kleinhaus and James W. Prichard. Dept. Neurol., Yale Med. Sch., New Haven, Ct. 06510.

Leech Retzius cells can fire prolonged Ca-dependent action potentials in response to transmembrane stimuli in Na-free Ringer containing tetraethylammonium chloride 25 mM (TEA), (Kleinhaus, A.L. and Prichard, J.W. J. Physiol. 246: 351, 1975). Under the same experimental conditions, Sr 1.5-13.5 mM was capable of substituting for Ca as a current carrier. $BaCl_2$ 2-25 mM could also substitute for Ca under these conditions, with the important difference that TEA was not necessary. Therefore, in addition to acting as a current carrier, Ba was able to perform the critical action of TEA, presumably depression of outward K current, which it is known to do in frog sartorius, chick heart, barnacle muscle and frog spinal neurones. The amplitude of the divalent cation action potential in the Retzius cell increased with increasing divalent cation concentration, the slopes being, respectively, 33, 40, and 70 mV per tenfold change in the external concentration of Ca, Sr, and Ba. Manganese chloride 3 mM (Mn) reversibly abolished all the divalent cation-dependent action potentials, but did not alter the Na-dependent action potential of the Retzius cell. These findings suggest that the divalent cations can carry current through one or several Mn-sensitive channels which are separate from the fast Na channel. At this stage of characterization, the properties of the divalent cation channel (or channels) closely resemble those found in widely different tissues, e.g. barnacle muscle (Hagiwara, S. et al, J. Gen. Physiol. 63: 564, 1974); frog sympathetic ganglia (Koketsu, K., and Nishi, S. J. Gen. Physiol. 53: 608, 1969) and mammalian ventricle (Kohlhardt, H. P. et al., Pfluegers Arch. 342: 125, 1973).

951 PREDICTION OF BURST WAVEFORMS FROM VOLTAGE CLAMP MEASURMENTS IN BURSTING PACEMAKER NEURONS. S.J. Smith*and S.H. Thompson* (Spon: C.F. Stevens). University of Wash. Seattle, Wa. 98105.

Voltage clamp studies of bursting pacemaker neurons of Tritonia reveal current transients relaxing on a time scale comparable to the burst rhythm. To see if these currents can explain the slow voltage oscillations characteristic of these cells, a quantitative model has been developed. Two variable conductances with distinct activation and reversal characteristics can account for the clamp currents seen between 500 msec. and 10 sec. after depolarizing steps. At shorter times, the currents are very similar to those observed in the somata of non-bursting nudibranch neurons by Connor and Stevens (J. Physiol. 213:21, 1971). Voltage clamp experiments provided the parameters for a system of equations extending the Connor and Stevens model to include the two slower components. The system was solved for voltage as a function of time at constant current by numerical integration. The solutions obtained reproduce the main features of bursting activity, with episodes of repetitive firing separated by silent intervals lasting 10 to 30 seconds. This provides strong evidence for a causal role of the two slow currents in pacemaker oscillation.

952 SLOW IONIC CURRENTS IN BURSTING PACEMAKER NEURONS. S.H. Thompson* and S.J. Smith* (Spon: J. Palka). University of Wash. Seattle, Wa. 98105.

Bursting pacemaker neurons in the ganglia of the nudibranch Tritonia fire groups of 2-10 spikes separated by hyperpolarized interburst intervals. Single driven spikes in bursters are followed by depolarizing afterpotentials (5 mv. in ampl. and 3 sec. in duration) which summate during repetitive stimulation and represent a depolarizing process capable of sustaining multiple discharge. Voltage-clamping to -50 mv. immediately after spikes or after short (20-100 msec.) depolarizing command pulses reveals an exponentially decaying inward tail current the amplitude of which is a function of the external Na^+ and Ca^{++} concentration. As the command pulse duration is increased, the inward tail current is gradually and smoothly diminished and finally at long durations, replaced by an exponentially decaying outward tail current which is sensitive to the external K^+ concentration and which reverses at about -60 mv. Evidently two slow conductance processes, kinetically distinct from other ionic conductances described in these cells, are activated sequentially during commands and can be separated by activation pulse duration. We used ionic substitution to separate the two processes in order to measure time- and voltage-dependent activation parameters for each. The slow inward current, B-current, begins to activate at voltages below those which cause significant activation of other currents and its activation time constant is short at depolarized voltages, but quite long at rest potential. B-current has only been seen in bursting cells in our preparation. The slow outward current, S-current, activates more slowly at all voltages but attains a higher $\bar{g}$ max. S-currents are observed in many non-bursting cells. The slow kinetics of these currents suggest a role in producing the cyclic voltages changes during bursting.

953 GLIAL CELLS AND EXTRACELLULAR POTASSIUM: THEIR RELATIONSHIP IN MAMMALIAN CORTEX. Timothy A. Pedley and Kin J. Futamachi*. Dept. of Neurology, Stanford University Sch. of Med., Stanford, CA 95305.

Simultaneous recordings were made of glial cell potentials and extracellular potassium concentration ($[K^+]_o$) in cat cortex in order to provide more quantitative information about the sensitivity of mammalian neuroglia to changes in $[K^+]_o$. A penicillin epileptogenic focus was used to generate both transient and sustained elevations in $[K^+]_o$, thus allowing measurements of glial membrane potential (V_m) at both resting and increased $[K^+]_o$ levels many times during the same experiment. Resting V_m averaged 92.6 ± 10.9 mV for 33 cells. Characteristic slow depolarizations mirrored increases in $[K^+]_o$ in most respects. During sustained elevations in $[K^+]_o$ such as occurred during ictal episodes ("seizures"), the glial membrane potential responded in a manner predicted by the Nernst equation with a slope close to the theoretical 60 mV. During focal and transient rises in $[K^+]_o$, however, significant discrepancies occurred between the measured rise in $[K^+]_o$ and the associated glial cell depolarization. For example, many glial cells depolarized more than would have been expected from the observed $[K^+]_o$ change at that site. Though such findings may be attributed in part to the different spatial relationships which exist between glial membrane, K^+-sensitive electrode tip and released K^+ under these circumstances, they suggest in addition the possibility of a glial synctitium which acts as a spatial buffer to increases in $[K^+]_o$, and/or a heterogeneous population of glial cell types.

954 DENDRITIC ACTIVITY IN IN VITRO HIPPOCAMPAL NEURONS. Philip A. Schwartzkroin, Institute of Neurophysiology, University of Oslo, Norway.

Indication of active dendritic spike generation has been seen in the in vitro hippocampal slice preparation. Recording intracellularly from CA1 pyramidal cell bodies in the slice, one finds low amplitude, fast rising potentials in one-third of the cells. In most cases, the amplitude of supposed dendritic spikes is not altered by hyperpolarizing current passed into the cell body, suggesting that the site of their generation is quite distal on the dendrites; hyperpolarization does increase the amplitude of the full soma spike and of most EPSPs. Electrode penetrations through the proximal dendritic zone have revealed sharp-rising potentials similar to those seen at the soma, but of larger amplitude. No slow-rising prepotentials were seen associated with these potentials, but depolarizing after-potentials were present.

The occurrence of dendritic spikes, although not necessarily coupled with the depolarizing after-potential (DAP) seen in most cells, may be a related phenomenon arising from peculiar dendritic membrane characteristics. Bursting activity of cells of hippocampus may be attributed to summation of DAPs. With repetitive stimulation of the cell, the DAP potentiates markedly in amplitude and duration; occasional broad spike-like potentials of graded amplitudes may arise from the DAP. The potentiation is facilitated by cell hyperpolarization, as is cell bursting activity. In some cases of cell bursting, full soma spikes do not arise from the DAP, but potentials similar to dendritic spikes are unmasked.

The mechanisms underlying dendritic spike generation and depolarizing after-potentials are still to be described. Careful analysis of these phenomena seems warranted given the similarity of hippocampal burst potentials and bursts generated by paroxysmal depolarization shifts in epileptogenic cortex.

955 SPIKE INITIATION BY WHITE-NOISE CURRENT IN APLYSIA NEURONS. Hugh L. Bryant and David Brillinger.* Anatomy Dept., Sch. Med., UCLA, Los Angeles, 90024 and Statistics Dept., Univ. Calif., Berkeley, 94720.

Spike initiation in Aplysia neurons was examined using Gaussian white-noise (GWN) transmembrane current. The following retrospective question was asked first: Given that a spike has occurred, what are the relevant features of the preceding current waveforms? It was concluded that: 1) a large variety of current trajectories can be effective; 2) that the average trajectory has a "preferred" status; 3) that many different stimulus parameters (trajectory amplitude, slope and acceleration, variability, correlations) precede spikes and that the absence of one may be compensated by the presence of others. We have recently asked also the following prospective question: What is the probability of a spike given that a certain current trajectory has occurred? A first approximation to this problem is based on the following simple model. We estimate for various values of τ the function $f_\tau(x)$ defined by:

$$f_\tau(x)=\lim_{h\to 0}\frac{1}{h}\,\text{Prob}\left\{\text{spike in the interval } [t+\tau,t+\tau+h] \,/\, x<X(t)<x+\Delta x\right\},$$

where X is the input current. That is, we have estimated the probability of a spike given that a certain value of the input current has occurred at a certain time in the past. From this analysis we have concluded that a simple threshold model of spike triggering is inadequate. An extension to the analysis of the probability of a spike given pairs of current values at two different times in the past led to the conclusion that certain input combinations can lead to enhanced spike probability. Finally, estimates of the linear coherence between the GWN input current and the output spike train showed high values (0.7) at low frequencies, decreasing to insignificant levels at 6 Hz. (Supported by USPHS grant to J.P.S. and by NSF grants to D.B. and J.P.S.)

956 THERMAL DEPENDENCE OF MEMBRANE POTENTIAL AND INPUT RESISTANCE OF CAROTID BODY CELLS. M. Baron* and C. Eyzaguirre. Dept. Physiol., Univ. Utah Coll. Med., Salt Lake City, Utah 84132.

The membrane potential (MP) and input resistance (Ri) of superfused carotid body cells were studied by intracellular penetrations with microelectrodes filled with 3 M KCl. At 35-37°C the mean MP was 25 mV (range 10-55) and the mean Ri was 40 MΩ (range 10-140). Dispersion of data may have been due to i) varying degrees of cell damage due to their small size (6-10 μ); ii) impalement of different cell types; iii) different experimental conditions due to different degrees of oxygenation at various tissue depths. The thermal response consisted of a marked depolarization and/or great decrease in Ri on cooling to 29-30°; simultaneous recordings of chemosensory discharges from carotid sinus nerve showed a parallel decrease in discharge frequency. Rewarming reversed the effect although, frequently, cells did not recover completely. The degree of depolarization seemed to be directly related to the rate of cooling (°C/sec). The decrease in Ri and depolarization were also directly related to the initial Ri and MP values at normal temperature. A reversal potential (RP) for the cooling effect was found at +3 to -8 mV. The ionic mechanisms underlying the thermal response were studied with the following results: 1) When $[Cl^-]_o$ was reduced to 10% of that in normal saline, both MP and Ri decreased while RP shifted toward a more positive level; also, depolarization and decreased Ri induced by cold were exagerated. 2) Replacing $[Na^+]_o$ with choline induced cell hyperpolarization, an increase in Ri and the thermal response was reduced. 3) Addition of ouabain (5×10^{-5} M) or strophantidin (10^{-6} w/v) to the bathing saline was rather ineffective in changing MP, Ri or the thermal response to cooling. 4) Ionic substitution experiments showed little or no effects of K^+ ions on MP, Ri or the response to cooling. 5) Lack of Ca^{++} tended to increase MP and Ri. 6) Elimination of Na^+ and Ca^{++} ions from the bathing medium potentiated the effects of lack of Na^+ alone. Attempts at morphological identification of impaled cells showing obvious thermal responses were made by iontophoretically ejecting 6% procion navy blue from the recording microelectrode. More than 90% of the cells so stained were identified as glomus type I cells. In some instances, the stained elements appeared isolated; in others, the stained type I cells were close to another stained element which seemed to be a type II cell; often, a glomerulus formed by 3-5 type I cells and some type II cells appeared simultaneously stained. Multiple staining with a single ejection of dye suggests the presence of membrane to membrane specializations (tight or gap junctions) that would allow the passage of dye from one cell to another. However, there is no electron microscopic evidence in support of this assumption. It is concluded that the majority of cells responding to temperature changes are type I cells, although it is possible that type II cells may show the same effect. The latter are, probably, more difficult to impale and, thus, less amenable to direct investigation. Temperature seemed to affect especially the membrane permeability to Cl^- ions which also appeared to be very important in the maintenance of MP and Ri. Na^+ and Ca^{++} also participated in these phenomena to an apparently lesser degree. K^+ ions were quite ineffective in the maintenance of MP, Ri and on the receptor response to cooling.

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957 ALTERED ELECTRICAL EXCITABILITY IN PAWNS, BEHAVIORAL MUTANTS OF PARAMECIUM AURELIA. Stanley J. Schein* (SPON: Herman Buschke). Dept. of Molecular Biology, Albert Einstein College of Medicine, N. Y., N. Y. 10461.

Reversal of swimming direction in paramecia follows the rapid influx of calcium which carries the inward current during regenerative active depolarization. Pawns, behavioral mutants of Paramecium aurelia that have lost their 'reversal response' to varying degrees, were isolated by their tolerance of the paralyzing and toxic effects of barium. Genetic studies on seven of the mutants place the pawns into three genes; crosses show that mutants isolated by Dr. C. Kung (using a behavioral selection) are of the same genes, pwnA, pwnB and pwnC. The majority of the barium selected mutants retain a weak reversal response; two, pwnB(314) and pwnC(320) have normal or nearly normal reversal behavior. Each of the mutants and the wild-type were immobilized in agar, impaled from above with two (4 M KAc filled) electrodes and current-clamped. Afterward, ionic current could be measured by subtracting capacitative current ($C \cdot dV/dt$) from total stimulus current; then active ionic current could be derived by subtracting the ionic current expected for a passive (RC) cell from the total ionic current. All mutants are normal with respect to 1) resting potential (-20 to -25 mV), 2) membrane time constant (ten to fifteen msec) and 3) delayed rectification. However, all mutants have reduced active inward ionic current (reflecting reduced calcium activation); the calcium current is smaller in those mutants displaying weaker reversal responses: While the pwnB(314) mutant has 25% of the wild-type active inward current and the pwnC(320) mutant has 50%, the other mutants have 5% or less. Anomalous rectification in the wild-type and in both pwnA and pwnC mutants is apparent when the cell is hyperpolarized to about -40 mV. Surprisingly, anomalous rectification is not observed until -60 mV in the partially excitable pwnB(314) and until -75 mV in two completely inexcitable alleles of the same pwnB gene. Thus in pwnB mutants, the magnitude of the defect in anomalous rectification correlates with the magnitude of the loss of calcium activation. Strains carrying mutations in two genes from two parental partial pawns exhibit even weaker reversal responses; they also have less calcium activation and show the defect in anomalous rectification if the double-mutant strain contains a pwnB mutation. The extremely inexcitable mutants should be useful in providing a tetrodotoxin-like separation of currents. The alteration of quantitative parameters of excitability in the partially excitable mutants may provide insight into the role played in excitability by each gene's product.

958 SURFACE LABELING OF INTACT SYNAPTOSOMES: GANGLIOSIDES. B.L. Hungund*, S.P. Mahadik*, and M.M. Rapport, Div. Neuroscience, N.Y. State Psychiatric Inst., and Dept. Biochem., Columbia Univ., New York, N.Y. 10032.

When intact synaptosomes of rat brain were labeled by oxidation with galactose oxidase followed by reduction with ^{3}H-borohydride (Steck, 1972), then disrupted and their membranes fractionated with diatrizoate (Tamir, Mahadik, Graf, Roizin & Rapport, 1975) to give fractions A to E, the specifically labelled glycoproteins and glycolipids were found predominantly in fractions A and B (Mahadik, Hungund, & Rapport, 1975) indicating that they originated in the exposed surface of synaptosomes. Gangliosides of these synaptosome fractions (isolated by dialysis of the upper phase of washed $CHCl_3$-MeOH extracts) have now been examined. The total ganglioside contents in μg/mg protein were: A, 45; B, 42; C, 26; D, 19; E, < 3. Specific radioactivity ratios, with and without enzyme treatment (+E/-E), were: A, 6.6; B, 3.1; C, 1.9; D & E, < 1.3, confirming selectivity of procedures. By visual estimation (TLC plates) the approx. ganglioside composition was: G_4, 15%; G_2 + G_3, 35%; G_1, 20%; other, 30%. Radioactivity was found almost entirely in G_4 (G_{M1}). With isolated membrane fractions, little selectivity was seen: 1) all ganglioside species were labeled; 2) much more label was present (even when fractions were pretreated with unlabeled $NaBH_4$); 3) specific activity ratios (+E/-E) for total gangliosides were: A, 7.9; B, 8.9; C, 4.6; D, 5.0. The results confirm the conclusion that membrane fractions A and B are derived from the exposed synaptosome surface, and also show that G_4 is the only ganglioside species available for reaction at that surface.

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959 PURIFICATION AND CHARACTERIZATION OF A GLUTAMATE-BINDING GLYCOPROTEIN FROM SYNAPTIC MEMBRANES. Elias K. Michaelis Dept. Human Development, Univ. of Kansas, Lawrence, Ks. 66045.

Synaptic membrane preparations have been shown to contain a glutamate-binding site that is Na^+-independent and has some of the characteristics of the physiologic receptor for this dicarboxylic amino acid (Michaelis, et.al, Biochim. Biophys. Acta 367, 338; Roberts, Nature 252, 399). The current studies were undertaken in an attempt to further purify and chemically characterize this binding macromolecule. A 200-fold purification of this synaptic membrane high-affinity glutamate-binding protein was achieved by a combination of affinity batch separation on glutamate-loaded glass fiber and affinity chromatography on concanavalin A sepharose. This purified protein is devoid of any glutamate dehydrogenase, glutamate decarboxylase, or glutamine synthetase activity. Its glutamate-binding activity differs from that of the glutamate transport system in that it is Na^+-independent, it is inhibited by both concanavalin A (70 μg/ml) and 2-mercaptoethanol (0.1 mM), and it is partially inhibited by glutamate diethyl ester. Glutamate binding to this glycoprotein has a dissociation constant K_D = 0.85 μM, and has a maximum binding capacity of 65.5 nmoles/mgprotein. Triton X-100 electrophoresis revealed only one small molecular weight glycoprotein bearing the glutamate binding activity. The molecular weight of this protein was estimated by SDS electrophoresis to be 13,800. It is felt that this glycoprotein may represent the glutamate physiologic receptor of brain synaptic membranes. (Supported by Univ. of Kansas Biomed. Grant #4082-5706, and NICHHD Grant HD-02528).

960 HETEROGENEITY OF ERYTHROCYTE SPECTRIN PROTEIN WITH REFERENCE TO DUCHENNE MUSCULAR DYSTROPHY. Allen D. Roses and Stanley H. Appel. Div. Neurology, Duke Univ. Sch. Med., Durham, N.C. 27710.

Homogenous purified membrane fractions from excitable tissue are difficult to isolate. Tissue from human disorders are also difficult to obtain and control. We have used erythrocyte membranes to study alteration of components of membranes in inherited disorders of excitable tissues. Phosphorylation of the erythrocyte membrane protein spectrin peak II (nomenclature of Fairbanks et al., Biochem. 10, 2606 [1971]) is increased in membranes from Duchenne muscular dystrophy (DMD) patients and some carriers compared to age and sex matched controls. Both the initial rate and the level of peak II phosphorylation are significantly increased. Other membrane protein components are not abnormally phosphorylated suggesting possible substrate differences in DMD. In order to evaluate possible substrate protein differences and their relationship to muscle abnormalities, a detailed investigation of spectrin was performed. Spectrin was extracted from ghosts that were incubated under conditions used for protein phosphorylation. The extraction method of Marchesi et al., (Biochem. 7, 50 [1970]) was used. Spectrin was purified and separated from extractable radioactive phospholipid on a .1% SDS Sepharose-4B column and subjected to SDS-polyacrylamide and isoelectric focusing (IF) analysis. SDS was removed prior to IF. Purified spectrin that migrates as a doublet (mw. 240,000 and 220,000) on SDS-polyacrylamide gels contains a multiplicity of Bromphenol blue stainable bands on IF. Autoradiography of IF spectrin demstrates additional labeled bands not seen by staining techniques. The exquisite substrate heterogeneity of spectrin and its implications for multiple functional capacities is fundamental to investigations of diseases using the erythrocyte as a model membrane. These IF techniques are being used to isolate the possible abnormal component of peak II in DMD.

961 SEPARATION AND CHARACTERIZATION OF TWO TYPES OF RAT BRAIN MITOCHONDRIA. H. Tamir, S. Mahadik*, A. Klein*, L. Roizin*, J.C. Liu* and M.M. Rapport. Div. of Neuroscience and Dept. Neuropathology, N.Y. State Psychiatric Inst. and Depts. of Biochemistry and Pathology, Columbia Univ. College of Physicians and Surgeons, New York, N. Y. 10032.

Two types of mitochondria were separated from rat brain by a two-stage gradient centrifugation in sodium diatrizoate: fraction P_2C originating primarily from neuronal and glial cell bodies (obtained as the pellet on a continuous gradient of 10 to 22%) and fraction E released from the osmotically shocked P_2B fraction and thus originating primarily from nerve endings (obtained as the pellet on a discontinuous gradient of 10%, 14%, 16%, 18% and 22%). Frs. P_2C and E were comprised almost entirely of mitochondria (electron microscopy): P_2C showed a predominance of electron dense matrices and cristae whereas in E they were predominantly light. The two types of mitochondria exhibited other differences. 1) ATP synthesis (DNP and Ca^{++} sensitive) was 50% higher in Fr. P_2C 2) Mg^{++}-ATPase was 25% higher in Fr. P_2C 3) MAO type A activity (serotonin substrate) was 25 to 30% higher in Fr. E 4) Pargyline (10^{-5}M) inhibited this MAO activity in Fr. P_2C by 70% with little effect on MAO in Fr. E. No Na,K-ATPase was detected in either fraction. MAO activity in both fractions was inhibited by harmaline (10^{-7}M) and chlorgyline (10^{-7}M), whereas deprenyl (10^{-4}M) had no effect. Heating (50°C, 20 min) destroyed 10% of the activity with serotonin and 90% of that with tyramine. Electrophoresis of SDS extracts of Frs. P_2C and E on gradient slab gels showed over 70 bands with Coomassie Blue, with minor qualitative differences in the patterns but significant quantitative differences. We conclude that significant biochemical and morphological differences can be demonstrated between mitochondria present in the terminal portions of axons and those in cell bodies of rat brain.

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962 TOPOGRAPHY OF THE SYNAPTIC PLASMA MEMBRANE. Y-J. Wang*, G. Crawford* and H.R. Mahler. Brain Research Group, Chemical Laboratories, Indiana University, Bloomington, IN 47401, USA.

Highly purified synaptic plasma membranes of rat cerebral cortex, after salt extraction, dissociation and electrophoresis in the presence of sodium dodecyl sulfate, exhibit a characteristic pattern of integral polypeptides (estimated masses in K daltons, major band in italics): *205;* 185 (doublet), *145*, 123, 118, *100, 92,* 83, 78, 66, *50, 42, 39,* 35, 31, 25, 21. Disposition on the external (junctional) surface can be inferred from presence of carbohydrate prosthetic groups (periodate-Schiff reaction and labeling with fucose *in vivo*) and susceptibility to enzymatic iodination on intact synaptosomes. Components migrating with the bands at 145, 118, 100(?), 66, 50 and 35 Kdaltons meet these criteria. Extraction with 0.2% Triton X-100 in the presence of ethylene diaminetetraacetate leads to the quantitative and preferential removal of the bands at 66, 42 and 21 Kdaltons. In contrast iodination of components at 205, 185, 92(?) and 39 requires prior lysis of synaptosomes; they are therefore localized on their interior (synaptoplasmic) surface. The inaccessibility of some of the remaining components suggests that they may be largely within the lipid bilayer. This pattern is qualitatively distinct from that characteristic of smooth-surfaced microsomes, the most likely contaminants, the components of which appear equally accessible to iodination before or after lysis. The question of the presence of actin and tubulin, suggested in earlier reports, their topographical localization, as well as their quantitative contributions to the bands at 50 and 40 Kdaltons is under critical examination. The synaptosomal surface carbohydrates can bind specific lectins, e.g. concanavalin A and lectins from lentil and wheat germ, but such binding does not affect high affinity uptake of choline, GABA, norepinephrine, 5-hydroxytryptamine, dopamine or L-glutamate. While mild trypsinization of the surface is equally without effect on the uptake of the first five of these transmitters, it reduces the maximal uptake velocity of L-glutamate by 50% without any significant affect on its K_M. (Supported by Grant NS 08309 from the National Institutes of Health.)

963 PROTON NMR STUDIES OF RABBIT SYNAPTOSOMES. Ruven Greenberg, Christopher Whalley,* and Daniel Fiat.* Physiol. Dept., U. of Ill. Med. School, Chicago, Ill. 60680 and Argonne Nat'l. Lab., Argonne, Ill. 60439. (Spon: V. Nair)

Proton magnetic resonance studies were carried out by means of a 220 MHz Varian Super Conducting spectrometer on rabbit cortical synaptosomes in 0.8 M NaCl in D_2O. Many well resolved lines some of them similar to those ascribed to membrane moieties in RBC membrane fragments (D. Chapman, 1970) were observed. These included CH=CH, CH_2-CH_2, CH_3, phosphatidyl choline, and sialic acid. No significant changes in the chemical shift values were observed with the addition of 60 mM K^+ and/or 4 mM Ca^{++} to the synaptosomes maintained at 0° and 37°C. The longitudinal relaxation times (T_1) were determined for the individual lines by using a sequence of 180°-90° pulses while observing the effect of the time interval between pulses on the Fourier Transform Spectrum. We observed a lengthening from 0.7 sec to 1.0 sec for the phosphatidyl choline and sialic acid moieties after 2-4 hours at 37°C, indicating a shorter correlation time presumably due to the less restricted molecular motion of the two moieties. No changes were observed after 2-7 days at 0°C. Neither was there a change with the addition of a depolarizing concentration of K^+ (60 mM) and/or Ca^{++} (4 mM) and presumably therefore, the lengthened T_1 is not correlated with depolarization per se. Interestingly, the lengthened T_1 correlated with the K^+ content of the synaptosomes which decreased from 80 mM at 0 time, to 20 mM at 2 and 4 hours, to 0 mM at 6 and 8 hours. Conceivably, therefore, the change in the lipid moieties is correlated with membrane changes associated with K^+ release.

964 MACROMOLECULAR STRUCTURAL TRANSITIONS IN HIGH ELECTRIC FIELDS - IMPLICATIONS FOR NEUROBIOLOGY. Chester T. O'Konski. Dept. Chem., Univ. Calif., Berkeley, CA 94720.

The control of Na^+ and K^+ permeabilities in nerve membrane may be achieved through the action of polyanionic macromolecules whose configurations - and therefore binding and ion transport properties - are dependent upon electric field intensity. A plausible control model would consist of a helical polymer with a hydrophobic exterior dissolved in the quasi two-dimensional lipid bilayer membrane, and with a structure such that negatively charged groups are located adjacent to a hollow core through which the cations may pass. As the electric field intensity changes during nerve depolarization, the helical structure could change pitch and diameter, or there may be more subtle changes in hydration, binding coefficients, or in potential energy barriers for cation transport. A gate-like steric mechanism involving the motion of side chains on proteins is still another possibility.

These speculations as to possible mechanisms are useful for developing molecular methods of study of the mechanisms of nerve impulse transmission and information storage in the CNS; similar considerations have already excited the attention of several scientists. Our interest developed from the studies of electric birefringence effects in solutions of DNA and other polyanions, where optical responses other than the usual macromolecule orientation type were found. New results indicate the occurrence of field-induced associations between the DNA macromolecules. The optical steady state signals and the dynamics of the field-free relaxation also indicate structural modification of the DNA by the electric field pulse. Ion mobilities appear to be modified by the process; this is shown by solution conductance changes.

Experiments designed to detect macromolecular structural transitions in macromolecules involved in nerve membrane are outlined and studies of Keynes, Cohen, and others on optical signals in nerve membrane are reviewed. A fast on-line electro-optic relaxation spectrometer developed here for solution studies is described. Such instrumentation should be useful in studies of macromolecular mechanisms of neural phenomena. Various optical configurations have been considered and exploratory experiments on membrane fragments and vesicles have been made.

965 REPRODUCTION IN VITRO OF MYASTHENIC SYNDROME BY AN EXTRACT OF BRONCHOGENIC CARCINOMA. Koichi Ishikawa*, John Kerch Engelhardt*, Takehiko Fujisawa*, and Tatsuya Okamoto* (SPON: Anant Dravid). COH, Duarte, CA 91010, and IPCR, Chiba Univ., Chiba, Japan.

Acetone extracts of a bronchogenic carcinoma from a patient with the Lambert-Eaton (L-E) syndrome reproduced in vitro the defect in neuromuscular transmission that is characteristic of this syndrome.

When the frog nerve-muscle preparation was exposed to frog Ringer containing the extract, both the isometric twitch tension and the EMG induced by nerve stimulation markedly decreased in amplitude. This effect was not observed with extracts of normal lung tissue or of lung cancer tissue from patients without the L-E syndrome.

The L-E extract did not affect action potential conduction in the desheathed frog sciatic nerve. Intracellular recordings indicated that the end plate potential was reduced by the L-E extract but the amplitude distribution and time course of the miniature end plate potentials were unaffected. In addition, the L-E extract reduced the quantum content of the end plate potential in bathing solutions containing high concentrations of magnesium.

It is concluded that the L-E extract blocks neuromuscular transmission in the frog by interfering with the acetylcholine release mechanism at the motor nerve terminal.

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966 CATIONIC INHIBITION OF QUANTAL RELEASE AT THE FROG NEUROMUSCULAR JUNCTION. Stanley Misler* and W.P. Hurlbut*, (SPON: J. Musacchio) Rockefeller University. (S.M. supported by NIGMS, GMO 1668. N.Y.U. Medical Center)

Recently, Muller and Finkelstein attributed Mg^{2+} inhibition of quantal content, "m", to "electrostatic screening" of Ca^{2+}. Generalizing this hypothesis, an antagonism between Ca^{2+} and a given cation should be predictable from diffuse double layer theory, assuming only a uniform density of fixed negative charge on the outer surface of the presynaptic terminal membrane, and using a relationship between the $[Ca^{2+}]$ nearest this surface and "m". Experimental data are compared with theoretical predictions from "screening" for two other effects. (1) Reduction of univalent cation concentration, $[U^+]$, on "m". A univalent cation, which would be impermeant during the nerve terminal action potential (A.P.), was sought, for use as a Na^+ substitute. The isosmotic substitution of sucrose for the impermeant univalent at constant $[Na]_o$ would then constitute the reduction in $[U^+]$ at a nearly constant A.P. waveform. Glucosamine (at pH 6.5) and arginine (at pH 6.9) were chosen as standards because (a) their post-synaptic effects were minimal; (b) estimates of the synaptic transfer function at the neuromuscular junction, using glucosamine or arginine to vary terminal A.P. amplitude, resembled those obtained at several synapses, by graded presynaptic depolarization; and (c) the effects of either on compound A.P. amplitude were nearly comparable to those of TMA or choline. Agreement, between the observed effect of $[U^+]$ reduction to that predicted from the effects of Ca^{2+} and Mg^{2+}, was ≥ 0.7, when $m \leq 10$, and at least qualitative at higher values of "m". (2) Mn^{2+} inhibition of "m". Since Mn^{2+} is 20X as effective as Mg^{2+} in inhibiting "m", most of its action cannot be due to "screening". Two possibilities for a non-screening action were considered (a) specific Ca^{2+} channel blockage and (b) surface charge binding. Experiments on Mn^{2+}-Mg^{2+} competition at low $[Mn^{2+}]$ were performed to evaluate each choice.

967 PARADOXICAL EFFECTS OF $[Ca^{++}]_o$ ON MEPP FREQUENCY IN MUSCLES TREATED WITH β-BUNGAROTOXIN (β-BuTX) OR La^{+++}. M. T. Alderdice* and R. L. Volle* (SPON: Edward G. Shaskan). Univ. of Conn. Health Center, Farmington, Conn. 06032.

β-BuTX (0.5 μg/ml) increases the frequency of miniature endplate potentials (mepp's) in frog sartorius muscle; after several hours, mepp frequency is depressed to virtually zero. Similar results have been reported with rat diaphragm (Chang, Chen and Lee; JPET 184:438, 1973). During the initial phase (prior to depression), increasing $[Ca^{++}]_o$ from 0.5 to 2.0 mM decreases mepp frequency, an effect opposite from that of untreated muscles. Disinhibition occurs when $[Ca^{++}]_o$ is reduced from 2.0 to 0.5 mM. La^{+++} (50 μM) also produces an increase in mepp frequency and, like β-BuTX, raising $[Ca^{++}]_o$ from 0.5 to 2.0 mM decreases mepp frequency, while decreasing $[Ca^{++}]_o$ increases mepp frequency. The increase in mepp frequency produced by β-BuTX is enhanced as $[K^+]_o$ is raised from 2.5 to 10.0 mM, whereas Mg^{++} (7 mM) abolishes the effect of β-BuTX and reduces mepp frequency to a low level. The late depressant phase of β-BuTX on mepp frequency appears to be due to depletion of transmitter (at least readily releasable stores) since La^{+++} (50 μM) exhibits no effect and hypertonic $[K^+]_o$ elicits only a small transient effect during this phase; however, inhibition of transmitter release by β-BuTX is also a possibility. (Supported by NS 07540-08).

968 THE MECHANISM OF ACTION OF β-BUNGAROTOXIN. Gail M. Wagner*, Peter N. Strong*, and Regis B. Kelly. Depts of Biochemistry and Physiology, UCSF, San Francisco, 94143.

Neurophysiological studies using the rat phrenic nerve diaphragm preparation have demonstrated an initial increase in neurotransmitter released (spontaneous, evoked and delayed) in response to the application of β-bungarotoxin followed by a progressive block of synaptic transmission. In searching for the biochemical basis of these physiological effects, we have discovered that β-bungarotoxin reduces ATP synthesis and is a non-competitive inhibitor of calcium uptake by brain mitochondria. It inhibits the extent but not the rate of uptake by rat brain mitochondria at very low toxin concentrations (50% inhibition at 8 pmoles toxin/mg protein). Sixty percent of uptake is inhibited at low toxin concentrations with complete inhibition being achieve only at very high toxin concentrations. Liver mitochondria, on the other hand, are only inhibited at very high toxin concentrations. In some way, the toxin can distinguish liver from brain mitochondria. We have also demonstrated that the toxin possesses phospholipase A2 activity. This activity does not account for the preferential inhibition of brain mitochondria by the toxin since the calcium uptake activities of liver and brain mitochondria are equally inhibited by phospholipase A2 from Crotalus adamanteus venom. Also, phospholipase A2 injected intraperitoneally into mice at 60 times the lethal concentration of β-bungarotoxin does not cause death. Although β-bungarotoxin has phospholipase activity this is insufficient by itself to explain its neurotoxicity or its specificity for brain mitochondria.

Possible mechanisms will be presented by which the biochemical character of the toxin may give rise to its physiological effects.

969 EFFECT OF BLACK WIDOW SPIDER VENOM ON NEURONS IN TISSUE CULTURE. Lee L. Rubin*, Karl H. Pfenninger and Alexander Mauro*(SPON: F. Brink, Jr.). Rockefeller Univ., New York, N.Y. 10021 and Yale Univ. Sch. Med., New Haven, Conn. 06510

Black widow spider venom (BWSV) induces, without observed synaptic membrane damage, massive release of acetylcholine in the myoneural junction. The effects of purified BWSV on synapses formed in tissue culture by chick embryo spinal cord neurons and muscle cells were examined using electrophysiological and morphological techniques. At venom doses of 0.05 μg/ml., greatly increased synaptic activity occurs and is rapidly followed by pronounced structural alterations and eventual dissolution of the entire axonal population while muscle cells remain completely intact. Studies of chick embryo spinal cord explant and dissociated cell cultures indicate that BWSV is remarkably specific; it disrupts axonal structure but does not affect neuronal soma, glial cells or fibroblasts. Axons from spinal cord explants of embryos older than day 14 become increasingly resistant to BWSV. Preliminary freeze fracture and thin section examination of rat spinal cord explants (whose axons are similarly disrupted) suggest that the venom initiates a redistribution of the axonal plasma membrane, producing, from axons of uniform size, alternating constrictions and varicosities. External membranes and internal organelles retain their integrity at this stage, and neighboring glial processes appear normal. Emphasizing the selective action of BWSV, these ultrastructural findings indicate that its effects are confined to the developing nerve fiber whose plasma membrane, as previously demonstrated, is characterized by a low density of intramembranous particles.

970 PURIFICATION FROM BLACK WIDOW SPIDER VENOM OF A PROTEIN CAUSING THE DEPLETION OF SYNAPTIC VESICLES. N. Frontali*, B. Ceccarelli*, A. Gorio*, A. Mauro*, P. Siekevitz*, M.C. Tzeng* and W.P. Hurlbut* (SPON: C. M. Connelly). Rockefeller Univ., New York, N.Y. 10021 and Inst. of Pharmacol., Univ. of Milan, Milan, Italy.

By conventional chromatography (Sephadex G-200; DEAE cellulose, pH 8.2) we have purified from black widow spider venom a protein that produces at frog neuromuscular junctions all of the physiological effects of the crude venom. The protein increases by several orders of magnitude the frequency of occurrence of miniature end-plate potentials, blocks neuromuscular transmission, causes the nerve terminals to swell and causes an almost complete depletion of synaptic vesicles. This evidence indicates that the protein acts directly on the mechanism controlling the release of neurotransmitter from nerve endings. The spider toxin thus acts presynaptically in contrast to the α-bungarotoxin of snake venom, which acts postsynaptically.

The protein is the major water soluble protein of the spider venom. It migrates as a single band on SDS gel, has a molecular weight of about 100,000, is not a glycoprotein and has virtually no lipolytic or proteolytic activity. It seems pure enough to warrant detailed study of its chemical structure and of its site and mode of action.

In addition to affecting the neuromuscular junction crude spider venom also depolarizes the crayfish stretch receptor, accelerates the beat of the cockroach heart and kills houseflies. These latter effects of the crude venom are not due to the purified protein but are due to other protein components of the venom.

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971 THE INTERACTION OF CALCIUM WITH BLACK WIDOW SPIDER VENOM AT THE FROG NEUROMUSCULAR JUNCTION. Janie Smith* and Allen W. Clark. Dept. Anat., Univ. Wisc., Madison, 53706.

Black widow spider venom (BWSV) produces a dramatic increase in the spontaneous frequency of mepp's at the frog neuromuscular junction and a concomitant depletion of synaptic vesicles from the nerve terminal. Clinically, the symptoms of BWSV in humans can be reduced by the infusion of calcium salts. We have investigated the effects of elevated [Ca^{2+}] on the activity of BWSV in isolated sartorius and cutaneous pectoris muscles from the frog, Rana pipiens. Perfusion with [Ca^{2+}] as low as 5mM can totally abolish the venom effect in one minute. Increasing [Ca^{2+}] to 50mM does not affect the onset of this abolition. The cutaneous pectoris is consistently both more sensitive to BWSV than the sartorius from the same frog and more resistant to the venom-abolishing effects of elevated Ca^{2+}. If both muscles are treated with the same dose of BWSV, however, the venom effect on either muscle is abolished by the same level of Ca^{2+}. Perfusion with elevated Ca^{2+} simultaneously produces an almost total loss of spontaneous mepp's at the same venom-treated junctions. In the absence of BWSV, these [Ca^{2+}] reduce mepp amplitude and increase mepp frequency. Preparations treated with BWSV and elevated Ca^{2+} are refractory to additional doses of venom. We believe these results suggest that BWSV and Ca^{2+} interact with the same site on the nerve terminal plasma membrane. With the doses of venom used we were unable to restore the venom effect by washing the terminal with either 0-Ca^{2+}-Ringer's or with EGTA-Ringer's.

972 RAPID UPTAKE AND RELEASE OF HORSERADISH PEROXIDASE (HRP) IN MOTOR NERVE TERMINALS OF THE SNAKE. U.J. McMahan* and A. Yee* Dept. Neurobiology, Harvard Medical School, Boston, Mass. 02115 (SPON: S.W. Kuffler).

During transmitter release, synaptic vesicles fuse with the presynaptic plasma membrane and then may be retrieved by endocystosis. One method of studying the recycling process has been to use HRP to mark endocytotic vesicles. We show by light microscopy that HRP can be taken up and then released by motor nerve terminals on electrical stimulation, which is consistent with electron microscopic histochemistry by others. This technique may aid studies of the kinetics and mechanism of transmitter release, and serve as a convenient anatomical method of demonstrating transmitter release sites, particularly in cases where the postsynaptic membrane is not available as a bioassay. We use the thin costocutaneous muscle of the snake, which permits all neuromuscular junctions to be seen in whole mounts and solutions to be changed rapidly. Following nerve stimulation in a bath containing HRP, muscles are fixed and treated by the method of Straus (1964) to demonstrate HRP. For release experiments, after initial loading of the terminal, the preparation is washed to remove extracellular HRP and the nerve restimulated before fixing and staining. Characteristics of HRP uptake are: 1) It is Ca^{++} dependent. 2) It is visible after as few as 600 stimuli (20/sec for 30 sec or 1/sec for 10 min). 3) Little uptake occurs when HRP is added immediately after stimulation, even though HRP is available for uptake within a few sec of addition to the bath. 4) Electron microscopy confirms that much of the HRP is in vesicles shortly after uptake. 5) HRP can be released, following uptake, by further stimulation. A cycle of uptake, washing, and partial unloading of HRP could be completed within 5 min. Thus, the recycling of some synaptic vesicles may be very rapid. (supported by NIH grants NS 02253, NS 05731, and NS 70606)

973 MOUSE MINIATURE END-PLATE POTENTIAL AMPLITUDE HISTOGRAMS SHOW MULTIPLE PEAKS INDICATING SUMMED RELEASE OF SUBUNITS. M. E. Kriebel* and D. R. Matteson* (SPON: J. B. Preston). Dept. Physiol., Upstate Med. Cen., SUNY, Syracuse, N.Y. 13210.

Isolated diaphragms from 10-14 day old mice were slightly stretched against the floor of a controlled temperature bath fixed to the stage of a compound microscope. Saline containing Prostigmin (10^{-7}-10^{-6}g/ml) and tetrodotoxin (2 x 10^{-6}g/ml) was circulated with a bubble lift (95% O_2 - 5% CO_2). Identifiable muscle cells (10-15μ dia) were penetrated near terminal nerve branches with micropipettes (3M KCl, 30-70 megohms) positioned orthogonal to the fiber axis. We found that miniature end-plate potential (MEPP) amplitude histograms showed multiple peaks and that the number of modes was dependent on MEPP frequency. Amplitude vs. time to peak graphs showed that all MEPPs fell on the same curve. We altered MEPP frequency with temperature (30^{o}-42^{o}) and found that low temperatures produced very low MEPP frequencies (8/min) and gave a relatively large percentage of small mode MEPPs (s-mode) (10-20%) and gave the largest major mode (12-14 times the s-mode). We recorded for up to 5 hrs from single cells (32^{o}, 15 MEPPs/min) and found that the mean of the s-mode (0.5 mV) did not noticeably change although the major mode spontaneously decreased to a steady state 8-12 times the s-mode within an hour. At 37^{o} (1 MEPP/sec) the major mode was reduced 6-8 times the s-mode which contains about 5% of the MEPPs. At slightly elevated temperatures (4 MEPPs/sec) the MEPP amplitude histograms have an overall normal shape showing peaks at integer multiples of the s-mode with the major mode 4 to 7 times the s-mode. A more severe temperature challenge which produced showers of MEPPs for several minutes skewed the MEPP histogram to s-MEPPs. During recovery, the skew distribution gave way to a flat distribution showing 3 or 4 modes. We propose that s-MEPPs represent quanta and that the larger MEPPs represent the summed release of s-MEPPs.

974 A STATISTICAL ANALYSIS OF SPONTANEOUS TRANSMITTER RELEASE AT THE INSECT NEUROMUSCULAR JUNCTION. H. M. Washio* and S. T. Inouye* (SPON: H. Kawamura). Lab. Neurophysiol., Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo, Japan.

The miniature excitatory postsynaptic potentials (m.e.p.s.p.s) were recorded intracellularly from the depressor muscles isolated from the metathoracic legs of cockroach, Periplaneta americana. The spontaneous potentials occurred at frequencies ranging from 1.0 to 3.0/sec in the standard saline containing 5mMCa. Most part of sequence of the potential was uniform in time. However, a short burst of high-frequency discharges was occasionally observed. The time intervals between the events and the counts of them were analysed, using a computer program to test for properties of a Poisson process.

A statistical analysis showed that the spontaneous transmitter release approximates a Poisson process at this tissue. This means that the release of each m.e.p.s.p. is independent and cannot be influenced by past or future events. It may be true that the distribution of the intervals does not fit the criteria for a Poisson process when the sequence containing a short burst of high-frequency discharges is selected for the data set. In a statistical analysis of the set, however, it was suggested that the primary process of such a distribution is Poisson which is occasionally contaminated by the burst phase of the release rates.

975 EFFECTS OF HISTRIONICOTOXINS ON THE ENDPLATE POTENTIAL AND ACETYLCHOLINE RESPONSE OF SKELETAL MUSCLE. John M. Sarvey*, Antonio J. Lapa*, Leona M. Masukawa*, Edson X. Albuquerque, John Daly* and Bernhard Witkop*. Dept. of Pharmacology and Experimental Therapeutics, Univ. of Maryland, School of Medicine, Baltimore, MD 21201 and Lab. Chemistry, NIAMDD, NIH, Bethesda, MD 20014.

The derivatives of histrionicotoxin (HTX) in which the hydrocarbon chains are progressively reduced (H_2-iso-HTX, H_4-iso-HTX, H_4-HTX, H_8-HTX and H_{12}-HTX) were studied for their effects on amphibian and mammalian skeletal muscles. These derivatives (70 μM) blocked the neurally evoked twitch of the frog sartorius muscles within 15 minutes after bath application. The time to block was dependent on the degree of reduction of the side chains, greater reduction producing slower onset of block. The endplate potentials (EPPs) of these muscles were also blocked at the same concentration. Since the HTX derivatives (3.5 μM) produced at least 70% blockade of the extrajunctional acetylcholine (ACh) sensitivity in chronically denervated soleus muscles of the rat, it appears that the origin of the block of neuromuscular transmission is postsynaptic. The fully reduced derivative of HTX, H_{12}-HTX (3.5 μM), inhibited the ACh induced contractures in the frog rectus abdominis muscle in a non-competitive manner, whereas d-tubocurarine chloride (2 μM) competitively inhibited this response. It was further shown that H_{12}-HTX (140 μM) did not inhibit the irreversible block of the EPP induced by α-bungarotoxin in the mouse diaphragm muscle (23°C), although d-tubocurarine chloride (14 μM) did. Moreover, all of the HTX derivatives (70 μM) produced a rapid decrease (70-80%) in EPP amplitude when trains were elicited at a stimulus frequency of 20 Hz at the frog neuromuscular junction. A similar effect was seen at the extrajunctional receptors of denervated rat soleus muscle with repetitive (1 Hz) iontophoretic application of ACh in the presence of HTX derivatives. Because HTX does not appear to bind to the ACh receptor itself, the decrease in ACh sensitivity observed here must necessarily be at some stage beyond the ACh receptor interaction site. Of the antimuscarinic agents and local anesthetics studied, we have found that the kinetics of interaction of the histrionicotoxins with the sites controlling ionic conductance at the frog neuromuscular junction resemble most closely those of atropine. It is concluded that the effects of HTX can be extended to its derivatives, and that no qualitative difference in their action can be seen when the hydrocarbon chains are reduced. This indicates that the acetylenic and allenic bonds of the hydrocarbon chains in HTX are not crucial during blockade of neuromuscular transmission. (Supported in part by U.S.P.H.S. Grant NS-12063).

976 CYTOCHEMICAL LOCALIZATION OF ACETYLCHOLINE RECEPTORS BY MEANS OF PEROXIDASE-LABELED α-BUNGAROTOXIN. Thomas L. Lentz, Jean Rosenthal*, and Joseph E. Mazurkiewicz*. Section of Cytology and Department of Pharmacology, Yale University School of Medicine, New Haven, Conn. 06510

The cytochemical localization of acetylcholine (ACh) receptors in newt (Triturus viridescens) triceps and sartorius muscles and mouse diaphragm was investigated with a procedure utilizing α-bungarotoxin (α-Btx) labeled directly with horseradish peroxidase (HRP). Purified α-Btx was conjugated with HRP with a modification of the method of Nakane and Kawaoi (1974, J. Histochem. Cytochem., 22:1084). α-Btx and HRP were reacted in a 1:1 mole ratio and that species of the conjugate mixture corresponding to a MW of ∿48,000 was isolated and used. This fraction should be composed predominantly of conjugate in which one molecule of peroxidase is coupled to one of α-Btx. Muscles were incubated for two hours in the HRP-α-Btx conjugate (∿10^{-6}M), rinsed for two hours, fixed in glutaraldehyde, incubated in hydrogen peroxide and diaminobenzidine for demonstration of peroxidase activity, fixed in osmium tetroxide, and processed for electron microscopy. The conjugate was tested physiologically for retention of activity. It produced a postsynaptic blockade at frog sartorius neuromuscular junction similar to that seen with α-Btx alone, except that considerably longer times were necessary for complete block of spontaneous miniature and evoked end plate potentials.

The product of the peroxidase reaction occurred on the junctional folds of the motor end plate. A uniform layer of reaction product ∿150A thick occurred over the apical portions of the junctional folds. Activity ended abruptly on the lateral portions of the folds and the membranes bordering the bases of the secondary synaptic clefts were unreactive. Non-junctional regions of the sarcolemma were unreactive. Activity was also observed on regions of the axon facing the muscle surface, often including the axolemma overlying the "active zones" of the nerve terminal. Such presynaptic activity was restricted to the plasma membrane of the axon without an overlying layer. The specificity of the presynaptic reaction is under investigation, but it was observed that fingers of Schwann cell cytoplasm which are interposed between reactive pre- and postsynaptic membranes were unreactive. A diffuse staining of the synaptic cleft was sometimes seen in intensely reactive preparations.

A number of controls demonstrated the specificity of the HRP-α-Btx staining procedure. Tissues were incubated in either Ringer's solution alone (to test for endogenous peroxidase), HRP alone (to test for non-specific binding of HRP), or preincubated in α-Btx prior to incubation in the conjugate (to block specific cholinergic binding sites). The tissues were subsequently reacted for peroxidase as described above. In no case was reaction product seen at the pre- and postsynaptic sites reactive after incubation in the HRP-α-Btx conjugate alone.

This technique reveals the localization of postsynaptic ACh receptors at the tips of junctional folds. The localization of activity is consistent with the location of receptors in the sarcolemma from which they project a short distance into the synaptic cleft. In addition, the possible presence of presynaptic receptors within the axolemma is indicated. This procedure represents a simple and convenient method for the high resolution localization of ACh receptors.

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977 CHOLINERGIC SITES IN SKELETAL MUSCLE: PROGRESSIVE CHANGES FOLLOWING DENERVATION. Richard R. Almon and Stanley H. Appel. Dept. Med., Div. Neurol., Duke Univ. Med. Ctr., Durham, N. C. 27710.

The bio-electric response of skeletal muscle to acetylcholine (ACh) is mediated through receptor macromolecules located on the surface membrane. The degree and distribution of sensitivity to ACh (as measured by the bio-electric response of the cells) varies as a function of innervation. Whereas innervated muscle is locally sensitive at the end plate, non-innervated embryonic and denervated muscle has a widely distributed sensitivity over the cell surface. As an approach to understanding the role of the ACh receptors in these sensitivity changes, we have examined the thermodynamics of the interactions of cholinergic ligands with macromolecules in systems derived from rat skeletal muscle. Using this approach, a set of sites in detergent extracted systems from slow and fast rat skeletal muscle has been defined (Biochemistry 13:5522, 1974). This set of sites binds ^{125}I diiodo-α-bungarotoxin (α-Bgtx) with characteristics indicating a homogeneous ligand population interacting with a single set of identical, independent sites. The association constant of this set ranges from 10^8 l/m to 10^9 l/m depending on the source and composition of the system. Equilibrium inhibition studies demonstrate that the set also binds d-tubocurarine, carbamylcholine, and decamethonium. The number of sites in this set is increased 10 to 20 fold in systems derived from 10-day denervated muscle compared to those derived from normal innervated muscle. This set of sites also localizes with surface markers following density gradient centrifugation of muscle membrane fractions (J. Biol. Chem. 249: 6163, 1974). Using serum IgG derived from patients with myasthenia gravis (Science 186:55, 1974), it was demonstrated that antigenic differences exist between the macromolecular receptor complex in systems derived from normal and denervated rat skeletal muscle (Biochim. et Biophys. Acta, 1975, in press). In the present report, cholinergic interactions in systems derived from rat skeletal mixed muscle are detailed. The isotherms of the binding of α-Bgtx over an extended range (10^{-10} M to 10^{-5} toxin) demonstrate the presence of two sets of sites. The first is a high affinity set of sites ($K_a \cong 10^9$ l/mole) corresponding to the set previously described in slow and fast muscle systems. The second is a lower affinity set ($K_a \cong 10^5$ l/mole) in the area of the isotherm not previously studied. Based on interactions and purification with concanavalin A, both these sets of sites appear to be either part of or closely associated with carbohydrate containing macromolecules. Following denervation the number of sites in the high affinity set drops slightly at day 1 (.036 pm/mg) then increases sharply at days 3-7 to a peak at day 8 (1.15 pm/mg) and begins a decline at day $8 > 9 > 10$. Similar results are obtained when sensitivity to the myasthenia antibody is examined. Sensitivity first appears at three days and progresses coincidentally with the increase in extrajunctional sites. The simultaneous appearance of sensitivity to the myasthenic IgG and the extrajunctional sites support our previous conclusion that only extrajunctional sites in these preparations interact with the globulin. In contrast to the high affinity set of sites, the number of sites in the low affinity set increases only about 5 fold following denervation. This increase also begins between day 2 and 3. The definition of this low affinity set of sites remains to be determined.

978 SKELETAL MUSCLE NECROSIS CAUSED BY CHOLINESTERASE INHIBITION: A FUNCTIONAL DISORDER OF THE NEUROMUSCULAR JUNCTION. Lynn Wecker* and Wolf-D. Dettbarn. Dept. Pharmacol., Vanderbilt Univ. Sch. Med., Nashville, TN, 37232.

The organophosphorus cholinesterase (ChE) inhibitor paraoxon (Px, diethyl p-nitrophenyl phosphate) has been found to produce a progressive myopathy in rat skeletal muscle. The earliest stage of lesion development is characterized histologically by heavy trichrome staining of central nuclei with splitting fibers. This progresses to enlargement of central nuclei, more intense splitting and an initial breakdown of fiber architecture. Final stages are characterized by total necrosis and phagocytosis.

There appears to be a marked difference in the susceptibility of muscle types to necrosis induced by Px. The myopathy is more severe in the diaphragm, with 60% tonic, slow fibers, than it is in the soleus with a majority of intermediate fibers or the gastrocnemius, a fast contracting, phasic muscle. Maximum lesions are seen in the diaphragm after 3 days' treatment with Px when 6.5% of all fibers were found to be affected. The number of lesions in the soleus and gastrocnemius at this time was 0.7% and 0.8%, respectively.

In order to understand the possible role of neural influences in this myopathy, the activities of involved enzymes were studied. In the diaphragm, soleus and gastrocnemius muscles, maximal inhibition of ChE activity occurred during the first 30 min after the initial Px treatment. Within 24 hours, diaphragm and soleus ChE had recovered to about 50% of control. The enzyme activity in the gastrocnemius at this time was 67% of control values. Administration of pralidoxime chloride (P-2-AM) to rats, a reactivator of phosphorylated ChE, 10 min after Px, totally prevented lesion formation. When administered 30 min after Px, lesions were present, but significantly reduced in number. It appears that the initial period of ChE inhibition may be critical for initiation of the lesions.

Ultrastructural examination of the diaphragm indicated that the pathology is primarily associated with the motor endplate region, suggesting neural involvement (Laskowski *et al.*, Exp. Neurol., in press). Electrophysiological analysis showed an increase in MEPP frequency and antidromic firing of the phrenic nerve when tested 30 min after *in vivo* Px administration (Laskowski and Dettbarn, JPET, in press). Furthermore, denervation of the left hemidiaphragm of rats was found to significantly decrease the number of lesions as compared to the intact right hemidiaphragm.

It appears that altered neuromuscular function by virtue of ChE inhibition is capable of inducing a myopathy in rat skeletal muscle. (This research was supported by a grant-in-aid from the Muscular Dystrophy Associations of America, Inc. and an NIH grant #12438-01 from the Institute of Neurological Diseases and Stroke.)

979 NERVE TERMINAL PLASTICITY IN NARCINE BRASILIENSIS ELECTRIC ORGAN. A.F. Boyne, Dept. Anatomy, Univ. of Iowa, Iowa City, 52242.

In vivo fatigue in Torpedo marmorata electric organ has been reported to be associated with a 50% loss of the synaptic vesicles (Zimmerman and Whittaker, J. Neurochem., 1973, 22:324) (1). It has also been shown that some of the cholinergic vesicles in another torpedine ray, N.Brasiliensis, have the capacity to bind calcium (Ca) during fixation so that an electron dense structure (EDS) is seen in vesicles in the final thin sections (Boyne, Bohan, Williams, J.Cell Biol., 1974, 63:780) (2). The fate of the vesicles with Ca EDS has now been examined in N.Brasiliensis electric organ fatigued by stimulation in vivo. Chemical analysis of vesicle bound acetylcholine (ACh) and ATP confirmed that these are reduced by 78 to 85% at the point of fatigue (n=4). Morphometric analysis was carried out on 3 stacks of electrocytes in each organ. The number of vesicles observed per micron length of electrocyte dropped to 51% of control levels ($n=5$; $p<0.01$). Vesicles with Ca-EDS fell to 24% of control levels ($n=4$; $p<0.001$). The EDS of the lost synaptic vesicles was not transferred to the nerve terminal membrane plasma membrane. The number of vesicles forming pentalaminar attachments to the nerve terminal membrane fell to 9.7% of control values ($p<0.001$). These changes are illustrated diagrammatically in Fig. 1. The results are consistent with the hypothesis that vesicular ATP provides the Ca binding site and that only those vesicles with ATP can attach to the terminal membrane so as to sustain neurotransmission.

The lost vesicle membrane was calculated to be equivalent to 0.096 square microns per micron length of electrocyte. Analysis of nerve terminal perimeter and area, cisternal perimeters and membranous aggregates showed that, although fatigue increased each measure, the variability was high and no significant changes were detected. On the other hand, fatigued terminals showed a significant increase (2600%; $p<0.001$) in the length of double, separated profiles of plasma membrane encircling a central region of the terminal cytoplasm. Synaptic vesicles were frequently seen in the central cytoplasm. The plasma membrane involved in these forms accounted for 55% of the lost vesicle membrane. The net increase in all the categories accounted for 95% of the lost vesicle membrane. Possible interpretations of the plasma membrane arrangements in the fatigued terminals are illustrated in Fig. 2; the diagrams represent terminals lying against the ventral face of the electrocyte cells.

Although these results are consistent with eventual exocytosis and incorporation of vesicle membrane into terminal membrane, they neither prove that this is the only mechanism nor that it is the major mechanism of neurosecretion. Further experiments are required to evaluate the relative importance of exocytosis and other possibly more selective secretion mechanisms (2).

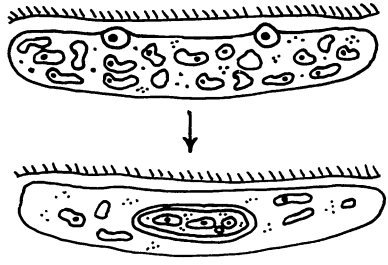

Fig. 1. Diagrammatic representation of fatigue effects on terminal cross sections.

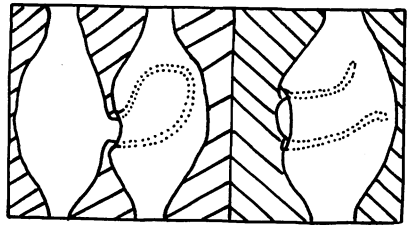

Fig. 2. Possible interpretations of fatigued terminal plasma membrane arrangements.

980 NEUROMUSCULAR TRANSMISSION IN THE DYSTROPHIC MOUSE (129/REJ-dy). Salvatore Carbonetto* (SPON: J.N. Weakly). The Neurobiology Program, Univ. of North Carolina, Chapel Hill, N.C. 27514

Standard electrophysiological techniques were used in an attempt to make a detailed examination of deficits in neuromuscular transmission in dystrophic mice. Such deficits have been suggested by several investigators (e.g., Experientia 15:155, 1974). Intracellular records were made from the EDL muscle of eleven dystrophic (dy/dy) and six normal (?/dy) mice at a well-defined stage of the disease determined by the age of the animals and the degree of motor impairment. Essentially all (98%) of the dystrophic and normal muscle fibers that showed spontaneous m.e.p.p.s. also showed evoked e.p.p.s. Thus, there was no evidence of "functional denervation" in dystrophic muscles. The mean e.p.p. amplitude tended to be larger and the mean quantal content (m) slightly smaller in dystrophic fibers, however these differences were not statistically significant. The m.e.p.p. amplitude and input impedance were larger than in normal muscle fibers. Dystrophic fibers showed a higher frequency of "giant" spontaneous potentials than normal muscles. These giant potentials were three to four times the modal m.e.p.p. amplitude, had prolonged rise times of up to 9 msec. and were not blocked by application of 10^{-6} M. tetrodotoxin. The frequency of m.e.p.p.s. was not different from normal in dystrophic muscles. It is concluded that synaptic efficacy in dystrophic neuromuscular junctions is essentially normal. (Supported by NIMH grant MH11107 and a grant from the Alfred P. Sloan Foundation)

981 A HISTOCHEMICAL CHARACTERIZATION OF REINNERVATED RAT SKELETAL MUSCLE FOLLOWING NERVE CRUSH AT BIRTH. F.M. Sansone[1] and J.J. McArdle.[2] Dept. Anat. Sci.,[1] SUNY, Buffalo, N.Y. 14214 and Dept. of Pharmacol.,[2] CMDNJ, N.J. Med. Sch., Newark, N.J. 07103

Reinnervated extensor digitorum longus(EDL) and soleus(SOL) muscles of the rat examined six months after the sciatic nerve was crushed at birth show morphological and histochemical alterations. The large succinic dehydrogenase(SD)-poor, phosphorylase(Ph)-rich fibers which are found on the surface and scattered throughout the control EDL are absent in the reinnervated EDL, which consists of moderate to deeply SD reactive fibers. Some Ph activity is found in all reinnervated fibers except a few extremely small fibers. All fibers in the control and reinnervated SOL muscles exhibit an intermediate to strong SD activity. Accordingly, Ph activity is moderate or high in only a few of the control and reinnervated SOL fibers. Myosin adenosine triphosphatase(MATPase)-rich fibers predominate in both the control and reinnervated EDLs. MATPase-poor fibers in the control EDL are small and randomly distributed. In contrast, in the reinnervated EDL, the MATPase-poor fibers are intermediate or large in size and appear in clusters throughout the muscle. MATPase-poor fibers predominate in both the control and reinnervated SOLs. There are fewer MATPase-rich fibers in the reinnervated SOL than in the control. (Supported by USPHS, NIH Grant NS11055 and a University of Buffalo Foundation Grant).

982 REINNERVATION OF RAT SKELETAL MUSCLE FOLLOWING NERVE CRUSH AT BIRTH. J.J. McArdle[1] and F.M. Sansone[2]. Dept. of Pharmacol.,[1] CMDNJ, N.J. Med. Sch., Newark, N.J. 07103 and Dept. of Anat. Sci.,[2] SUNY, Buffalo, N.Y. 14214

Miniature end-plate potentials(mepps) could be recorded in the extensor digitorum longus(EDL) as early as 11 days after crushing the sciatic nerve at birth. At 18 days, all of the muscles twitched when stimulated indirectly. Six months after nerve crush at birth, the EDL did not contain muscle spindles and the number of extrafusal fibers was 15% of control. At this time, the mean $\pm$ S.E. of the mepp frequency (22°C) was 1.08 ± 0.09 (N=64) as compared to the control value of 2.41 ± 0.13 (N=57). The mean quantal content of end-plate potentials(m) and the size of the readily releasable pool (32°C) were 62% and 58% of controls, respectively. Increasing $[Ca]_o$ to 5mM increased m and the probability of transmitter release(p) for control EDL, while p alone was increased for experimental EDL. Resting membrane potential, specific membrane resistance, and time characteristics of the action potential were equivalent to control. Electron microscopic examination of reinnervated muscle revealed fibers having structurally normal-appearing neuromuscular junctions and sarcoplasmic reticulum. However, in some fibers, the Z bands were wavy and incomplete in spots so that the myofibrils extended for two sarcomere lengths. (Supported by USPHS, NIH Grant NS 11055 and a University of Buffalo Foundation Grant).

983 EFFECTS OF MEMBRANE-ACTIVE DRUGS ON SEROTONIN TRANSPORT AND BINDING IN RAT SKELETAL MUSCLE: RELATION TO EXPERIMENTAL MYOPATHIES. Stephen M. Stahl & Herbert Y. Meltzer, Departments of Psychiatry and Pharmacological & Physiological Sciences, University of Chicago, Chicago, Ill. 60637.

Recent investigations have shown that serotonin (5HT) in combination with ischemia, tricyclic antidepressants or antihistamines can produce an experimental myopathy in rats which shares some histological characteristics with Duchenne muscular dystrophy. Although the histopathology of the muscle lesions has been characterized, little is known about the biochemical actions of 5HT in this myopathy, or whether 5HT acts upon the muscle or the vasculature.

We have investigated the characteristics of 5HT transport in rat skeletal muscle utilizing the isolated intact extensor digitorum longus (EDL) muscle, or in some cases 2 mm chopped cubes of that muscle. Tissue was incubated in Krebs-phosphate buffer pH 7.4 at 37°C containing 1.25×10^{-5}M nialamide and C^{14}-5HT or C^{14}-urea (10^{-4} - 10^{-6}M) for varying time periods up to 2 hours. Radioactivity was determined in both supernatant and tissue and tissue: medium ratios calculated. Counts represented 95% unmetabolized 5HT. No evidence was found for active transport of 5HT into the EDL preparation either via an Uptake 1 process into presynaptic sympathetic neurons or via an Uptake 2 process into postsynaptic elements such as blood vessels: transport was unaffected by ouabain, lack of sodium, metabolic inhibitors or 5-methoxytryptamine, was not saturable and was dependent upon the external concentration of 5HT. However, C^{14}-5HT transport was temperature sensitive, and C^{14}-5HT tissue accumulation was 2-3 fold greater than that of C^{14}-urea. These results suggest that 5HT transport into the EDL preparation is mediated via passive diffusion into skeletal muscle followed by tissue binding. Both C^{14}-5HT and C^{14}-urea accumulation were enhanced 10-35% by high *in vitro* concentrations (1mM) of tricyclic antidepressants (imipramine, chlorimipramine, or iprindole), chlorpromazine, and procaine, presumably due to increased passive diffusion secondary to the membrane lytic effects of these drugs at high concentrations. C^{14}-urea accumulation was unaffected by low *in vitro* concentrations (1μM) of these drugs whereas C^{14}-5HT accumulation was slightly (10%) inhibited by low *in vitro* concentrations (0.1 μM) of imipramine as well as by 1μM - 1mM chlorpheniramine.

In order to investigate the biochemical nature of the myopathy produced by *in vivo* 5HT plus various membrane-active drugs, we studied the *in vitro* accumulation of C^{14}-5HT and C^{14}-urea in rats pretreated *in vivo* with 25 mg/kg/day chlorpheniramine, 10 mg/kg/day imipramine, or 25 mg/kg/day imipramine I.P. for 3 days and sacrificed on the fourth day. Each of these *in vivo* treatments decreased C^{14}-5HT accumulation by about 10% without affecting C^{14}-urea accumulation. These *in vivo* treatments potentiated the inhibition of C^{14}-5HT accumulation by the same drugs at low concentrations *in vitro*.

In summary, 5HT accumulation into rat skeletal muscle is most likely mediated by passive diffusion followed by tissue binding and not by active transport via an Uptake 1 or 2 process. Certain membrane-active drugs which in combination with 5HT produce a myopathy in rats also inhibit the accumulation of 5HT in the EDL preparation both *in vivo* and *in vitro*. The fact that chlorpheniramine and low concentrations of imipramine inhibit C^{14}-5HT accumulation without affecting C^{14}-urea accumulation is consistent with the possibility that these drugs inhibit 5HT binding without affecting passive diffusion. This inhibition of 5HT binding may play a role in the action of some membrane active drugs in mediating myopathic changes.

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984 ANTI-SARCOPLASMIC RETICULUM ANTIBODIES: DIFFERENTIAL EFFECTS ON SR FUNCTIONAL PROPERTIES. Barry W. Festoff, W. King Engel and N. Bojji Reddy*. Medical Neurology Branch, NIH, Bethesda, Maryland, 20014.

Sarcoplasmic reticulum vesicles (SRV), isolated from human gastrocnemius obtained at time of surgical amputation, efficiently accumulate $^{45}Ca\ Cl_2$. Simultaneously-measured Ca^{++}-Mg^{++}ATPase is present at relatively high specific activity. In addition, an active, SRV-bound adenylate cyclase (AC) is also present and appears distinct from AC activity associated with an enriched fraction of surface membrane sarcolemma. SRV AC is stimulated 30-40 fold by NaF and possesses B-adrenergic receptor properties demonstrated by catecholamine and guanosine trinucleotide stimulation. Antibodies to intact human gastrocnemius SRV were produced in rabbits. IgM and IgG immunoglobulins were produced and at least 3 separate precipitin bands were obtained on immunodiffusion and immunoelectrophoresis. These antibodies were then used in the various functional assays to gain further information on the Ca^{++}-uptake system in SRV.

Incubation of SRV and antisera took place in the cold for 3-4 hours. At a concentration of anti-SRV of 0.5mg/ml, $^{45}Ca^{++}$-uptake was inhibited 60-70%. Simultaneously assayed (utilizing [λ32P-ATP] and Cerenkov radiation) $Ca^{++}Mg^{++}$ATPase, however, was significantly stimulated at the lowest concentration of (Ca^{++}) added (5μM), and plateaued at higher concentrations. At the same antisera concentrations AC activity was inhibited 80-90% compared to normal sera controls. There was no dissociation of the AC inhibition as basal, fluoride and hormone activities were all inhibited proportionately.

This apparent discrepancy between $^{45}Ca^{++}$-uptake and ATP hydrolysis could possibly be explained by an "uncoupling" usually considered to be due to an exaggerated increase in efflux from the SRV (i.e., an enhanced "leakiness"). If this were so one might expect that anti-SRV antibody would be lytic and hence, complement-dependent (Martonosi: BBRC 60: 382, 1974) and in addition, an enhanced efflux should be demonstrable in appropriate experiments. However, heating at 60°C for 45 minutes to destroy complement had no effect on the inhibition of SRV $^{45}Ca^{++}$-uptake, nor did adding guinea pig complement to pre-immune or specific antisera have any additive effect. In addition, in several passive efflux experiments, no increase in "leak" was obtained with anti-SRV, with or without added complement, when compared with buffer or pre-immune sera controls.

Thus it appears that specific binding antibody(ies) to some component(s) of the Ca^{++}-uptake system of these SRV, not dependent on increased SRV "leakiness", have been produced. Using SDS-polyacrylamide electrophoresis of SRV proteins followed by double immunodiffusion in agar we have demonstrated that antibody has been made to the large subunit (phosphorylated intermediate). This appears to enhance rather than inhibit ATP hydrolysis under identical conditions where inhibition of Ca^{++}-uptake occurs. What role, if any, SRV AC, and indirectly, cyclic AMP, have in Ca^{++}-uptake is presently not clear. In separate experiments, an inhibitory effect of cyclic AMP on Ca^{++}-uptake was observed at 1μM in the absence of added protein kinase. Suggestive stimulation of transport was observed when cAMP and kinase were added together though not as great as reported for cardiac SRV (Tada et al.: JBC 249:6174, 1974). The data suggest, however, that the accepted localization of these various functions and their respective roles in the SR membranes may require further examination.

985 THE EFFECT OF EXPERIMENTAL REINNERVATION ON MUSCLE FIBER TYPE DISTRIBUTION AND TERMINAL INNERVATION RATIO. D. H. Beermann, R. G. Cassens, C. Couch, F. Nagle (SPON: S. Kornguth) Muscle Biology Lab., University of Wisconsin, Madison, WI 53706

Reinnervation was studied in 18 pigs following crush of the upper sciatic nerve. Pig muscle has an unique arrangement of fiber types. Type I fibers occur in the middle of the muscle bundle and are surrounded by type II fibers; one or several areas of type I fibers may be present in each bundle. Normal superficial semitendinosus muscle has type II predominance with 1-3 type I fibers per bundle; deep semitendinosus has 10-70 type I fibers per bundle. At 1 week post-crush, stimulation of the sciatic nerve proximal and distal to the crush failed to elicit muscle contraction; marked demyelination of the nerve and persistence of histochemically demonstrated acetyl cholinesterase were also observed. Muscle and nerve samples were taken from 7 to 31 weeks post-crush. Loss of the normal spatial arrangement of fiber types and the presence of large islands of type I and type II fibers were observed in deep semitendinosus following nerve crush. Functional and absolute terminal innervation ratios of normal superficial and deep semitendinosus muscles were approximately 1.00 despite the presence, in normal pig muscle, of "type grouping". Functional and absolute terminal innervation ratios were markedly increased in muscle where fiber type islands occurred. Up to 7 muscle fibers were observed to be innervated by a single subterminal axon. This study further defines the role of neural regulation of histochemical muscle fiber type and provides direct evidence that type grouping is a result of subterminal axon sprouting or branching. (Supported by Muscular Dystrophy Association of America.)

986 EFFECTS OF CORTICOSTEROIDS ON RABBIT MUSCLE EXCITABILITY. Michael Seider* and Raphael Gruener. Department of Physiology, University of Arizona College of Medicine, Tucson, Arizona 85724.

Chronic administration of corticosteroids produces muscle weakness, wasting and type II fiber atrophy. In addition, the membrane potential depolarizes and excitability decreases (Arch. Neurol. 26:181:1972). Muscle weakness is particularly severe in rabbits after steroid administration. Therefore, we injected rabbits with 1.5 mg/kg/day Prednisone (SQ) for 4 weeks. The extensor digitorum longus (EDL) was isolated, mounted in a chamber and continually perfused with a balanced salt solution at pH 7.4 and 35°C. Conventional microelectrode techniques were used to monitor membrane potentials and to elicit action potentials (APs).

The resting potential decreased from a normal of -78.2 ± 1.8 ($\bar{X}$ ± S.E.) (n=200;8) to -69 ± 1.8 mV (n=400;16) in EDLs from treated animals. A shift towards larger MEPP amplitudes was found in experimental muscles, but no significant difference between experimentals and controls was seen in either inter-MEPP interval distribution or mean MEPP frequency.

Spontaneous fibrillation-like potentials, having amplitudes of 2-40 mV were observed in 13/16 muscles from treated animals only. The fibrillation-like spikes were irreversibly blocked by 10^{-6}M tetrodotoxin within a few minutes but were unaffected by 1.5 mg/l tubocurare for 10 minutes. Recovery of regenerative activity, after TTX blockade, was studied as a function of time. Complete AP blockade occurred within 5 minutes in all muscles. Recovery of excitation occurred in less than 15 minutes in normals, but required at least 45 minutes in experimentals.

The data suggest that steroid administration in rabbits causes alterations in the Na^+ conductance channel which result in the appearance of fibrillation-like potentials and a prolonged binding by TTX. Supported by NIH grant (NS-10417 to R.G.) and Training Grant HL-05884.

987 CHLORIDE CONDUCTANCE IN RAT DIAPHRAGM FIBERS. P.T. Palade* and R.L. Barchi, Dept. of Neurol. & Biochem., Sch. of Med., U. of Pa., Phila., Pa. 19174

Membrane conductances were determined in single fibers of rat diaphragm at 35°C using a 2-electrode technique. Hyperpolarizing constant current pulses were injected via a citrate-filled microelectrode, and electrotonic membrane responses recorded at three points along each fiber using a KCL-filled microelectrode. Distance measurements were determined with a calibrated ocular micrometer. Fibers were rejected for resting potential (RP) below 50 mv at any time, or for ΔRP of >10 mv.

Assuming a myoplasmic resistivity of 125 Ω cm, analysis of 832 control fibers from 125 preparations gave the following values for selected parameters; RP = 70.3 ±6.2 mv, specific membrane resistance (R_m) = 366 ±108 Ω cm^2, specific membrane conductance (G_m) = 2.94 ±0.76 mmho/cm^2, diameter (d) = 45.1 ±11μ and space constant (λ) = 0.57 ±.17 mm. Similar analysis of a selected group of fibers (N=201) with RP >75 mv indicated average values of; RP = 78 ±2.8 mv, R_m = 388 ±93 Ω cm^2, G_m = 2.71 ±0.6 mmho/cm^2, d = 47.6 ±8.8μ, and λ = 0.601 ±0.09 mm, confirming the validity of overall fiber selection criteria. In some cases calculated fiber diameters were compared with those determined histologically in the same preparation and these were found to be in good agreement.

Analysis of 74 fibers from 17 preparations in Cl^--free solution (methylsulfate substitution) gave RP = 69.2 ±7.3 mv, R_m = 3196 ±2158 Ω cm^2, G_m = 0.41 ±.20 mmho/cm^2, d = 62.2 ± 17μ, and λ = 1.93 ±0.74 mm. Assuming that $G_m = G_{Cl} + G_K$ in normal Ringers and $G_m = G_K$ in Cl^--free solution, G_{Cl}/G_K = 6.2. Calculations using experimental values of G_{Cl}, RP, $[Cl_o]$, estimated values of $[Cl_i]$ and constant-field assumptions suggest that P_{Cl} = 5.25 x 10^{-5} cm/sec at 35°.

Membrane current-voltage relationships were determined in several solutions, including Rb^+ substituted (K-free) Ringers. Calculated curves for pure Cl^- current (I_{Cl}) were obtained over the range of -50 $< V_m <$ -125 mv. I_{Cl} varied with V_m in a manner predicted by the Goldman constant field equation.

The relationship of G_{Cl} to extracellular pH was studied over the range of pH 4.0 to pH 10.0. In each of 24 experiments performed on separate preparations at 35°, an average of 5 fibers were sampled at pH 7, and at a second test pH in normal and Cl^--free solution. Both G_{Cl} and G_K remained constant between pH 7 & pH 10. G_{Cl} dropped markedly between pH 7.0 (3.3 mmho/cm^2) and pH 4.5 (0.5 mmho/cm^2) suggesting the involvement of a titratable group having a pK near 5.5. G_K rose slightly over this same range.

Analysis of temperature dependence was performed in 7 preparations, sampling 5 fibers at each of 3 temperatures (range 6°-40°) in Cl (+) and Cl (-) solutions. G_{Cl} revealed a definite biphasic variation with temperature, remaining constant between 25° and 40° (Q_{10} = 1.05) and dropping markedly below 25° (Q_{10} = 1.8). G_K varied with an average Q_{10} of 1.1 throughout the range studied.

Several experiments were performed to ascertain effects of certain divalent cations on G_{Cl} and G_K. Raising Ca^{++} to 10 or 20 mM or addition of 1 mM Mn^{++} increased G_{Cl} and reduced G_K slightly; removal of Ca^{++} had little effect. 1 mM Zn^{++} or 0.2 mM Co^{++} had no effect on G_{Cl} but raised resting cation conductance. 0.2 mM UO_2^{++} or 0.05 mM Cu^{++} decreased G_{Cl} and Cu^{++} also increased resting cation conductance, causing depolarization.

Preliminary experiments on glycerol-treated (detubulated) fibers suggest that chloride conductance is a characteristic of both surface and T-tubular membranes in this preparation.

988 JUNCTIONAL AND NON-JUNCTIONAL MEMBRANE ULTRASTRUCTURE OF FAST AND SLOW TWITCH MUSCLE. Mark H. Ellisman* and John E. Rash* (SPON: S.K.Sharpless) Dept. of M.C.D.Biol., U. of Colo., Boulder and U. of Md. Med Sch., Balt.

The neuromuscular junctions and extra-junctional excitable membranes of rat fast twitch extensor digitorum longus (EDL) and slow twitch soleus muscle fibers are compared by freeze-fracture, conventional, and high voltage electron microscopy. Thin sections confirm the existence of morphological differences between synaptic complexes of fast and slow twitch fibers. However, in freeze-fracture preparations, no fiber-specific distinctions can be made between the EDL and soleus junctional specializations associated with transmitter release or ACh receptivity. Both fiber types reveal similar paired doublet rows of 100 A particles which are associated with synaptic vesicle-fusion. Furthermore, both fiber types reveal similar numbers and distributions of the 110-140 A particles thought to represent ACh receptor complexes. It is hypothesized that this arrangement of putative receptor complexes in irregular rows may be dictated by a network of 100 A filaments and ladder-like bridges observed in high voltage stereo micrographs of the juxtaneural portions of the folds. Thus, the differentiation of fast and slow twitch fibers is not reflected in the micromorphology of their synaptic membranes. However, differences are observed in the macromolecular organization of their non-junctional electrically excitable sarcolemmas. All EDL fibers are characterized by specific membrane markers--orthogonal arrays of 60 A particles which occur at densities of 5-40/μm^2. In contrast, slow twitch soleus fibers usually fail to exhibit these specializations in 90% of fibers, revealing them at low density in the remaining 10%. Thus, differences in the macromolecular architecture of the membranes of fast and slow twitch fibers occur in the electrically excitable non-junctional sarcolemma but not in the synaptic membranes.

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989 ISOMETRIC MUSCLE PERFORMANCE IN MEDIAL GASTROCNEMIUS MOTOR UNITS OF THE CAT. J. Young and F. Zajac. Elec. Eng. Dept., Univ. of Maryland, College Park, Maryland 20742.

It was previously reported by us (4th Annual Meeting, Soc. for Neuroscience) that short stimulus trains ($\leq$ 4 pulses) to single units must contain an initial "doublet" (2 pulses with a short interpulse interval; $\leq$10 msec) with subsequent longer intervals to maximize tension area, or the average active state of the muscle unit. Discharge bursts from many cat extensor motor units during slow walking contain many more than 4 pulses occurring at rates presumably less than fusion (Zajac and Young, this meeting). We have therefore studied the tension output characteristics in units to long pulse trains.

Defining T_n as the interval between the n^{th} and $n+1^{th}$ pulses and T_1^*, $T_2^*, \ldots, T_n^*$ as the best n intervals in maximizing tension area, all trains of m intervals consisting of $T_1^*, T_2^*, \ldots, T_m^*$ ($m \leq n$) produce near maximum tension area for m+1 pulses. The "optimal" pulse train is always characterized by an initial doublet (10 msec in our study), followed by a transitional period of no more than four intervals to attain a steady state interval T^*. The transitional period decreases for units with higher twitch-tetanus ratios. Lengthening of the first and transitional intervals from the optimal train caused a much more pronounced reduction in area than similar lengthening of steady-state ones. We conclude that the regulation of initial discharge rate is important in controlling tension output and might be critical for maximizing energy efficiency.

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990 ACTION OF CARDIOTOXIN ON FROG SKELETAL MUSCLE AND CULTURED EMBRYONIC CHICK HEART CELLS. Karen Arms, D.Bruce Gray, Stanislaus Lai-Wo Leung* and Daniel McPheeters* Section of Neurobiology and Behavior, Cornell University, Ithaca,N.Y.14853.

Cardiotoxin (CTX), a basic polypeptide fraction from venom of the cobra Naja naja siamensis, was prepared by Sephadex CM-50 fractionation from whole venom. When clumps of embryonic chick heart cells in culture were exposed to 15-20 μg per ml of CTX in Ringer or Tyrode saline solution, most of them stopped beating. Cells cultured from early embryos were more resistant to the toxin than those from older embryos. After 30 min exposure to the toxin, 42% of beating clumps from 4-day embryos were still beating. 32% of clumps cultured from 7-8 day embryos, and only 16% of clumps from 12-13 day embryos continued to beat after this treatment. Electrophysiological recording showed that embryonic heart cells were irreversibly depolarized by the toxin.

CTX at 24 μg per ml in saline solution irreversibly depolarized the membrane of frog sartorius muscle. Neither the presence of $3x10^{-5}$M tetrodotoxin (TTX) nor the absence of Na^+ from the solution bathing the muscle significantly reduced the rate at which CTX depolarized the muscle. When sartorius muscles were exposed to CTX in the presence of 18mM Ca^{2+} or 10mM Mn^{2+}, no depolarization occurred over a 60 min period; if the muscle was then washed and replaced in normal saline solution, it depolarized immediately. If it was washed in saline containing 18mM Ca^{2+}, before it was replaced in normal saline, no depolarization occurred. 10 mM Mg^{2+} was ineffective in preventing depolarization by CTX.

High concentrations of Ca^{2+}, Mn^{2+} and Mg^{2+} preserved cultured heart cells from 7-day chick embryos from the cessation of beating which otherwise occurred in the presence of 24 μg per ml CTX.

Experiments with saline solutions containing ^{22}Na showed that 30 min exposure to CTX caused a 44-73% increase in the intracellular sodium content of 12-day embryonic heart cells. The presence in the incubation mixture of $7x10^{-5}$M TTX completely abolished this increase but did not prevent membrane depolarization of the cells.

The evidence presented here suggests that CTX depolarizes cells by increasing membrane permeability to a number of ions. Depolarization occurs even when sodium permeability is greatly reduced. CTX apparently acts in two stages. Only the second stage of CTX action is irreversible and it is prevented by high concentrations of Ca^{2+}, Mn^{2+} and, in embryonic heart cells but not in frog sartorius, by Mg^{2+}.

991 NEUROTROPHIC REGULATION OF ACETYLCHOLINESTERASE IN REGENERATING SKELETAL MUSCLE. Stephen R. Max, David H. Rifenberick, Edson X. Albuquerque and Mabel Zelle*. Depts. Neurol., Ped., and Pharmacol. and Exper. Therapeut., Univ. of Md., Sch. Med., Baltimore, Md. 21201.

The activities of total cholinesterase (ChE), acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) were measured in homogenates of rat anterior tibial muscles. These muscles underwent degeneration and subsequent regeneration secondary to the intramuscular injection of the myotoxic local anesthetic Marcaine (Bupivacaine) plus hyaluronidase. In the first series of experiments, ChE decreased to 40% of control by the first day after injection of Marcaine. As regeneration progressed in drug-treated muscles, ChE exhibited an "overshoot" and increased to 130% of control by the 7th day. By day 19 AChE returned to the control level (Max and Rifenberick, J. Neurochem., 24:771, 1975). To determine whether the increase in ChE is subject to neural regulation, we measured the activities of AChE and BuChE in Marcaine-treated muscles after denervation, and after application to the sciatic nerve of a silastic cuff containing 0.1% (w/w) vinblastine. This procedure, which suppresses axoplasmic flow, causes the development of some signs of denervation, such as membrane depolarization, extrajunctional ACh sensitivity, and tetrodotoxin-resistant action potentials, without affecting muscular activity per se (Kauffman et al., Exp. Neurol., 44:404, 1974). Denervation prevented the Marcaine-induced increase in AChE. Chronic application of vinblastine (days 3 and 7) prevented the increase in AChE. Although partial recovery of physiological changes was observed after 14-21 days of application of vinblastine, AChE returned to the control level by day 14. BuChE remained unchanged in these experiments. These results suggest that the production of AChE in regenerating skeletal muscle is subject to neural regulation. (Supported by USPHS Grants NS-11342-01, NS-12063, a grant from the Muscular Dystrophy Association, Inc., and NIH fellowship NS 54205-02.)

992 THE EFFECTS OF DRUGS ON MECHANICAL THRESHOLD IN MYOTONIC AND NORMAL GOAT MUSCLE. Kathleen G. Morgan* and S. H. Bryant. Dept. Pharmacology and Therapeutics, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Threshold of mechanical activation was studied at 38° C in external intercostal fibers from myotonic and normal goats in vitro using the method of Adrian et al. (J.Physiol.204:207,1969). Tetrodotoxin-blocked fibers were depolarized in steps from a holding potential of -90mV by a two-electrode point voltage-clamp. The membrane potential necessary to produce barely visible contractions near the current electrode was recorded for several pulse durations. The resulting potential(strength)-duration curves were analyzed and compared. Under all conditions the threshold membrane potential decreased to a rheobasic value at long durations. The rheobase for myotonic fibers was unexpectedly higher than that for normal fibers, -44±1mV and -58±1mV, respectively. In contrast, the myotonia-inducer, anthracene-9-carboxylic acid, decreased the rheobase in a dose-related manner to as low as -83mV at a concentration of 1.4×10^{-2}M. Normal fibers in a chloride-free sulfate medium more closely resembled the congenitally myotonic fiber in having a similarly elevated rheobase. The uncoupling agent, dantrolene sodium (4.5×10^{-5}M), also raised the rheobase of normal fibers to as high as -30mV; possibly there may be partial uncoupling in the myotonic fiber. The duration-dependent part of each curve was fitted to kinetic models of mechanical activation. In general, the rate constants describing the release of activator were essentially the same in myotonic and normal fibers, and in normal fibers either treated with anthracene-9-carboxylic acid or in a sulfate medium. On the other hand, dantrolene sodium markedly decreased the rate constants of release. Supported by USPHS Grant NS-03178.

993 A REPOLARIZING POTENTIAL TRANSIENT DURING DESENSITIZATION OF FROG SKELETAL MUSCLE IN CHLORIDE-DEFICIENT SOLUTIONS. Terry M. Mikiten. Dept. Physiol., Univ. Tex. Health Sci. Ctr., San Antonio, Texas 78284.

The time-course of desensitization of frog sartorius muscle exposed to carbamylcholine (carb) in vitro was investigated using conventional intracellular microelectrode techniques. Prolonged (15-30 min.) recordings at the endplate regions of single muscle fibers were made after the initial period of mechanical activity. In normal Ringer solution, the return of membrane potential (Em) in the presence of 40-100 μM carb followed a simple exponential time course, often with superimposed potential transients occurring 10-20 min. following carb application.

These experiments were repeated using Ringer solution in which all but 3.6 mM of the extracellular chloride was replaced by either K-methylsulfate/Na-methylsulfate, or K-methylsulfate/Na-isethionate. After 60-120 min. equilibration in Cl^--deficient solutions, the mean resting Em was 85.3 ± 1.2(S.E.)mV. Addition of 40 μM carb to the bathing solution caused a depolarization of the post-junctional membrane which declined in several distinct phases. The initial phase was roughly linear and slow [dEm/dt=1.7 ± 0.8(S.E.)mV/min]. This was followed by the abrupt onset of a sigmoid-shaped repolarizing potential transient (RPT), 10-30 mV in amplitude [mean=22.9 ± 2.6(S.E.)mV], during which time the repolarizing dEm/dt increased roughly 50-fold. Following this, Em either repolarized slowly along an exponential time course to a plateau level, or was steady. RPT had a threshold-like character; the mean Em at the onset of RPT in 24 experiments was 30.2 ± 2.9(S.E.)mV, while the mean Em at its completion was 52.1 ± 2.9(S.E.)mV. The mean time of onset of RPT in these experiments was 13.3 ± 1.0 min. following application of carb.

Tetrodotoxin (10^{-7}g/ml - 5 x 10^{-7}g/ml) had no effect on either the magnitude or time-course of RPT. Ten-fold reduction of the extracellular Ca^{++} delayed the onset of RPT (mean onset time = 21 ± 2.3 min. after adding carb), reduced the dEm/dt of the initial phase and decreased the magnitude of the RPT excursion (mean=17.8 ± 1.6(S.E.)mV) without altering the Em at the outset. Two-fold elevation of the extracellular Ca^{++} concentration had no effect on the initial slow phase of membrane repolarization, but did affect RPT, increasing dEm/dt: it had the effect of increasing the slope of the relation between dEm/dt and the amplitude of the RPT excursion.

Since RPT was associated with a decrease in effective membrane resistance, it seems possible that RPT arises from a sudden increase in membrane permeability, driving Em in the direction of E_k. Although these experiments do not clarify the exact mechanism underlying RPT, they suggest: (1) that in the normal Ringer solution, the time-course of repolarization in the presence of bath-applied carb may indeed depend on chloride redistribution as suggested by Jenkinson and Terrar (Br. J. Pharmac. 47:363, 1973), (2) smaller voltage transients seen during repolarization in Cl^--containing solutions may be related to RPT, and (3) the possibility that RPT reflects some interaction between cellular Ca^{++} depots and Cl^-. (Supported by USPHS Grant No. NS09560).

994 PHARMACOLOGICAL SPECIFICITY AND THE EFFECTS OF d-TUBOCUARINE ON A DEPOLARIZING DOPAMINE RESPONSE. Paul R. Myers, David Livengood and William Shain
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The pharmacological sensitivity of a depolarizing dopamine response to selected adrenergic and cholinergic antagonists has been investigated using the vertebrate neuronal somatic cell hybrid TCX11, a subclone of NX31.NX31 was derived from the fusion of the neuroblastoma N18TG2 and cells from the sympathetic nervous system of 13 day old mouse embryos. 4 days prior to experiments, TCX11 was grown in the presence of dibutyryl cyclic AMP to stop cell division and promote differentiation of neuronal characteristics.

Standard electrophysiological techniques employing a bridge circuit were used to simultaneously record membrane potential and pass intracellular polarizing current pulses. Iontophoretically applied dopamine elicited a depolarizing, conductance increase response. The reversal potential was determined to be -15mV, suggesting the depolarization resulted from increased conductances to both Na^+ and K^+. Rapid repeated dopamine applications desensitized the receptor. Equal quantities of noradrenaline elicited a much smaller depolarization and also desensitized the membrane to dopamine.

d-Tubocuarine added to the bath reversibly blocked the dopamine response with an I_{50} of $8x10^{-8}$M and total blockade at $4\text{-}5x10^{-7}$M. The cholinergic antagonists hexamethonium(100μM) and α-bungarotoxin(μM) had little or no effect on the response while the dopamine antagonists bulbocapnine and chlorpromazine blocked the response at concentrations greater than 50μM. Iontophoretically applied acetylcholine and 3-methoxytyramine had no effect. Collectively the data demonstrate that the receptor is dopaminergic. These results support the view that antagonism by d-Tubocuarine is not limited to acetylcholine receptors but instead may be selective for other sites common to receptor complexes for a variety of neurotransmitters, possibly those structures mediating specific ion conductances.

995 $PGF_{2\alpha}$(PROSTAGLANDIN $F_{2\alpha}$)INHIBITION OF CORTICAL EVOKED POTENTIALS INDEPENDENT OF LOCAL pO_2. Mary Ann Marrazzi and John H. Daugherty*. Dept. Pharmacology, School of Medicine, Wayne State University, Detroit, Mich. 48201 and University of Missouri Institute of Psychiatry, St. Louis, Mo. 63139

$PGF_{2\alpha}$ is the predominant naturally occurring PG in brain. We have previously shown it inhibits synaptic transmission, using both transcallosally evoked field potentials (Fed. Proc, 33:286, 1974) and intracellular recording from the pericruciate cortex (Fed. Proc, 34:764, 1975). These cerebral effects were not correlated with changes in blood pressure. Others, using angiography and electromagnetic flow meters, have suggested that PGs selectively regulate cerebral blood flow. Nevertheless, we find that the reduction in transcallosally evoked potentials could not be accounted for by reduced oxygen availability. Instead, this actually increases or does not change, as measured by an oxygen electrode adjacent to the field electrode. Furthermore, this $PGF_{2\alpha}$ inhibition of synaptic transmission is the same as we previously showed for norepinephrine (NE) including reduction in field potentials, inhibition of unit firing, membrane hyperpolarization, and increased membrane resistance. Siggins et al. previously showed cerebellar PG-NE antagonism. Such antagonism would suggest localization of PG action at NE receptors. We find $PGF_{2\alpha}$ does antagonize NE reduction of cortical evoked potentials, suggesting a competitive inhibition, in which the weaker PG occupies the site and precludes the similar, more intense NE inhibition. Again, there is no reduction in cortical pO_2. These studies were all carried out with flaxedilized cats in which $PGF_{2\alpha}$ (25-45 μg/kg expressed as the free base but administered as the tromethamine salt) was given close-arterially (intracarotid) while recording potentials transcallosally evoked by submaximal stimuli of 1 per 2 sec. A vehicle blank was without effect. Similarly, arachidonic acid had little or no effect at equivalent concentrations. ($PGF_{2\alpha}$ was generously donated by Upjohn.)

996 EFFECTS OF LEAD, MERCURY AND CADMIUM ON CALCIUM UPTAKE OF PRESYNAPTIC NERVE TERMINALS IN BULLFROG SYMPATHETIC GANGLIA. T. E. Kober* and G. P. Cooper. Dept. Environ. Health, Col. Med., U. Cincinnati, Cincinnati, OH 45267.

Pairs of sympathetic ganglia from bullfrogs (Rana catesbeiana) were bathed in vitro in frog Ringer's solution with and without the addition of $PbCl_2$, $CdCl_2$, $HgCl_2$, d-tubocurarine, or mecamylamine. After a 30 minute equilibration period 10 microcuries of ^{45}Ca was added to the bath. One ganglion was then stimulated supramaximally for 20 minutes at 6/sec., rinsed, and prepared for scintillation counting. Stimulated ganglia in control solution took up 2 to 5 times as much ^{45}Ca as unstimulated ganglia. Mecamylamine and d-tubocurarine did not affect ^{45}Ca uptake nor did antidromic stimulation of the postganglionic nerve, indicating that most of the ^{45}Ca was taken up due to presynaptic nerve activity. These data are in agreement with those of Blaustein (Science 172:391, 1971). $PbCl_2$ (50 μM) blocked not only the 45 Ca uptake related to preganglionic stimulation but also decreased the uptake by unstimulated ganglia. $CdCl_2$ (50 μM) blocked ^{45}Ca uptake in stimulated ganglia but not that of unstimulated ganglia. $HgCl_2$ (250 μM) had the apparently paradoxical effect of decreasing ^{45}Ca uptake by stimulated ganglia and increasing the uptake by unstimulated ganglia. The data support the conclusion that these metals block ganglionic transmission by interfering with the role of calcium in transmitter release. (Supported by EPA contract #68-03-0429 and NIEHS grant #ES-00159).

997 CALCIUM IS ESSENTIAL FOR CHEMICAL SYNAPTIC TRANSMISSION IN THE FROG CEREBELLUM IN VITRO. J. T. Hackett, Dept. Physiol., Univ. of Virginia, Charlottesville, VA. 22901

At chemically operating synapses, Ca^{2+} is required for depolarization-evoked neurosecretion. Sr^{2+} and Ba^{2+} are able to restore synaptic transmission after it has been blocked in low Ca^{2+} solution, whereas Mg^{2+}, Co^{2+}, and Mn^{2+} antagonize Ca-dependent transmitter release. The question is raised whether the two excitatory synaptic inputs to cerebellar Purkinje cells are similarly affected by divalent cations.

All excitatory synapses in the isolated frog cerebellum can be maintained in vitro for about one week (Hackett, Brain Res. 48, 385, 1972). Parallel fiber-Purkinje cell (PF-PC) transmission was increased as Ca^{2+} was raised from 0.125 to 2 mM. The maximum slope of log (PF-PC synaptic field potential) against log $[Ca^{2+}]$ was 1.6. This value is 1.25 to 2.5 times lower than those obtained at the neuromuscular junction (Cooke, Okamoto and Quastel, 1974). Synaptic transmission was not further enhanced by higher concentrations of Ca^{2+} (2 to 8 mM); concentrations lower than 0.125 mM could not be used because these affected the electrical excitability of PC. Mg^{2+}, Sr^{2+}, and Ba^{2+} (0.5 to 5 mM) did not affect PF-PC transmission and did not restore transmission when it was depressed in low Ca^{2+}. Mg^{2+} (5 to 20 mM), Co^{2+} (0.5 to 2 mM), and Mn^{2+} (0.125 to 1 mM) antagonized Ca-dependent PF-PC transmission. Mn^{2+} was the most potent blocker of synaptic transmission. Climbing fiber-Purkinje cell (CF-PC) transmission was also dependent upon Ca^{2+} and was blocked by Mg^{2+}, Co^{2+}, and Mn^{2+}.

Compared with peripheral and other central synapses, synaptic transmission in the frog cerebellum appears to have a less steep Ca^{2+} dose-response curve, and to have a more selective requirement for Ca^{2+}, namely Ca^{2+} can not be substituted by Sr^{2+} or Ba^{2+}.

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998 SPECIFIC DECREASE OF PTP DURATION BY ETHANOL AT A SYNAPSE IN APLYSIA EXHIBITS PROLONGED TOLERANCE. M. Elaine Traynor*, P.B.J. Woodson*, J.P. Tremblay*, W.T. Schlapfer and S.H. Barondes. Depts. Neuroscience and Psychiatry, UCSD, La Jolla, Ca. 92037 and V.A. Hospital, San Diego, Ca. 92161.

The effect of bath application of ethanol on the monosynaptic, unitary and presumably cholinergic EPSP in cell R15, obtained by stimulation of the right visceropleural connective of the abdominal ganglion of Aplysia californica was studied. At concentrations of less than 2% there were no detectable effects. 5% ethanol specifically decreased the duration of post-tetanic potentiation (PTP) subsequent to a train of 100 stimuli at 1/sec, in 10 of 11 animals. The average rate constant of PTP decay was 8.6 (range 2-16) times greater in the presence of ethanol than in the absence of the agent. 5% ethanol did not reduce the amplitude of an isolated EPSP at this synapse, and also had no effect on the size of an iontophoretic ACh potential evoked in the soma of R15. Similarly, neither the synaptic depression observed between a pair of pulses 1 sec apart, nor the frequency facilitation during trains of 100 pulses at 1/sec, was affected by 5% ethanol. With continued exposure to 5% ethanol for 3 hours 5 of 6 preparations developed tolerance to the specific effect of ethanol. In the tolerant state, the rate constant of PTP decay was the same as in the control state. After the development of tolerance the ethanol was removed by perfusing with normal medium. Readministration of 5% ethanol 4 hours later had no effect on the rate constant of PTP decay in all three animals treated in this manner. Two of these preparations were then perfused with normal medium for an additional 8 hours at which time the administration of 5% ethanol was again without effect.

999 CHANGES IN THE PARAMETERS OF A FLOW MODEL OF TRANSMITTER ECONOMICS DURING AND AFTER REPETITIVE STIMULATION AT A PLASTIC SYNAPSE IN APLYSIA CALIFORNICA. P.B.J. Woodson*, W.T. Schlapfer, J.P. Tremblay* and S.H. Barondes. (SPON: W. Wiederholt). Dept. Psychiatry, UCSD, La Jolla, Ca. 92037 and V.A. Hospital, San Diego, Ca. 92161.

Changes in the parameters of a flow model of transmitter economics (Schlapfer et al., this meeting) during and after trains of stimuli were determined for the monosynaptic, unitary and presumably cholinergic EPSP obtained by stimulation of the right visceropleural connective in the cell R15 of the abdominal ganglion of Aplysia californica. The model parameters are: A, the size of the pool of transmitter immediately available for release; F, the fraction of A released by an action potential; M, the rate of transmitter mobilization into A; D, the rate constant of demobilization from A. Interpretation of five observations according to this model led to the development of techniques for determining the magnitudes of the changes in the model parameters: 1) during repetitive stimulation a steady state facilitated EPSP is reached; 2) when stimulation is stopped there is immediately manifest a period of PTP, with no intervening period of post-tetanic depression; 3) with 300 or more pulses at 1/sec the amplitude of the PTP is constant; 4) the PTP decays with a single exponential time course; 5) synaptic depression during the PTP period decays with a similar time course. Using these techniques, we found in 33 animals that during a train of 300 pulses at 1/sec, F increased on the average by a factor of 2.3 (range 1.5-3.4), while M also increased, on the average, by a factor of 5.97 (range 4.38-9.50). The magnitudes of these changes increased with increasing number or frequency of stimuli in the train. Animals with large resting values of F and M (Schlapfer et al., this meeting) had small stimulus dependent changes in these parameters. Among the different animals it was found that animals with large changes in F had large changes in M. A decreased upon 300 stimuli at 1/sec, on the average by a factor of 0.74. After repetitive stimulation, A and M returned quickly (over seconds) to their stable resting values (Schlapfer et al., this meeting), but F was found to decay slowly (over minutes). The rate constant of PTP decay was inversely proportional to both the number and frequency of stimuli in the preceeding train over a fairly wide range and reached an asymptote. We conclude from these findings that the amplitude of frequency facilitation is limited by changes in M, while determined by changes in both F and M, but the amplitude and duration of PTP at and after its peak is determined solely by changes in F. The correlations among the model parameters appear to underly the observation that the magnitudes of all the plasticities of the transmission process at this synapse correlate with each other and with the size of an isolated EPSP.

1000 RESTING VALUES OF THE PARAMETERS OF A FLOW MODEL OF TRANSMITTER ECONOMICS AT A PLASTIC SYNAPSE IN APLYSIA CALIFORNICA. W.T. Schlapfer, P.B.J. Woodson*, J.P. Tremblay* and S.H. Barondes. Dept. Psychiatry, UCSD, La Jolla, Ca. 92037 and V.A. Hospital, San Diego, Ca. 92161

A flow model of transmitter economics has been applied to the quantitative analysis of transmitter release at the monosynaptic, unitary and presumably cholinergic EPSP obtained by stimulation of the right visceropleural connective in cell R15 of the abdominal ganglion of Aplysia californica. The model is based on the assumption that synaptic depression is the result of a transient depletion of the pool of available transmitter. This assumption is grounded in our observations that the magnitude of the depression is an increasing function of the amplitude of the first EPSP of a pair of EPSPs, when the size of the EPSPs is varied by manipulations known to vary transmitter release (Ca^{++}, Mg^{++}, post-tetanic potentiation), but not when the size of the EPSPs is varied postsynaptically (postsynaptic receptor blockers, hyperpolarization level). The model parameters are: A, the size of the pool of transmitter immediately available for release; F, the fraction of the pool A released by an action potential; M, the rate of transmitter mobilization into A; D, the rate constant of demobilization from A; and q, the postsynaptic conversion factor (millivolts per millimoles of transmitter released). An interpretation of the time course of synaptic depression according to this model yields estimates of the resting (equilibrium) values of these parameters. In 33 preparations the following average values and ranges were found: F = 0.209 (0.125-0.309), D = 0.065 sec^{-1} (0.031-0.155), qA = 54 mV (19-148), and qM = 3.5 mV/sec (1.2-9.5). A positive correlation between the magnitude of the synaptic depression (independent of q) and the size of the first of a pair of EPSPs (dependent on q) among many different preparations suggests that q has a narrow distribution (Schlapfer et al., BRAIN RES. 76:267, 1974). In the resting state the parameters were found to have stable values, in accord with the observation that each preparation has a unique amplitude of isolated EPSP. The values of F and A were negatively correlated, i.e., animals with large Fs tend to have small As. The values of F estimated for different preparations correlated with the size of the isolated EPSP, i.e., animals with large EPSPs tend to have large values of F. Increasing the calcium concentration in the bathing medium increased the resting values of both F and A. The increase in A is caused by an increase in the net rate of transmitter mobilization (M - DA) in elevated calcium. The action of calcium on F and on the net rate of transmitter mobilization is similar to the increase of these parameters observed during repetitive stimulation (Woodson et al., this meeting).

1001 EFFECTS OF COOLING ON THE MAUTHNER FIBER - GIANT FIBER SYNAPSE OF THE HATCHETFISH. S.M.Highstein, P.G.Model* & M.V.L. Bennett, Dept. of Neuroscience, Albert Einstein Col. of Med., N.Y., N.Y. 10461.

Hatchetfish were submerged in a temperature controlled Ringer bath. Mauthner fibers, excited by spinal stimulation, and giant fibers were recorded from intracellularly near the synapses in the medulla. 10/sec stimulation for 10 min at 24-26°C depletes Mauthner terminals of vesicles, and PSPs and quantal size are reduced. Small quanta are ascribed to release of incompletely filled vesicles. Many irregular membrane compartments appear in the terminals, which are replaced by vesicles during 1-2 hours rest. 10/sec stimulation while cooling to 12-14°C still causes depletion of vesicles, but there are few internal compartments. Instead the external surface of the terminal is markedly increased by many invaginating whorls of paired parallel membranes with large numbers of attached coated vesicles. We interpret these data as follows: Transmitter is released by exocytosis and addition of vesicle membrane to the external surface. At 24-26°C membrane is rapidly reclaimed by coated vesicles which fuse to form the irregular compartments. New vesicles are formed from the compartments in a process that is slow even at room temperature. At lower temperatures membrane internalization is prevented, because coated vesicles are unable to function normally. At 12-14°C spike, PSP and mPSP duration and synaptic delay are prolonged 2-3 fold. PSP amplitude is greatly reduced at low stimulus rates, probably largely due to reduced quantum number. At 10/sec PSPs are much further reduced and in some cases fail. This reduction occurs after too few stimuli to deplete the total vesicular store, and low temperature may interfere with mobilization.

1002 CHANGES IN AXON TERMINALS OF THE PERFORANT PATH AND IN DENDRITIC SPINES OF THE DENTATE GRANULAR CELLS FOLLOWING STIMULATION OF THE ENTORHINAL AREA. Eva Fifkova and Anthony Van Harreveld. Dept. Psych., Univ. Colo., Boulder, Co. 80302 and Calif. Inst. Technol., Pasadena, Ca. 91109.

A monosynaptic pathway, which originates in the entorhinal area and terminates on dendritic spines of granular cells of the dentate gyrus, exhibits a long lasting potentiation, as was shown electrophysiologically by Bliss and Lømo (J. Physiol., 232:331, 1973). Swelling of dendritic spines and the consequent decrease of electrical resistance of spines was suggested as a possible mechanism of the increased synaptic efficacy in this pathway. Recently we demonstrated such a spine swelling following stimulation of the entorhinal area (Fifkova and Van Harreveld, Anat. Rec. 181:355, 1975; Van Harreveld and Fifkova, Proc. Kon. Ned. Akad. Wet., C, 78:21, 1975). This swelling was observed 2 min after stimulation and persisted for 1 hr. The aim of the presented paper was to investigate as to whether a change occurs also in axon terminals of the stimulated pathway and if so, how does it correlate with the swollen spines. In 14 mice the entorhinal area was stimulated at 30/sec for 30 sec. Stimulated animals were divided into two groups with respect to the period of survival. The short survival group included animals sacrificed 2, 4, 6 and 10 min following stimulation and the long survival group animals sacrificed 30 and 60 min following stimulation. Unstimulated mice (11) with or without sham procedure were used as controls. Since the entorhinal pathway terminates exclusively on dendritic spines of the dentate granular cells, only those axon terminals were measured, which contacted dendritic spines; the dendritic spines in contact with axon terminals were measured as well. The synaptic complexes were measured in the distal third of the dentate molecular layer, in which the lateral entorhinal pathway terminates. In the stimulated group of short survival axon terminals became smaller by 25% than those of the controls. In the stimulated group of long survival axon terminals had returned to values of the controls. Changes in spines followed a different path. In the stimulated short survival group spines became larger by 16% as compared to those in the controls. This difference increased to 21% in the stimulated long survival group. Changes in axon terminals and spines of stimulated preparations were highly significant as compared to the controls. A transient change in synaptic terminals upon stimulation of the entorhinal area seems to induce a more endurable change in dendritic spines contacting these terminals. Swelling of dendritic spines may be caused by glutamate released from axon terminals and axons of the stimulated pathway similar to that described in other neural structures (Wienreich and Hammerschlag, Brain Res., 85:137, 1975). Glutamate released in the vicinity of a spine will make its plasma membrane permeable to Na, which will enter the spine accompanied by Cl and water for electrical and osmotic reasons. As a result of this the spine will swell. The shrinkage of axon terminals following stimulation of the lateral entorhinal path is more difficult to explain, since the loss of glutamate and water (for osmotic reasons) does not seem to be large enough to account for the marked shrinkage of these structures. That the swelling of spines becomes independent of changes of presynaptic terminals suggests a self-perpetuating process in the spine swelling, which could explain the long lasting potentiation observed in this pathway.

1003 SYNAPTIC TRANSMISSION IN AN ACOUSTICO-LATERALIS RECEPTOR. J.H. Teeter* and M.V.L. Bennett (SPON: J. Engel, Jr.). Dept. of Neuroscience, Albert Einstein Col. of Med., N.Y., N.Y. 10461.

Tonic (ampullary) electroreceptors of the transparent catfish (Kryptopterus bicirrhus) signal low voltage low frequency fields by acceleration or deceleration of the resting discharge in afferents of the lateral line nerve. The initial sensory transduction is mediated by neuroepithelial cells that synapse with these fibers. Many data indicate that transmission at this synapse is chemical and that stimuli act directly on presynaptic membrane to modulate a tonic release of transmitter. To investigate the role of divalent ions and putative transmitters, pieces of fin with ampullae were removed from the fish and perfused with various solutions. These preparations remain as sensitive as in situ receptors for at least an hour. Ringer containing 15 mM Mg, 0 mM Ca slows and blocks the resting discharge and synaptically mediated responses to stimuli, but 15 mM Ca, 0 mM Mg does not block the resting discharge and occasionally causes a transient acceleration. The Mg effect is presynaptic since the threshold for direct excitation of the nerve is not affected. L-glutamate (1μM) markedly increases the nerve discharge whether or not Mg has been used to block resting activity. L-aspartate at 0.1 mM evokes a much smaller response. D-glutamate (0.1 mM) and GABA (0.1 mM) produce either no change or a small deceleration. These results support the suggestions that 1) synaptic transmitter release in acousticolateralis receptors is mediated by an influx of Ca as at other chemical synapses and 2) the transmitter at these synapses is L-glutamate or a related compound.

1004 AN ANALYSIS OF SOME EFFECTS PENTYLENETETRAZOL HAS ON THE ISOLATED FROG SPINAL CORD. Robert A. Davidoff. Neurology Service, VA Hosp. and U. of Miami Sch. of Med., Miami, FL

Pentylenetetrazol (PTZ) has been used to induce epileptiform discharges in the mammalian CNS for almost 50 years, but since its precise mode of action on neurons is unknown, clarification of its synaptic effects was sought. Experiments were performed on the isolated frog spinal cord superfused with oxygenated Ringer solution. Electrical responses recorded from dorsal (DR) and ventral (VR) roots revealed the actions of PTZ upon different parts of the spinal cord. The addition of PTZ to the superfusate (10^{-4} to 7×10^{-2}M) consistently depressed monosynaptic and potentiated polysynaptic VR activity evoked by lateral column and DR volleys; spontaneous motoneuron discharges frequently resulted. In the same concentrations PTZ significantly decreased primary afferent depolarization (PAD) measured either by DR terminal excitability testing or by dorsal root potentials generated by stimulation of an adjacent DR (DR-DRP) or a VR (VR-DRP). Concurrent with this, in normal Ringer or in Ringer containing 20mM $MgSO_4$ (which blocks synaptic transmission), PTZ anatagonized the depolarizing effects on DR terminals both of glutamate and of GABA--the transmitter thought responsible for PAD. PTZ altered neither high affinity uptake by cord slices nor K^+-evoked release of [^{3}H]GABA from them. Furthermore, the convulsant did not affect the concentration of GABA in isolated cords. It thus appears that the excitatory action of PTZ upon the spinal cord arises from its ability to block PAD and to antagonize the actions of depolarizing amino acids on DR terminals. The exact mechanism of this antagonism requires further investigation.

1005 EFFECTS OF DIPHENYLBARBITURIC ACID ON NEUROMUSCULAR AND SPINAL CORD FUNCTION IN THE CAT. Anthony Zavadil, III*, Kenneth L. Dretchen and Arthur Raines. Georgetown University, Schools of Med. and Dent., Washington, D.C. 20007

Diphenylbarbituric acid (DPB) is an anticonvulsant which may be viewed as a derivative of both diphenylhydantoin and phenobarbital. In cats anesthetized with 70 mg alpha-chloralose per kg, DPB administered i.v. as the soluble sodium salt in doses of 10-80 mg/kg depresses posttetanic potentiation (PTP) in the cat soleus neuromuscular junction. PTP in this system is produced by high frequency repetitive after-discharges originating in the motor nerve terminals. DPB also suppresses the twitch augmentation produced by physostigmine, which also is due to high frequency repetitive discharges originating in nerve endings. With doses greater than 20 mg/kg DPB enhances twitch strength. Experiments in curarized cats with muscles directly stimulated indicate that this latter effect is due to a direct action on muscle.

In spinal cats DPB depresses mono- and polysynaptic reflex discharges but fails to exhibit any specific PTP suppression. Effects on the two systems examined indicate a prolonged duration of DPB action. Determinations of plasma DPB concentrations by gas-liquid chromatography indicate a prolonged plasma half-life for this agent. Therefore with respect to effects at the neuromuscular junction DPB resembles diphenylhydantoin; however, with respect to influences on spinal cord function, DPB resembles barbiturates. DPB exhibits the prolonged duration of action characteristic of both of these materials.

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1006 BINDING PROPERTIES OF A PRE-SYNAPTIC BUNGAROTOXIN TO RAT BRAIN HOMOGENATES. Mildred A. Donlon*, Gene S. Tobias*, William G. Shain and George N. Catravas*. Neurobiology Dept., Armed Forces Radiobiology Research Institute, Bethesda, Md. 20014.

The β-bungarotoxin proteins in the venom of the banded krait (Bungarus multicinctus) have potential significance in the study of synaptic physiology since they inhibit pre-synaptic transmitter release. We have separated the protein components of the crude venom using carboxymethyl Sephadex C-25 chromatography and have labeled the most basic bungarotoxin protein with ^{125}I in order to examine its binding properties. The molecular weight of this protein, based on analytical centrifugation and amino acid composition analyses, is 11,000 $\pm$ 1,000. The minimum lethal intravenous dose for mice is 120 µg/kg. The radiolabeled toxin exhibits saturable binding with time and with increasing toxin concentration to rat brain homogenates. The binding has an apparent Km of 10^{-9} M, and there are approximately 25 fmoles of toxin bound per mg protein. Binding assays of toxin to subcellular fractions of rat brain have demonstrated non-saturable binding to myelin and saturable binding to both crude mitochondrial and synaptosomal fractions. The toxin binds equally well on a per mg protein basis to re-purified mitochondrial and synaptosomal fractions. At saturation of binding to synaptosomes and mitochondria (~ 20 min), myelin bound 10% of the radioactivity while synaptosomes and mitochondria bound about 45% each (47% and 43%, respectively). Both Ca^{+2} and Mg^{+2} inhibit binding to synaptosomes, with I_{50} values of 0.1 mM and 1.0 mM, respectively. These findings suggest that this β-bungarotoxin may antagonize the role of Ca^{+2} in pre-synaptic transmitter release.

1007 THE EFFECT OF HALOTHANE ON BRAIN ACTOMYOSIN-LIKE PROTEIN AND ON SYNAPTOSOMAL UPTAKE AND RELEASE OF NEUROTRANSMITTERS. John J. Getzow*, William J. Nicklas, C. Mahendran*, and Soll Berl. Dept. Neurology, Mt. Sinai Sch. of Med., C.U.N.Y., New York, New York 10029.

Halothane has been demonstrated to produce muscle relaxation and also inhibition of synaptic transmission. The latter has been suggested to be at the presynaptic site. The proposed role of neural actin, myosin, and calcium-sensitive proteins in neurotransmission (Berl, et al., Science, 179, 1973; Mahendran et al., J. Neurochem., 23, 1974) provides a basis for combining these multiple effects into a cohesive theory. Halothane (1.8 - 9.0mM) caused an inhibition (14-86%) of the MgATPase activity of the actomyosin-like protein (NS) isolated from rat brain synaptosomal preparations. There was a significant but lesser inhibition of CaATPase activity. Halothane in the presence of EGTA 10^{-3}M (with or without excess Ca^{2+}) inhibited the MgATPase activity to a lesser degree than did halothane alone. Halothane inhibition of the MgATPase activity was non-competitive; K_i = 5 to 7mM.

These studies were carried out in glass stoppered test tubes at 37.5°. Halothane was added by saturating various components of the assay system. Halothane concentrations were determined spectrophotometrically after extraction of assay media with hexane and reading at both 210 and 230 nm.

Effects of halothane on the uptake and release of GABA, glutamate, DA, and NE were also studied. At 6mM halothane, nearly complete inhibition of the uptake of GABA and glutamate occurred. At 1.37mM halothane there was a 30% decrease in uptake of GABA; there were significant but lesser effects on uptake of DA, glutamate, and NE.

These effects of halothane on NS ATPase activity and on transmitter uptake and release may play a role in the mechanism of general anesthesia. (Supported in part by NIH Grant NS-11824).

1008 EFFECTS OF ETHANOL ON COLLATERAL INHIBITION OF THE GOLDFISH MAUTHNER CELL. D.S. Faber and M.R. Klee*. Res. Inst. on Alcoholism, Buffalo, N.Y. 14203, and Dept. of Physiol., SUNYAB.

Intra- and extracellular recordings were obtained from the goldfish Mauthner cell (M-cell) before and after addition of 1-2% ethanol to the water respirating the fish. Electrophysiological parameters measured included the M-cell's membrane and action potentials, the collateral inhibition evoked by antidromic stimulation, and the postsynaptic responses to stimulation of the ipsi- and contralateral VIIIth nerves. Low concentrations of ethanol (3-5 μg/mg brain weight, as determined by gas chromatography) specifically blocked, over a period of 1 hr, both components of collateral inhibition, i.e., the initial phase of electrical inhibition and the subsequent chemically mediated IPSP. Only with appreciably higher concentrations was M-cell excitability altered; the safety factor for impulse transmission to the axon hillock was reduced and its action potential failed. Common inhibitory interneurons mediate both collateral and afferent inhibitions of the M-cell. The latter were not affected by ethanol. Therefore, we conclude that the block of collateral inhibition occurs at the excitatory synapses between the M-cell axon collaterals and these interneurons. Additional observations suggest that ethanol acts pre-synaptically at that site. In control recordings, the IPSP fatigues if stimulus frequency is greater than 0.5-1.0/sec. As the ethanol action progresses, fatigue occurs at frequencies as low as 0.1/sec. Furthermore, when the IPSP is abolished at higher frequencies, rest periods of 1-5 min will restore it transiently. In the final stages such rest periods are ineffective and the IPSP is completely abolished. These results are consistent with the interpretation that ethanol acts presynaptically to impair transmitter release. (Supported by NIH Grant No. NS-12132-01.)

1009 SYNAPTIC COUPLING INTO THE PRODUCTION AND DISRUPTABLE STORAGE OF AN ENDURING CHANGE IN NEURONAL RESPONSIVENESS.

Benjamin Libet, Haruo Kobayashi* and Tetsuro Tanaka*. Dept. Physiology, Sch.Med., Univ.California, San Francisco, San Francisco,Calif. 94143.

The rabbit superior cervical ganglion exhibits a unique heterosynaptic interaction in which one presynaptic (dopaminergic) input produces an enduring enhancement in the postsynaptic response (the slow EPSP) to another (cholinergic) input (Libet and Tosaka, Proc.Natl.Acad.Sci. 67, 667, 1970). This modulatory action of dopamine (DA) begins within 30 sec and is retained for hours. The retention, but not the initial enhancement, can be disrupted by a treatment with 50 μM guanosine 3'5'-monophosphate (cyclic GMP) for 8 min. The disruptive effect is relatively specific for cyclic GMP, either dibutyryl or nonbutyryl; 5'-GMP was somewhat effective, while guanosine and cyclic AMP were ineffective. Disruption is fully effective if cyclic GMP is begun up to 4 min after DA, but retention is complete if onset of cyclic GMP is delayed for > 10-15 min. This indicates the existence of a disruptable storage process which produces a nondisruptable form of the neuronal change that is responsible for the enduring enhancement (of the slow muscarinic depolarizing responses to ACh).

Treatment with cyclic AMP (1mM for 6-10 min) could fully reproduce the modulatory action of DA, in its magnitude, duration (hours) and time-dependent disruptability by cyclic GMP. The cyclic AMP action appeared to be a postsynaptic one; it could occur in ganglia already depleted of functionally-releasable DA. This full similarity of the modulatory actions, and the previous findings by the Greengard group (Science 171, 1156 and 174, 1346, 1971; J.Pharmacol. 188, 676, 1974) that DA or suitable orthodromic input can stimulate a substantial production of cyclic AMP, supports our hypothesis that cyclic AMP mediates the intracellular production and storage of the enduring neuronal change induced synaptically by DA.

The DA-modulating action provides a model in which a "memory trace" is initiated in the postsynaptic unit by one synaptic input (DA), and is "read-out" as a specifically enhanced responsiveness to another (ACh) input delivered later. It possesses the additional striking similarities to classical memory phenomena, - an initial "shorter term" change, a disruptable "memory storage process" and an enduring nondisruptable "memory trace". Such a neuronal memory trace would not in itself provide for specificity of engram, but it could provide an enduring molecular alteration in neurons which participate in larger neuronal network complexes, with specificity of an engram provided by the behavior of the whole complex or ensemble.

1010 SYNAPTIC TRANSMISSION WITHOUT ACTION POTENTIALS. Katherine Graubard, Dept. of Biology, Univ. of Calif., San Diego, La Jolla CA 92037

Non-spiking inhibition (NSI), defined as postsynaptic inhibition following presynaptic depolarization without intervening action potentials, has been described for synaptic connections in lobster stomatogastric ganglion (STG) (Maynard 1972, Maynard and Walton, 1975). This study of the EX1 to GM synapse confirms the following findings of Maynard and Walton: 1) there is no evidence of electrical coupling between EX1 and GM neurons; 2) action potentials can not be produced in EX1 by polarizations of the cell soma; 3) depolarization of EX1 causes hyperpolarization from the GM resting potential; 4) depolarization of EX1 reduces the firing rate of GM cells during ongoing activity; and 5) effects 3) and 4) are graded with EX1 voltage.

New findings are: 1) 10^{-7} M TTX abolishes action potential activity in the STG but causes at most a slight reduction in the size of NSI; 2) a depolarizing current step in EX1 produces NSI with a slow rise ($\sim$100 msec) to a peak of inhibition followed by a lower plateau. The NSI lasts for the duration of the depolarizing step (at least 25 sec) and a depolarizing rebound in GM (of up to 1 sec) frequently follows cessation of the EX1 depolarizing step; 3) conditioning EX1 with a small hyperpolarizing pulse has no effect on the test NSI. Conditioning with a large depolarizing pulse causes the test NSI to have a slower rise to peak and a reduced or abolished peak NSI response. A conditioning pulse reduces the test NSI over a time course of at least 4 sec. 4) Following the turn-off of large hyperpolarizing pulses in EX1, the EX1 cell undergoes a depolarizing rebound and the GM cell receives NSI which can last 3 sec. 5) The reversal potential for NSI and for the Interneuron I IPSP in GM are approximately the same. (Supported by USPHS NS10629, NS09222, NSF 39945 and the Alfred P. Sloan Foundation).

1011 FINITE DATA WINDOW DISTORTION IN DIGITAL SPIKE TRAIN SPECTRAL ANALYSIS. Robert J. Peterka*, Dennis P. O'Leary and Arthur C. Sanderson*. Univ. of Pittsburgh, Eye & Ear Hospital and Carnegie-Mellon Univ., Pittsburgh, Pa. 15213.

Digital sampling of spike trains introduces distortion due to finite data records. The French-Holden sampling algorithm (FHA) [Kybernetik 8: 165 (1971)] results in evenly spaced spike train samples for input to Fast Fourier Transform (FFT) algorithms. Care must be taken when the FFT is used in order to avoid distortion of the frequency spectrum due to aliasing. The efficient implementation of the FHA requires a sampling rate equal to twice the specified cutoff frequency. However, distortion in the frequency spectrum occurs due to aliasing since the finite length time signal derived from the spike train contains frequencies which are greater than one half the sampling rate. The extent of this distortion is dependent on (1) the record length of the original pulse train and (2) the proximity of a significant peak in the frequency spectrum to the specified cutoff frequency. The magnitude of the aliasing problem has been quantified for both a regular and a Poisson pulse train, and the implications of these results are demonstrated for sinusoidally modulated spike trains from the vestibular system of an Elasmobranch fish. For example, the integral of the power spectrum outside a specified cutoff frequency of a regular 10 Hz pulse train with record length of 10 sec can be as large as 12% of the integral of one complete cycle of the power spectrum. The aliasing problem can be reduced by (1) the use of data records of adequate length and (2) the application of standard time windowing techniques (e.g., Hanning window) or their equivalent frequency domain representations.

1012 EXPLICIT PERIODIC SOLUTIONS OF A NONLINEAR INTEGRAL EQUATION FOR LATERAL INHIBITION IN THE LIMULUS RETINA. Bernard D. Coleman, Carnegie-Mellon University, Pittsburgh, and George H. Renninger, University of Guelph.

For lateral inhibition in the Limulus retina both the delay τ in onset and the relaxation time δ for decay are independent of the distance between ommatidia. In previous work [Proc. Nat. Acad. Sci. 71, 2887-91 (1974)] we showed that if A, the sum of the inhibitory coefficients, is sufficiently large and if δ can be taken to be zero, then the response of the Limulus retina to a constant and uniform excitation e should be a sustained synchronous oscillation of period 2τ. When the relaxation time δ is not zero, the response r in impulses per second is governed by the integral equation

$$r(t) = m\left(e(t) - \frac{A}{\delta}\int_0^\infty e^{-s/\delta} r(t-\tau-s)\,ds\right), \qquad (1)$$

with m defined by $m(x) = \frac{1}{2}(x + |x|)$. We here show that if A and τ/δ are in appropriate ranges, then, for e held constant, (1) has a non-constant periodic solution which can be written in closed form in terms of elementary functions. This solution shows bursts of activity alternating with rest periods, as do the solutions we previously reported for the limiting case $\delta = 0$. For $\delta > 0$, the period of the oscillations exceeds 2τ and depends more strongly on τ and δ than on either e or A. Some analytical solutions of (1) for e not constant are also presented.

1013 IDENTIFICATION OF NEURAL ASSEMBLIES. George L. Gerstein and K. N. Subramanian*. Dept. of Physiology, School of Medicine, Univ. of Penna., Phila., Penna. 19174

When recording extracellularly from 20-50 neurons simultaneously but separably, it is inefficient to seek functional neural assemblies by cross correlation spike train analysis methods applied to neural pairs. We describe a new algorithm that allows rapid search for related groups of neurons, assuming that approximately correlated firing within such groups is higher than chance levels. The recorded spike trains are sliced into approximately 20 msec successive windows. Counts are made for each neuron's firings N(A), N(B),..., for various joint firings of two neurons N(A,B),..., for various joint firings of three neurons N(A,B,C,),..., etc. We then test whether any joint firing probabilities are significantly different from the product of the individual component firing probabilities. Using a criterion related to the Chi Square distribution, we start from any one neuron, and successively add on, one at a time, those neurons which tend to fire in a related manner. The end result is a Venn diagram which clusters related neurons, but gives no insight into the physiological connectivity involved. Such connectivity must still be assessed by traditional pair cross correlation methods; however, the number of pairs needing detailed calculation has usually been drastically reduced by the clustering algorithm. The sensitivity and accuracy of the neuron clustering algorithm is demonstrated on spike train data generated by computer simulation of several nets involving about 20 neurons. This approach is particularly applicable to experimental designs involving plasticity or several different behavioral states, and should allow study of the dynamic aspects of neuronal assemblies.

Supported by N. I. H. Grant #NS 05606.

1014 THE EFFECT OF CHANGES IN ISI DISTRIBUTION PARAMETERS ON FREQUENCY CODING. R. C. Schreiner*, G. K. Essick* and B. L. Whitsel. Dept. Physiol., Univ. of North Carolina, Chapel Hill, N. C., 27514.

A method has been developed to describe the capacity of neural spike trains to signal stimulus information (assuming a frequency code). The method is based on the amount of overlap in the interspike interval distributions (ISI distributions) obtained at different levels of the response continuum. The ISI distributions are computed from the impulse activity contained within "time slices" - samples of activity taken from periods (tested to be stationary) of the response evoked by adequate stimulation.

The ISI distributions of neurons located in somatic sensory areas I and II in the cerebral cortex of unanesthetized macaques are described (1) by the observed relationship SD = b + m (MI), where b may be equal to, less than, or greater than zero; and (2) by the observed changes in the symmetry of the distributions over a range of firing frequencies.

Assuming a frequency code is in effect, the nature of the SD - MI relationship and the change in the symmetry of the ISI distributions are shown to determine the capacity of somatosensory cortical neurons to signal stimulus information. Application of this approach to data obtained from a population of somatosensory cortical neurons indicates that neurons located in the different cortical laminae operate best at different firing levels.

Supported in part by USPHS Grants NS-10759 and NS-10865.

1015 A COMPUTER SIMULATION OF MECHANISMS UNDERLYING SYNCHRONIZED AND DESYNCHRONIZED ACTIVITY IN THE SEPTAL AREA. T.W. Calvert and J.J. Miller, Kines. Dept., Simon Fraser Univ., and Physiol. Dept., Univ. British Columbia, Vancouver, B.C., Canada.

Electrophysiological studies have suggested that the characteristic synchronized and desynchronized discharge patterns of medial septal cells is a consequence of hippocampal neuronal activity (Miller, J.J. and McLennan, H. Frequency related activity in the septum of the rat, Can. Physiol. 4: 191, 1973 ; McLennan, H. and Miller, J.J. The hippocampal control of neuronal discharges in the septum of the rat, J. Physiol. 237:607-624, 1974). In the present investigation a digital computer simulation has been used to determine the interaction of postulated hippocampal and septal mechanisms involved in the production of the discharge patterns of medial septal neurones.

This simulation is based upon the neuronal circuitry presented in Fig. 1 which closely approximates the pathways demonstrated by anatomical and electrophysiological experiments. There are two loop circuits present between hippocampal pyramidal cells (HP) and medial septal burster cells (MB): (i) a 'direct loop' mediated by medial septal inhibitory neurones (MI) and (ii) an 'indirect loop' mediated by lateral septal relay cells (LR). In addition there are local recurrent feedback loops in the hippocampus and the lateral septum mediated by inhibitory basket neurones (HI) and lateral septal inhibitory neurones (LI). The principal excitatory inputs influencing HP and MB neurones arise from entorhinal (ER) and hypothalamic (HY) regions respectively.

In order to examine the manner in which this circuitry results in oscillatory activity in the septal area attempts were made to mimic the relative cell populations: this simulation uses a pool of 10 HP neurones with 1 HI inhibitor and a pool of 10 LR neurones with 1 LI inhibitor; there is one of each of the MI and MB neurones. The simulation of each cell accounts for spatial and temporal summation of inputs as well as absolute and relative refractory periods. The entorhinal inputs of an independent random, poisson distributed spike train to each of the 10 HP neurones, and the hypothalamic input consists of the same to 1 MB neurone. The strengths of the synaptic connections were adjusted to give realistic firing rates.

The behaviour of the model indicated that the 'direct' loop without the lateral septal cells (LR and LI) connected, could produce a rhythmic bursting activity of MB neurones in the range of 2-10/sec. This frequency was dependent upon the strength of the hypothalamic input to MB neurones. When the 'indirect' loop through the lateral septal circuit was used to replace the MI cell, it was found that bursting in MB could again be evoked but that the conditions for this were critical. Also, the LI has the property of dropping out when LR activation reaches a certain frequency. This has the effect of disinhibiting LR cells and abolishes the bursting in MB. When both the 'direct' and 'indirect' loops were intact, MB neurones exhibit bursting in the range of 4-10/sec. but the bursting alternates with irregular activity every 1.0-1.5 sec. The results of this simulation suggest that the burst pattern of MB neurones is determined by the 'direct' loop mediated via MI neurones whereas the 'indirect' loop, via the LR neurones, has the function of either reinforcing this basic rhythmical activity or producing an irregular firing pattern depending on the level of neuronal activity in the hippocampus.

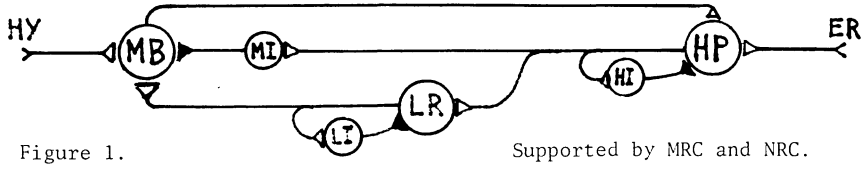

Figure 1.

Supported by MRC and NRC.

1016 ELECTRICAL SYNAPSES AND THE GENERATION OF COMPLEX MOTOR PATTERNS.

Brian Mulloney, Ruben Budelli*, and Donald H. Perkel. Dept. Zoology, Univ. California, Davis, Ca. 95616 and Dept. Biological Sciences, Stanford Univ., Stanford, Ca. 92037.

Many motor patterns which drive cyclic movements involve not only alternating bursts of impulses in antagonistic neurons, but also partially overlapping bursts in other neurons. The fractional overlap of such bursts is nearly constant through a wide range of cycle periods. While analyzing the neural mechanism generating one such motor pattern - the gastric rhythm of the stomatogastric ganglion of Panulirus interruptus - we simulated the known electrical and chemical synapses between neurons in this ganglion which ordinarily fire with such overlapping bursts, using RODNEY[1], a Fortran program which allows us to model networks of neurons using parameters based on physiological data. We find that low-pass electrical synapses of moderate strength (coupling coefficients of 0.1 to 0.4) between two neurons in otherwise independent networks of bursting reciprocally inhibitory neurons[2] will couple the bursts of these two neurons, and so couple the patterns of the two networks. However, the fractional overlap of the bursts changes with period. Unilateral inhibition of one neuron by a neuron of another network will couple the two motor patterns in antiphase; even very weak inhibition will not generate overlapping bursts. An electrical synapse in parallel with a weak chemical inhibitory synapse which becomes depressed during a train of impulses will couple the bursts of two neurons in different networks; the bursts of these neurons overlap by a nearly constant fraction through a wide range of periods. This is the pattern observed in stomatogastric neurons with this synaptic organization during spontaneous gastric rhythms.

Supported by NIH grant NS12295 to BM and NIH grant NS09744 to DHP.

[1]Perkel, D.H. and R. Budelli. In preparation.
[2]Perkel, D.H. and B. Mulloney. Science 185: 181-183 (1974).

1017 EXPERIMENTAL AND SIMULATED INPUT-OUTPUT RELATIONS FOR THE DORSAL COLUMN NUCLEI. D.Whitehorn,H.B.Lipson*,J.P.Walsh*,A.B. Campbell* and M.C. Mackey*
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The relationship between activity reaching a neural population(input) and that leaving (output) defines a functional state for the population. The input-output (IO) relations may also reflect the internal structure of the population. We have measured the IO relations for the dorsal column nuclei of cat using integrated response activity as the primary measure. Computer simulation was helpful both in defining parameters of population structure and function and determining their influence upon IO form.

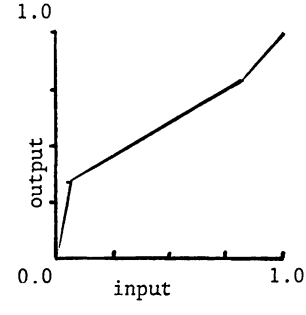
Experimental IO plots were obtained by applying brief electrical stimuli of varying intensity to the dorsal columns at C1. Area under the evoked potential in the contral. medial lemniscus was taken as output and area under the antidromic potential in ipsilat. cutaneous nerves as input. Most IO plots (figure 1) displayed two (or sometimes three) linear regions. In the first, output rose to 0.3 max. as input increased from 0.01 (threshold) to 0.1 max. In the second, output rose more slowly with increasing input.

Computer simulation of transmission within the nuclei involved relating activity in ten output groups to that of ten input groups. Each group represented fibers of a different specified diameter range. The level of activity in each output group at time t was determined by equation 1. Normalization yielded the fraction of each output group which was active. From this value and the number of fibers in each group, the total evoked potential for input and output was simulated (Gasser and Grundfest, AJP, 127:393:1939).

Parameters requiring definition in the simulation are: fiber size distributions for input and output tracts; order of recruitment of input groups; connection rules between input and output groups; magnitude of impact (c in eq.1) as a function of input groups; and excitability time constant (d in eq. 1). Fiber distributions (Lemniscus: Tsumoto,Ex.Br.Res. 21: 211:74; Nerve:Ekholm,Acta Phys. Scand.Sup. 297:1967) were held constant while the influence of the other parameters on IO form was examined.

With input recruitment ordered from large to small,connections established between input and output groups of equal fiber size and impact proportional to input fiber size, simulated IO plots closely matching the experimental (Fig.1) were obtained. Assuming impact equal for all input groups only slightly altered the IO form, while assumptions relating impact to the number of fibers in input or output groups produced dramatically different IO plots. Randomization of recruitment or connections yielded an IO plot linear over the entire range. Simulated IO relations were insensitive to changes in the excitability time constant. The results indicate the usefulness of IO analysis and population models in identification and quantification of neural population parameters. (Supported by NS09472, NINDS).

Fig.1: Form of IO plots (norm.) obtained experimentally and with best fit simulation.
X-axis:Fraction max. input area.
Y-axis:Fraction max.output area.



Equation 1:

$$\text{Output}(t)=\sum_{t_n} c(t-t_n)\,\text{Exp}(d(t-t_n)^2)$$

where $t-10 \leq t_n < t$ and the appropriate input is active at t_n.

1018 APPLICATIONS OF COMPUTER NETWORKING IN NEUROSCIENCE. Thelma Estrin, Robert J. Sclabassi*, and Richard Buchness*. Data Processing Laboratory Brain Research Institute, UCLA, Los Angeles, California, 90024.

Our Data Processing Laboratory has interconnected three available computers, a DEC PDP-12, an IMLAC PDS-1D, and an IBM 360-91, to form a network of distributed processors for interactive graphics programs. Two neuroscience applications using this system will be described.

This netowrk utilizes the strengths of each of the processors. The PDP-12 laboratory computer is well-suited for acquisition and preprocessing of neurophysiological data and for the generation of edited data sets. The IMLAC graphics terminal, with keyboard and light pen, provides a control processor for the transmission of edited data sets from the PDP-12 to the large scale IBM 360-91, as well as being an interactive display terminal for the PDP-12 or the 360-91. The 360-91 provides the most efficient processor for performing complex calculations on large volumes of data, and for storing data in a common base, accessible to all users of its time-sharing system.

A simulation program, called BRAINMAPS, designed to aid the user of a stereotaxic device, utilizes this network. Cross-sectional brain maps which have been digitized from a standard atlas and stored on PDP-12 or 360-91 disc can be displayed on the IMLAC terminal. The terminal user selects a map for display, specifies a target location, and enters angles of approach by the probe in the stereotaxic device; projection of the probe on the chosen map is then displayed. Various approach paths to the target can be simulated, the structures encountered along them examined, and the most favorable approach to a target from a point on the skull selected.

The program also includes subroutines to: scale the standard atlas based on body weight, calculate the length of probe required, and retrieve brain structure information. Large and complex data bases can be linked to the anatomical region from which they are derived. Stimuli or response records obtained at different times and from different animals can be combined for display.

A second utilization of the network is by a modeling and analysis program called NEURAL, which tests hypotheses about spike train data. The network allows interspike intervals of a spike train to be acquired by the PDP-12 and transmitted as edited data sets to the 360-91, where a large number of analyses routines for calculating statistical measures of spike trains are resident.

NEURAL has a modeling phase in which a network structure and initial parameters are specified from the terminal, and a computer simulation with appropriate cell parameters and synaptic connections is then generated. The model produces an output spike train which is also characterized by statistical measures of its simulated interspike interval distributions. The program includes a parameter optimization algorithm for adjusting structural parameters of model cells so that error between a simulated data set and experimental data set is minimized. The program also includes routines to test the hypothesis that the model generated data and the experimental interspike intervals are drawn from the same underlying distribution.

NEURAL illustrates the feasibility of creating and storing files of spike train data for common access by any user of the time-shared computer in which this data base is stored. If this computer is part of a national computer network, collaboration among laboratories in the acquisition, modeling, and analysis of neurophysiological data would be possible.

This work is supported by NINDS Grant No. NS 02501. Computing assistance was also obtained from the Health Sciences Computing Facility, UCLA, supported by NIH Special Research Resources Grant RR-3.

1019 PATTERNS OF INTERNEURAL CONNECTIVITY IN RELATION TO DIFFERENT CONDITIONS OF NEURAL ACTIVITY. - P. Anninos*, S. Zenone, Concordia University, Physics Dept., Montreal, Que.

An experimental study on the effect of the pattern of interneural connectivity is in progress in our laboratories based on our previous theoretical findings. This experimental study consists of the following techniques. 1) We choose certain structures in the brain which may or may not have laminar organization and using intracellular standard techniques we are going to investigate which of these structures exhibit sustained activities according to our previous theoretical work. 2) Then we proceed to do histological studies of such structures. These will consist in freezing the brain structure under study in different conditions of sustained activity and in slicing such structure in thin sections by using our microtome. This will be followed by appropriate staining techniques that will enable us to observe the structures under electron microscope. 3) Finally from these studies we will construct a histogram which will fit with a probability distribution function and calculate the variance and the mean of such distribution. These results will then be compared with our theoretical findings (P. Anninos and R. Elul, Bioph. J. 14, 8, 1974, B. Leake and P. Anninos, Journal of Theoretical Biology, in press, 1975). These results we expect to give us a complete topology of the brain in terms of structure and functions. This information will be very useful to clinical neurophysiologists, as well as to other related disciplines.

The above work is supported by NRC Grant # 240-222.

1020 MECHANISMS RESPONSIBLE FOR THE STATISTICAL PROPERTIES OF THE FIRING PATTERNS IN CLARKE'S COLUMN NEURONS. E. J. Muñoz-Martínez* (SPON: H. Aréchiga). Dept. de Neurociencias. CIEA del IPN. Apartado Postal 14-740. México 14, D. F.

The majority of Clarke's neurons respond with irregular discharges to static stretch of hindlimb muscles. These discharges usually show a strong negative dependency between succesive interspike intervals, i.e., short intervals are followed by long ones and viceversa. This report deals with the neural mechanisms responsible for the statistical parameters of the firing patterns. Intracellular recordings and simple computational techniques were used.

The negative dependency between interspike intervals depends on two factors. 1) The afterhyperpolarization (AHP) which follows the action potential. When the interval between two succesive spikes is shorter than the AHP, the AHP's of both spikes add to each other. This produces a greater afterhyperpolarization than that associated to a single spike and decreases the probability of firing for longer periods. 2) The distribution of amplitudes of the epsp's elicited by muscle afferents. Usually, cell firing is induced by series of large, regularly timed epsp's elicited by a single muscle afferent. The predominant influence of these large epsp's can be detected in the distribution of interspike intervals. In addition, smaller epsp's elicited by other afferents also induce cell firing depending on their temporal coincidence. When cell firing is consistently induced by the large epsp's, there is a negative dependency between intervals. If some of the large epsp's fail to reach the firing voltage, the negative dependency is decreased or abolished.

1021 DETERMINATION OF NEURONAL FEEDBACK PARAMETERS FROM SPIKE TRAIN ANALYSES.
J. F. Fohlmeister*, R. E. Poppele and R. L. Purple. Lab. of Neurophysiol., University of Minnesota, Minneapolis, 55455.

Inhibitory feedback in repetitively firing sensory neurons was studied through a synthesis of neuronal data collection, phenomenological modeling and electronic simulation. Negative feedback phenomena both intrinsic and extrinsic to the impulse producing neuronal membrane were studied experimentally. An electrogenic sodium pump which produces a hyperpolarization in the tonic stretch receptor of the crayfish, and axon collaterals with self-inhibitory synapses on the Limulus eccentric cell were the respective examples chosen for the experimental study. In both cases each nerve impulse produces an inhibition to subsequent impulse production which is characterized by a fixed reduction in stimulus effectiveness. The reduction decays exponentially in time and further sums from impulse to impulse. These cells adapt to step inputs and show a low frequency cutoff in their response to small amplitude sinusoidal drives. The leaky integrator model was used in connection with the low frequency cutoff behavior and a generalization of that model was used to correct errors arising from use of the leaky integrator in determining the adaptive response. A dynamic analysis indicates the appropriateness of the models for their respective applications in isolating feedback parameters. Explicit mathematical derivations have been carried out which relate feedback factors to the parameters of the leaky integrator model under conditions of steady state and sinusoidal drive, and to its generalization for the step stimulus input. The combination of techniques and models then permitted very accurate determination of the magnitude and time course for the neuronal feedback.
Supported by USPHS Grants NS11695 and EY0293.

1022 CEREBRAL CORTICAL RESPONSES TO CEREBELLO-THALAMIC SIGNALS: A MATHEMATICAL APPROXIMATION. Howard J. Wing* and Samuel H.H. Chan. Department of Physiology, Faculty of Medicine, University of Hong Kong, Hong Kong.

An incident stimulus delivered to the cerebellar deep nucleus produces a transient change in the electric field of the nucleus ventralis lateralis of the thalamus and subsequently creates another transient electric dipole in the cerebral cortex, with the negative pole at layer-4 and the positive pole at the cortical surface. This phenomenon is analogous to the temperature (V_n) distribution over a distance R ($R \geqslant 0$) from a focus on a crystal lattice which is heated by a pulse of time-dependent coherent radiation from a ruby laser at intensity I(t), one which can be thermodynamically represented by:

$$V_n = \int_0^{t_n} I(t_n - \lambda) e^{-R^2/t_n} \cdot \frac{d\lambda}{\lambda^{\frac{1}{2}}} \qquad (1)$$

Based on this analogy, the cortical response CORTEX(t_n+L,R,x) at a distance R ($R \geqslant 0$) from a cerebral focal point, at a depth x ($0 \leqslant x \leqslant 500\ \mu$) from the cortical surface and at any time t_n + L, to a thalamic signal THAL(t_n), can be mathematically approximated by the form:

$$\text{CORTEX}(t_n+L,\ x,\ R) = \delta \cos\left(\frac{\pi x}{2x_o}\right) \cdot \int_{t_n-E}^{t_n} \text{THAL}(t_n - \lambda) e^{-R^2/t_n} \cdot \frac{d\lambda}{\lambda^{\frac{1}{2}}} \qquad (2)$$

where L is the latency lapsed between the onset of cortical response and the onset of thalamic signal, E is the effective range ($E \geqslant L$) during which the cortex is under significant influence from the thalamus at time t_n, λ is the time variable of integration and δ is the normalization constant.

When reported amplitudes of thalamic signals evoked by a cerebellar stimulation were applied to the equation (2), the experimentally recorded and theoretically computed cortical responses were found to have a tight correlation both in amplitude, time and wave configuration. The best correlation coefficient for 40 corresponding experimental and theoretically generated cortical amplitudes at corresponding times was calculated to be 0.9293.

Computerized optimization of the constants in equation (2), performed by selecting the best correlation coefficient for 40 iteration points in time, between the theoretical and experimental cortical responses, resulted in L=2.6 msec, E=5.3 msec and R=0. The value for latency reasonably corresponds to the thalamo-cortical conduction time, synaptic delays and the conduction time from layer-4 to the cortical surface. The value for effective range can be interpreted that at any time t_n, the response recorded at the cortical surface is a summated field response of a given number of neurons which are under the influence of thalamic activities. Judging from the fact that the active portion of an action potential is within the range of 5-5.5 msec, the computed 5.3 msec is therefore an acceptable time range within which the cortical neurons are under direct thalamic influence. Furthermore, a bell-shaped distribution of the amplitudes of cortical responses, with a maximal signal at R=0 is understandable when any input to the cortex has a focal point of projection.

The similarity between thermodynamic reaction and neural response, as seen in the present mathematical approximation of cortical responses to cerebello-thalamic signals, is striking and suggests further use of general physical equations to describe neuronal phenomena.

1023 STRUCTURE AND ARRANGEMENT OF SYNAPSES IN THE CNS OF THE LEECH UNDER NORMAL AND EXPERIMENTAL CONDITIONS. Juan H. Fernández*, Faculty of Sciences, University of Chile and Dept. of Anatomy, Case Western Reserve University Medical School, Cleveland, Ohio 44106 (Spon: Marcus Singer).

The majority of the synapses in the abdominal nerve cord of the leech Macrobdella decora are confined to the neuropile of metameric ganglia. This is composed of fiber tracts and a central synaptic zone. Axons lying at the inner borders of the tracts make synapses and also send off short spines and beaded side branches which interweave with one another to form the synaptic neuropile. Synapses mostly occur in repetitive clusters formed by one or a few usually large presynaptic fibers profiles and several smaller postsynaptic ones. In this manner, various types of synaptic combinations between the nerve processes are established. They may include divergent, convergent and serial synapses. Specialized junctions between the postsynaptic fibers may be loci for electrical coupling. They have a narrow extracellular space (50Å in diameter) and a regular array of conic particles at the inner leaflet of one of the unit membranes. At the site of a lesion in a longitudinal connective, a new neuropile gradually develops (Fernández and Fernández, Nature 251, 428, '74). This also comprises fiber tracts and a synaptic zone which includes many synaptic clusters bearing a striking structural resemblance to those in the ganglia. The stereotyped character of the synaptic connections and the factors involved in the formation of synaptic clusters are discussed.

1024 SYNAPSES OF SPECIFIC SENSORY AND MOTOR NEURONS IN THE LEECH

Kenneth J. Muller and Uel J. McMahan* Dept. of Embryology, Carnegie Inst. of Wash., Baltimore, MD 21210 and Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Synapses made by functionally identified sensory and motor neurons in leech segmental ganglia have been examined microscopically by using injected horseradish peroxidase as an intracellular marker (Muller & McMahan, Anat. Rec., 1975). Electron micrographs of serial sections through injected processes provide a three-dimensional model of synapses seen throughout the neuropil. Synapses typically include agranular vesicles in the presynaptic element and two or more conjoint postsynaptic processes. The synaptic cleft is widened to approximately 30nm between pre- and postsynaptic membranes. An intracellular band of dense material is situated on the inner surface of the presynaptic membrane along the boundary between conjoint postsynaptic processes. Adjacent to this band vesicles fuse with the presynaptic membrane. Each sensory neuron synapses with hundreds of postsynaptic processes. These synapses occur in clusters along sensory neuron processes. In such regions sensory neurons receive synapses from other cells as well as make them. Each cluster may act independently of the others on the same cell.
(supported in part by USPHS Training Grant NS 05591 (KJM), Career Development Award NS 70606 (UJM) and Program Project Grant NS 02253)

1025 STRUCTURAL PRESYNAPTIC CHANGES FOLLOWING REPETITIVE ACTION POTENTIAL ACTIVITY IN GIANT RETICULOSPINAL AXONS OF LAMPREY. Warren O. Wickelgren, Dept. of Physiology, Univ. of Colo. Sch. Med., Denver, CO 80220.

Spinal cords of adult lamprey were stimulated electrically at 10/sec for periods up to 1 hr at 4-8°C. Intracellular recordings from giant Muller axons (reticulospinal elements) monitored evoked action potentials which followed the stimulation one-to-one. At the end of stimulation the cords were fixed immediately in 3% glutaraldehyde and prepared for transmission electron microscopy. Presynaptic areas from stimulated Muller axons were compared with those from control cords which were treated identically but not stimulated. The number of clear-centered, round vesicles was reduced and the number of coated vesicles increased in stimulated presynaptic areas. There appeared to be an increase in presynaptic "cisternae" in stimulated synapses but their number and appearance were highly variable. It was not possible to determine if there was an increase in presynaptic membrane area in stimulated axons due to a large inherent variability in presynaptic morphology. The large number of coated vesicles seen in contact with the presynaptic membrane in stimulated axons suggests that "vesicle recycling" in these axons is relatively slow under these experimental conditions. These results at vertebrate central nervous system synapses are in substantial agreement with findings at the frog neuromuscular junction (Heuser, J.E. and Reese, T.S., 1973, J. Cell. Biol., 57, 315-344). (Supported by NIH Grants NS-09661 and NS-50295).

1026 DYE DIFFUSION IN THE RABBIT BRAIN. Dennis R. Hafemann and Jerome R. Wujek.* Dept. Biol., Marquette University, Milwaukee, WI 53233

A quantitative study of dye diffusion in the rabbit brain was carried out. Stainless steel cannulas were implanted into the brain. One month after implantation, an aqueous solution of the dye, bromophenol blue, was injected into the brain through the cannulas. The dye was allowed to diffuse in the brain for two hours. Diffusion was stopped by perfusion with formaldehyde and the brain was removed. The brain was frozen and sectioned and the sections were mounted on glass slides. The brain tissue was then scanned photometrically with a Leitz microdensitometer. The data were assumed to describe simple diffusion of the dye in the brain, with no binding or other reactions between the dye and the brain tissue. On this assumption the diffusion coefficient of bromophenol blue in rabbit brain was estimated by the method of least squares to be 4×10^{-8} cm^2/sec.

1027 ULTRASTRUCTURAL CHANGES PRODUCED BY HYPOXIA AND HYPERCAPNIA IN CHEMORECEPTIVE NERVES IN THE RAT CAROTID BODY.

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Hypoxia and hypercapnia increase the firing rate of chemoreceptive axons whose terminals are in the carotid body. In previous selective denervation studies we found that in the rat carotid body sensory nerves end next to (Type I) glomus cells. Our ultrastructural evidence indicates that some regions of sensory nerve endings are presynaptic to glomus cells, some are postsynaptic, and some form reciprocal synapses. Consistent with their being presynaptic structures, sensory nerve endings on glomus cells contain synaptic vesicles. In the present studies we examined the ultrastructure of sensory nerve endings under conditions known to stimulate these endings: we determined the effect of hypoxia and hypercapnia on the number and distribution of their synaptic vesicles. In rats ventilated with 100% 0_2 for 20 min the concentration of synaptic vesicles in sensory nerve endings was 18.1 $\pm$ 1.3 (mean $\pm$ S.E.M.) vesicles μm^{-2} of section (approximately 70 nm thick). The concentration of synaptic vesicles for rats ventilated with air was similar. However, after ventilation with 10% 0_2 for 10 min, the concentration of synaptic vesicles in sensory nerve endings was reduced by 16% compared to the value for 100% 0_2. This reduction is statistically significant. After ventilation with 100% N_2 for 6 min, the reduction was 48%. Ventilation with 10% $C0_2$ for 10 minutes reduced the concentration of synaptic vesicles by 27% even though the 0_2 concentration of the inspired gas was the same as that of air. The reduction in synaptic vesicle concentration was reversed by restoring $Pa0_2$ and $PaC0_2$ to normal.

Hypoxia and hypercapnia also changed the distribution of synaptic vesicles in sensory nerve endings. Synaptic vesicles were most densely packed in rats ventilated with 100% 0_2 (73.4 $\pm$ 4.7 (mean $\pm$ S.E.M.) vesicles μm^{-2} of section in the region 0.25 μm^2 in area in which vesicles were most densely packed). Compared to this value, the maximal packing density of synaptic vesicles was 15% less for rats ventilated with air, 30% less for those ventilated with 10% 0_2 for 10 min, 37% less for those ventilated with 10% $C0_2$ in 20% 0_2 for 10 min, and 54% less for those ventilated with 100% N_2 for 6 min. Under these conditions similar changes did not occur in preganglionic sympathetic nerve endings on glomus cells, nor in preganglionic nerve endings on ganglion cells, nor in postganglionic sympathetic or parasympathetic nerve endings on blood vessels in the carotid body.

We infer from these data that hypoxia and hypercapnia which cause the increase in activity of chemoreceptive axons also cause the dispersion and depletion of synaptic vesicles in terminals of chemoreceptive axons. Presumably the number of synaptic vesicles is reduced during nerve stimulation when the rate of transmitter release from synaptic vesicles by exocytosis exceeds the rate of new vesicle formation. Synapses interconnecting sensory nerve endings and interneuron-like glomus cells complete a local feedback loop which may modify chemoreceptor sensitivity. (Supported in part by NIH grants HL-06285 and Pulmonary SCOR HL-14201 from the USPHS.)

1028 ULTRASTRUCTURAL CHARACTERIZATION OF SEROTONIN-CONTAINING AXONS IN ADULT RAT CEREBRAL CORTEX. Laurent Descarries and Alain Beaudet*. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada.

Serotonin-containing axons have been identified by means of high resolution radioautography in the fronto-parietal cortex of adult rats pretreated with a monoamine oxidase inhibitor. Prolonged topical superfusion with relatively high concentrations of 5-HT-^{3}H (10^{-5} and 10^{-4} M) allowed the detection of a maximal number of reactive terminals in the upper layers of cortex. In electron microscope radioautographs, the serotonin fibers corresponded to tenuous, unmyelinated processes (0.1-0.5 µ in diameter), bearing small boutons (0.7 µ in mean diameter) spaced at frequent intervals (1-3 µ). These varicosities contained round, agranular "synaptic" vesicles (350-550 Å), as well as large granular vesicles (800-1200 Å). The latter were not necessarily visible in random cross-sectional profiles but a topometric analysis of serial thin sections demonstrated that they were potentially detectable in every 5-HT varicosity. An average number of 7 large granular vesicles per labeled bouton could be extrapolated. Very few serotonin varicosities were engaged in genuine synaptic relationships. Despite extensive sampling, only 5% of the labeled boutons showed junctional complexes, as opposed to 50% of unlabeled nerve endings in the surrounding neuropil. The overall configuration, fine structural features and intercellular relationships of cortical serotonin fibers lead to the suggestion that 5-HT varicosities may be submitted to incessant translocation and/or reshaping.
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1029 SEROTONIN AXONS OF THE SUPRA- AND SUBEPENDYMAL PLEXUSES IN THE VENTRICLES AND SUBARACHNOID SYSTEMS OF RAT AND MONKEY.

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The supra- and subpendymal plexuses in the lateral ventricles, third and fourth ventricles and the aqueduct of Sylvius were studied with light and electron microscopic autoradiography as well as conventional electron microscopy. Tritiated serotonin labelling in these axons demonstrates the presence of active uptake systems for this transmitter. The uptake is enhanced by stimulation in the dorsal and median raphe neurons suggesting that they are the cells of origin of many of these fibers. Control studies with administration of tritiated norepinephrine produced no such labelling. Experiments with treatment of the animal with 5-6 dihydroxytryptamine produced characteristic electron density in the synaptic vesicles of supra- and subependymal axons with their ultimate degeneration. In the same experiment, subsequent labelling with tritiated norepinephrine confirmed that these axons contain an indoleamine, e.g. serotonin, and have no uptake systems for norepinephrine and dopamine. The fine structure of these axons was studied in detail in their various locations in the brains of rats and rhesus monkeys. Their role in assaying and maintenance of the transmitter composition of cerebrospinal fluid, and the significance of this will be discussed.

1030 COMPARATIVE SCANNING ELECTRON MICROSCOPY OF THE AREA POSTREMA IN THE SQUIRREL MONKEY, CAT AND DOG. Peter M. Klara* and Kenneth R. Brizzee. Tulane Univ. Med. Sch., New Orleans, La., 70112 and Delta Regional Primate Research Center, Covington, La., 70433.

Recent transmission electron microscopic (TEM) studies in this laboratory have revealed morphological variation in the area postrema (AP) of several mammalian species. The AP is related to the median eminence as well as the other circumventricular organs; however, the function of the AP remains unclear. In an attempt to elucidate AP function, scanning electron microscopy (SEM) was employed in an attempt to extend the knowledge acquired by TEM.

All animals were sacrificed by intracardiac perfusion with phosphate buffered 1% glutaraldehyde and 1% paraformaldehyde pH 7.3. Specimens were dehydrated through graded ethanols, critically point dried using liquid carbon dioxide and coated with gold-palladium. The specimens were examined with a AMR scanning microscope at 20 KV and 15^{o} tilt.

Morphological similarities between the three species included the absence of kinocilia either partially or completely over the AP surface. This is in contrast to the abundant kinocilia covering the surrounding ventricular floor. Microvilli were obvious on the AP surface of all three species but their numbers and distribution were distinctly different. The squirrel monkey demonstrated scattered microvilli which appeared to be randomly arranged over the surface. Microvilli on the dog AP were less numerous, smaller in length and also randomly positioned. Small patches of long kinocilia were observed on the dog AP surface. This was the only species examined which showed this characteristic. The dog also exhibited depressions of various sizes over the surface of the AP. Sometimes what appeared to be choroid plexus could be seen in these crater-like structures. Again, only the dog possessed this feature. The cat was unique in that the boundary of the AP was sharply delineated by the absence of kinocilia; microvilli were more numerous and seemed to be concentrated at the junction between ependymal cells. This resulted in a distinct hexagonal pattern not seen in other species. Similar patterns, however, have been observed in other CNS areas of known secretory activity. Supraependymal cells were present in all species examined, being more numerous in the dog and squirrel monkey. These cells were stellate in the dog and more globular in the squirrel monkey where they seemed to be concentrated at the lateral margin. This is reminiscent of cells present in the infundibular recess.

From these studies, as well as those of other investigators, it has been shown that mammals which possess an emetic response (eg. human, rhesus monkey, squirrel monkey, dog and cat) share some common morphological features with non-vomiting mammals (eg. rat, rabbit and mouse). Only the cat is significantly different. Whether this morphological difference indicates functions other than (or in addition to) those associated with emesis remains to be determined.

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1031 EXPERIMENTAL COLCHICINE ENCEPHALOPATHY IN DEVELOPING RAT CEREBELLUM. James W. Cosgrove* and M. del Cerro. Center for Brain Research, Univ. of Rochester School of Medicine, Rochester, New York 14642.

Studies on the effects of colchicine on the adult nervous system and on neurons in tissue culture have demonstrated this drug's ability to elicit striking changes in neuronal morphology, particularly on microtubules and microfilaments. In addition, similar effects have been demonstrated in a variety of other cell types. These studies have prompted an investigation of the effects of this drug on the developing rat brain. Colchicine in amounts ranging from 0.5 to 10 µg was injected into the cerebellar cortex of 8 to 10 day old rats and the effects examined from one to 72 hours after injection by light and electron microscopy. The observed effects included 1) the blocking of cell division especially in the external granular layer; 2) the disruption of the endoplasmic reticulum and the Golgi apparatus; 3) a moderate increase in the number of 10 nm neurofilaments with the appearance of some neurofibrillary tangles; 4) an increase in lamellar bodies in astrocyte end feet and their appearance in other cell types; 5) changes in the ultrastructure of the cerebellar growth cones and their subsequent disappearance; 6) changes in the cerebellar vasculature which included formation of perivascular spaces, reactivity of pericytes and disruption of the basement membrane; and 7) the eventual formation of a focus of necrosis and cell lysis.

The data demonstrate that colchicine produces some of the same morphological changes in neurons of the developing rat cerebellum as are seen in neurons in the adult nervous system and in neurons in tissue culture. However, there does appear to be a clear difference in the quantity of neurofibrillary tangles seen in the postnatal developing cerebellum after colchicine treatment as compared to mature neurons similarly treated. This difference could reflect differences in the intracellular pool of the protein(s) that form the cytoplasmic 10 nm filaments, or in the degree of maturation of the assembling mechanism.

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1032 ULTRASTRUCTURE OF INSECT CENTRAL SYNAPSES. Malcolm R. Wood*, Karl H. Pfenninger, Peter C.D. Pelikan*, Melvin J. Cohen. Dept. of Biology and Section of Cell Biology, Yale University, New Haven, Conn. 06520.

In the neuropile of the metathoracic ganglion of the cockroach Periplaneta americana we most frequently find one presynaptic terminal associated with several postsynaptic elements. The presynaptic membrane takes an undulating configuration which follows the convexities of the associated postsynaptic elements. Freeze-fracture reveals that individual synaptic vesicle attachment sites (VAS) are similar to those found in vertebrate synapses. However the distribution of the VAS is irregular in the cockroach and does not resemble the regular hexagonal array seen in central synapses of vertebrates. At one extreme, clusters of VAS are restricted to the crests of the undulating presynaptic membrane directly opposed to a postsynaptic element. At the other extreme, a linear array of VAS is adjacent to a trough in the undulation which opposes the intercellular space between 2 postsynaptic elements. In thin section, bismuth iodide and osmium impregnation reveal 2 configurations of presynaptic membrane densities. There are discrete presynaptic dense projections similar to those found in the vertebrate CNS. These occur on the crests of the presynaptic membrane presumably in the same area as the clusters of VAS. A second dense configuration is a solid band which is seen in only a small proportion of presynaptic terminals. These data suggest the existence of 2 synaptic types in the cockroach CNS. The clustered distribution of VAS and the location of discrete dense projections both resemble the configuration of these components in the vertebrate central synapse. The other type of synapse contains a presynaptic dense band and is perhaps associated with the linear distribution of VAS. This latter synaptic type would then appear similar to that previously described in the vertebrate neuromuscular junction. (Supported by NIH grant NS08996 to M J C)

1033 MORPHOLOGICAL EVIDENCE FOR EXTRAJUNCTIONAL ACETYLCHOLINE RECEPTORS IN TORPEDO ELECTROPLAX. Jack Rosenbluth. Depts. of Physiology & Rehabilitation Medicine, New York University School of Medicine, New York, 10016.

In vertebrate skeletal muscles acetylcholine receptors are normally concentrated in the sarcolemma immediately under axon terminals. An ultrastructural specialization of the postjunctional membrane in this region has been identified consisting of a granular substructure visible in the thickened outer dense lamina of the plasma membrane (JCB 62:755). Since the distribution of this specialization corresponds to that of the receptors and the concentration of the granules corresponds approximately to that of bungarotoxin binding sites in the postjunctional region it has been postulated that the granules visible are the acetylcholine receptors and that this morphologically specialized membrane can be equated to the receptive surface. Examination of thin sections of Torpedo electric organ by the same methods shows that the same specialization occurs in the innervated face of the electrocytes. The outer dense lamina of the plasma membrane of this face is distinctly thickened and ~70A granules can be resolved within it. However, in this case the specialized plasma membrane occurs not only under nerve terminals but extends over the entire innervated face. Postjunctional and extrajunctional portions of the innervated membrane are thus not visibly different from each other and presumably both are rich in receptors. In contrast, the plasma membrane of the noninnervated face is distinctly different in that it does not exhibit this specialization of the outer dense lamina. The cytoplasmic surfaces of the innervated and noninnervated membranes are similar and both are coated with an amorphous dense material into which "decorated" filaments that abound in the cytoplasm insert. The innervated and noninnervated membranes are also similar in replicas of freeze-fractured specimens in that the A faces of both contain high concentrations of large intramembranous particles. (Supported by grants NS07495 and NS09331 from the NIH).

1034 A FREEZE-FRACTURE STUDY OF THE MEMBRANES OF THE NORMAL AND WEAVER MOUSE CEREBELLUM. R.B. Hanna*, A. Hirano* and G.D. Pappas. Albert Einstein College of Med., Bx., NY and Montefiore Hospital, Bx., NY.

It has been generally assumed that a presynaptic input is necessary for the development of dendritic spines. In the cerebellum of the murine mutant "weaver", however, the dendritic spines are characterized as being "unattached" since the synaptic input from the parallel fibers is absent. The spines are surrounded only by astrocytic processes. Electron microscopy of thin sectioned and freeze-fractured preparations indicated that the unattached dendritic spines in the weaver, which have developed in the absence of any presynaptic input, are morphologically identical to the dendritic spines found in the normal littermates. Thin sectioned material exhibits the characteristic densely stained material which is adjacent to the cytoplasmic surface of the postsynaptic membrane in both the weaver and the normal littermate. The outer fracture face (B face) of the normal dendritic spine contains aggregations of 10 nm wide particles in the immediate postsynaptic region. Similar particle aggregations occur in the unattached spines of the weaver.

The freeze-fracture preparations also reveal rectilinear arrays of particles in the glial membranes. These rectilinear arrays, which have a center-to-center spacing of 7nm, are apparently distributed throughout the the astrocyte membrane, including the regions where glial gap junctions are found.

1035 DENDRITIC ATROPHY FOLLOWING DEAFFERENTATION OF NUCLEUS LAMINARIS IN THE CHICKEN: AN EM MORPHOMETRIC ANALYSIS. Francine M. Benes*, Thomas N. Parks and Edwin W Rubel. Depts. of Anatomy and Psychology, Yale University, New Haven, CT. 06520.

Nucleus laminaris (NL) of the chicken brainstem is composed of a monolayer cellular lamina receiving polarized binaural innervation: dorsally from second-order neurons of the ipsilateral nuc. magnocellularis (NM) and ventrally via the crossed dorsal cochlear tract from the contralateral NM. Midline transections of the crossed dorsal cochlear tract produced degenerative changes largely confined to the ventral neuropil of NL. The volume density and the frequency of primary dendrites in dorsal versus ventral neuropil were assessed at the EM level 60 and 96 hours after surgery. By 60 hours, the volume density of dendrite in the ventral area is 52% that of the dorsal and by 96 hours there is approximately a 75% reduction. These results are in contrast to those observed in sham-operated controls, where little difference in volume density of dendrite in dorsal versus ventral neuropil was observed. In each case, the differences were highly statistically significant. Atrophy of dendrites following deafferentation was also evidenced by a 70% reduction over controls in the frequency with which primary dendritic branches continuous with the soma were encountered in the samples. The rapidity and magnitude of the changes, as compared to previous investigations of dendritic atrophy, are probably due to the relatively complete interruption of innervation to the ventral dendrites. The present data suggest that the cytological integrity of dendritic processes can be profoundly compromised by removing their afferent input and that a tonic influence may be exerted by the presynaptic terminal. Supported by USPHS Training Grant 5-T01-CA5055-15 and NSF GB 31934 to EWR. Facilities and support provided by Dr. Russell J.Barrnett.

1036 ORTHOGRADE DEGENERATION OF NERVES INNERVATING MUSCLE SPINDLES. Mark De Santis and Wesley P. Norman*. Dept. Anat., Georgetown University, Washington, D.C. 20007.

Intrafusal muscle fibers of the muscle spindle are discretely innervated by sensory nerve terminals along the equatorial portion of the spindle and by motor nerve endings along the polar length. Degenerative changes following axotomy have been reported for sensory[1] and motor[2] endings of vertebrate spindles. The purposes of the present study are to compare: 1)freezing the nerve as a method of axonotmesis with cutting the nerve (axotomy) to a muscle, and 2)the ultrastructural changes of degenerating sensory with motor nerve terminals.

Spindles in the tenuissimus muscle of adult cats were studied. In eight animals the nerve to the muscle was frozen with dry ice on the left side and cut with a scissors on the right side. Nerve lesions were produced within 1 cm of the nerve entry into the muscle. Survival times were 0.75, 1.25, 2, 3, 4, 5, 6 and 7 days. The effect of a sustained denervation for five and 23 weeks was studied in spindles from two other cats. Spindles from 13 normally innervated tenuissimus muscles were studied for comparison. Tissues were fixed by immersion and processed for transmission electron microscopy.

There is a clear relationship between the location of sensory or motor terminals and the area enclosed by the outer capsule of the muscle spindle. When the cross-sectional area exceeds approximately 9,000 um^2, only sensory terminals are found; when the area is less than this value, only motor endings are seen, if any are present at all.

Freezing and severing the nerve to the muscle result in degeneration of sensory and motor nerve terminals innervating intrafusal fibers. Changes are seen in sensory terminals as early as 18 hours after the lesion and in motor endings by 30 hours after the lesion. No terminals are found five days after either type of lesion. Ultrastructural changes are more obvious in sensory than in motor endings. The most noticeable changes in sensory endings are: mitochondrial swelling and lysis, the presence of large dense structures and multivesicular bodies, and fragmentation of the neurolemma. Neurofibrillar hypertrophy is seen occasionally. There is no clear change in glycogen concentration of these endings. Debris of degenerating sensory terminals appears either to enter the intracapsular space or to be engulfed by the intrafusal muscle. Degenerating motor terminals are characterized by neurofibrillar hypertrophy and glycogen accumulation. Mitochondrial and neurolemmal changes are minimal. Degenerating motor terminals appear to be phagocytized by the overlying Schwann cell. Even five weeks after denervation, junctional gutters are present and surmounted by a cell resembling a Schwann cell.

Degenerative changes in nerve terminals are more pronounced at an earlier stage (two-three days) than those in myelinated nerve fibers within the spindle capsule. Intracapsular myelinated nerve fibers are disrupted at five to six days, a time when no terminals can be found on intrafusal fibers.

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1037 MORPHOLOGICAL CHARACTERIZATION OF ISOLATED BRAIN NUCLEI: DEPENDENCE ON PREPARATIVE TECHNIQUES. Steven J. Smith, Patricia J. McLaughlin* and Ian S. Zagon. Depts. Pharm. and Anat., M.S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033.

In the process of isolating nuclei from rat brain, we have noted the importance of preparative techniques for their identification. Nuclei were isolated from the brains of young male adult rats (180-200 g) by homogenization in 1.4 M sucrose containing 1-5 mM magnesium chloride; the homogenate was layered over a discontinuous gradient of 1.8/2.1/2.4 M sucrose, and centrifuged to yield a 2.4 M fraction (2.4P). After the 2.1/2.4 M interface was removed and adjusted to final sucrose concentration of 1.4 M, it was layered over a discontinuous gradient of 1.8/2.2 M sucrose and centrifuged to yield the 2.2 M fraction (2.2P). Isolated nuclei examined by light (phase contrast and azure C staining) microscopy were extremely condensed, frequently unidentifiable, and did not resemble *in vivo* counterparts. When these nuclei were washed 2 to 5 times with 0.25 M sucrose, a full spectrum of nuclear morphology became visible and identification was possible. Nuclei were round to oval in shape, nucleoli were visible, heterochromatin clumping could be distinguished, and nuclei resembled those in *in vivo* preparations. Continued washing in 0.25 M sucrose resulted in disintegration of nuclei.

Using this procedure, preparations of whole brain (WB), cerebellum (CB), and whole brain minus cerebellum (WB-CB) were compared using the 2.4P. The CB yield was large and almost entirely composed of small, round/oval nuclei, 5-8 μm in diameter, that had a characteristic stippling pattern of heterochromatin. The WB was slightly larger than the CB and comprised of nuclei resembling those in the CB. WB-CB was considerably reduced, with many nuclei identical to those in the WB and CB. Examination of the 2.2P from WB and WB-CB also showed some small nuclei, but their occurrence was less frequent. Small nuclei in the 2.2P and 2.4P were morphologically similar. Evidence established from *in vivo* studies have shown the major portion of cells in the cerebellum to be internal granule neurons and morphological comparison to *in vitro* nuclei was favorable.

In light of these experiments, the nuclear isolation technique of Lovtrup-Rein and McEwen (*J. Cell Biol.* 30:405, 1966) was repeated using WB and WB-CB; nuclei were washed as above. Numerous small nuclei that were identified by earlier investigators as oligodendrocytes and/or microglia, were identical to the nuclei of granule neurons.

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1038 THREE-DIMENSIONAL COMPUTER RECONSTRUCTION OF NERVOUS SYSTEM ELEMENTS. J. Mazziotta*, B. Hamilton and H. Huang*. Dept. Anatomy, Georgetown Univ., Med. Sch., Washington, D.C. 20007

Computer hardware and software are combined in a system that analyzes serial cross-sections of nervous system elements obtained on either an ultrastructural, light microscope, or gross level and generate three-dimensional images on a TV monitor.

Serial sections of the specimen are photographed at a suitable magnification and these standard 35mm negatives are scanned by a computer with the proper input device and stored as numerical data. The program automatically finds the boundaries of predetermined objects of interest in each section, thus eliminating tedious tracing and photographic manipulations. The object outlines are then automatically aligned and stored for future use.

Any plane of section passing through the object can now be displayed on a TV monitor. In addition, the three-dimensional image of the object can be displayed so the viewer may readily visualize the steric configurations and surface contours as viewed from one side. The three-dimensional image may be rotated or tilted to allow visualization of all surfaces.

Not only does the speed of this technique allow for the reconstruction of large numbers of objects but statistical comparisons of many three-dimensional objects can be performed numerically by the computer on objects previously scanned and stored on magnetic tape.

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1039 TRANS-NEURONAL TRANSFER OF RADIOACTIVE MATERIAL IN THE HIPPOCAMPAL FORMATION OF THE RAT. Oswald Steward. Depts. of Neurosurgery and Physiology. Univ. of Va. Sch. Med., Charlottesville, Va., 22901.

The autoradiographic tracing method has been an extremely useful and sensitive tool for the elucidation of projection pathways of the brain, and has led to the discovery of some relatively slight projections which had not been observed with orthograde degeneration methods. In the present study, however, a potential artifact of the method is investigated. Injections of 10μCi of 3H proline into the entorhinal area of the rat result in the labeling of the massive projection system from the entorhinal area to the distal dendrites of the dentate granule cells. However, with a 6 day post-injection survival, silver grains indicative of radioactive material may also be observed in the layer of the granule cell somatae, and in a distinct band in the zone of termination of the granule cell axons, the mossy fibers. Light microscopic and electrophysiological evidence from a variety of sources clearly indicate that this zone of termination of the mossy fibers receives no direct projection from the entorhinal area. Thus, the selective labeling of the terminal field of the mossy fibers must result from trans-neuronal transfer of radioactive material from the entorhinal afferent fibers to the post-synaptic granule cell. Observations in a variety of sites in the hippocampal formation indicate that the radioactive material is taken up by dendrites of both dentate granule cells and hippocampal pyramidal cells, and transported to the somata. However, labeling of the second order synapse has only been observed in the case of the dentate granule cells. Trans-neuronal transfer of radioactive material, which has heretofore only been reported in the retino-geniculate system, may be a characteristic of many synaptic systems in brain, and while the phenomenon may enable the tracing of second order neuronal projections (as in the case of the geniculo-striate system), caution is necessary in the interpretation of relatively minor projections.

1040 NEURAL ARGYROPHILIA INDUCED BY PUROMYCIN. R.C. Switzer. Dept. of Anat., University of Virginia, Charlottesville, Va. 22903.

Puromycin dihydrochloride (Sigma) in 0.9% saline was injected intracerebrally as a potential lesioning agent of perikarya but sparing fibers of passage. Variable length puromycin-peptidyl chains are produced instead of complete proteins. Injections were made with a 30 gauge Hamilton syringe or a micropipette (30-50um tips, pressure or iontophoresis) in rats with survival times of 4h-32d. Sections of the brains were processed with the cupric silver method of de Olmos. The effects in the injection site were primarily dependent on concentration and secondarily, on the rate of injection. A high concentration (0.2ul of 0.2M) yielded a "burned" out center zone. Beyond this zone selected perikarya exhibited intense argyrophilia rendering them as in a Golgi stain; with axon, dendrites and dendritic spines sharply defined. Lower concentrations of the injection (0.2 ul of 0.08M) eliminated the central burned zone. Injection rate seemed optimal at 0.01ul/min. Injections which surrounded Wilson's pencils in the striatum, the anterior limb of the anterior commissure and regions of the external capsule appeared to leave fibers untouched at the light microscopic level. While caudate projections to globus pallidus exhibited intense argyrophilia of terminals, the Nissl appearance of affected caudate perikarya looked nearly normal. Evidence exists suggesting that the transported puromycin-peptidyl products have rendered the neurons argyrophilic. Flexner et.al. (1971, Exp. Neur. 34:223) showed this transport for axonal elements using labeled puromycin. Preliminary results using cycloheximide and Actinomycin-D do not present the same picture as does puromycin. Further development of the use of puromycin in intracerebral injections seems to hold promise as: 1) a directed Golgi stain (on the slide Golgis), 2) a lesioning agent not affecting fibers of passage for tract tracing studies and experimental psychology studies. NIH Fellowship F22 NS02558-01

1041 TRANSPORT OF HRP BY DORSAL ROOT GANGLION CELLS IN THE KITTEN. Eric Proshansky* and M. David Egger. Dept. Anat., Rutgers Med. Sch. - C.M.D.N.J., Piscataway, N.J. 08854

In 1-3 week old kittens, we injected 20% horseradish peroxidase (HRP) solutions into the skin and muscle of the hindfoot, in order to observe whether intact sensory fibers were capable of uptake. Within 36 hours of HRP injections, the ipsilateral dorsal root ganglia, L7 and S1, were found to contain cells labelled with HRP reaction product. No dorsal root ganglion (DRG) contralateral to the injection sites contained labelled cells. With small injections (0.1-0.5 mg HRP), reaction product was visible only within DRG cell bodies. With larger injections (1.0-5.0 mg HRP), reaction product was visible within both the peripherally and centrally directed DRG fibers as a small number of discrete granules. Following injections into individual muscles, we found HRP labelled cells distributed in L7 and S1 dorsal root ganglia without any apparent somatotopic organization. However, after subcutaneous injections limited to the central foot pad (plantar cushion), labelled cells were concentrated in the rostral one-third of the L7 ganglion, suggesting some somatotopic organization of cutaneous afferents within the DRG. In addition, following HRP injections into the intrinsic plantar muscles of the foot, labelled motor neurons were found dorsolaterally in the ventral horn of the S1 spinal segment, in the motor cell column previously described by Romanes.

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1042 IN VIVO AND IN VITRO RABBIT BRAIN CONVERSION OF 2-PHENYLETHYLAMINE TO TYRAMINE AND VICE VERSA. R. Silkaitis*, S. Cann*, M. E. Wolf* and A. D. Mosnaim. Dept. Pharm., Chicago Med. Sch./Univ. Hth. Sci., Chicago, 60612 and Dept. Psychiatry, Cook County Hospital, Chicago, 60612.

There is increasing evidence that the endogenous neuroamines 2-phenylethylamine (PEA) and p-tyramine (TRM) are involved in the modulation of synaptic transmission. Brain PEA has been shown to proceed mainly from the in situ or peripheral decarboxylation of L-phenylalanine (PEA readily crosses the blood-brain barrier)(Borison et. al. 1974) whereas brain TRM apparently is mostly derived from the in situ decarboxylation of L-tyrosine. Using a procedure based upon the selective extraction of PEA in n-hexane (Mosnaim and Inwang, 1973) and TRM in 1-butanol, followed by further purification by TLC, we have studied the in vivo and in vitro rabbit brain tissue conversion of PEA to TRM and vice versa. Ten minutes after the intraventricular injection of labeled PEA (traces) significant amounts of radioactive TRM were recovered from brain homogenates, which as expected, were tripled when the animals had been pretreated with pargyline. Similarly, labeled PEA was recovered ten minutes after intraventricular injection of 1-^{14}C TRM (traces). These recoveries were doubled after pargyline pretreatment. Incubation of 1-^{14}C TRM (traces) with rabbit brain homogenates allowed the recovery of significant amounts of labeled PEA only in the presence of a MAO-inhibitor. The actual MAO-inhibitor and its concentration were crucial to the amounts of recovered PEA. In view of previous work reporting the in vivo rat brain conversion of dopamine to TRM and vice versa (Boulton and Quan, 1970) our results would complete a naturally occurring central nervous system pathway linking PEA to catecholamines.

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1043 BLOOD-BORNE PEROXIDASE ENTRY INTO NEURONS ASSOCIATED WITH THE CIRCUMVENTRICULAR ORGANS AND THE CRANIAL AND SPINAL MOTOR SYSTEMS. R.D. Broadwell and M.W. Brightman. NINCDS, NIH, Bethesda, Md. 20014.

Within the CNS, circumscribed regions contain fenestrated capillaries through which proteins, such as horseradish peroxidase (HRP), and probably hormones pass between brain and blood. These regions include the choroid plexus and the circumventricular organs (CVO): the median eminence (ME), neurohypophysis, area postrema (AP), subfornical organ (SFO), organum vasculosum (OV), and subcommissural organ (SCO). All but the choroid plexus and AP are thought to contain neurosecretory axons that terminate around the fenestrated capillaries.

In this study the origins of the axon terminals in the CVO of the mouse were traced with light microscopy after vascular infusion of HRP. The protein leaked through the fenestrated capillaries, was taken up by the adjacent axon terminals and transported intra-axonally back to the parent cell bodies. Thirty female mice received tail vein injections of HRP in divided doses of 30-120mg, depending upon the post-injection survival times which ranged between 1/2-24 hours. Within 2 hours the HRP had spread maximally to a radius of about 0.6-0.9mm from the midline into the area surrounding each of the CVO. Neuronal somata within each area of spread became diffusely labeled by the protein. By 4 hours, the intracellular diffuse labeling had disappeared, and the extracellular concentration was markedly diminished. By 8 hours, all visible extracellular HRP was gone while specific populations of hypothalamic neurons were labeled with particulate HRP reaction product indicative of intra-axonal retrograde transport. These neurons, projecting their axons to the ME or neurohypophysis, consisted of the supraoptic, paraventricular, accessory magnocellular, and infundibular nuclei and the periventricular stratum extending into the preoptic area. No other hypothalamic cell groups appeared labeled. A few labeled somata were adjacent to the OV, but it could not be determined whether they were related to the OV, ME or neurohypophysis. No labeled perikarya near the SFO could be identified and no leak could be discerned at the SCO. The origin of afferent input to the AP was unclear. By 8 hours, the cell groups immediately ventral to the AP were labeled retrogradely: the hypoglossal, dorsal motor vagus, and medial half of the tractus solitarius nuclei. According to Cajal, only the nucleus solitarius sends axon collaterals to the AP. By 12 hours, cranial motor nerve nuclei III, IV, V, VI, VII, mesencephalic nucleus of V, and the ambiguus nucleus were also retrogradely marked as were ventral motor horn cells of the spinal cord. It is presumed that the motor nuclei were labeled by HRP that had passed across muscle capillaries to the myoneural junctions. Sensory endings of neurons innervating muscle spindles also take up HRP as manifested by labeling of the mesencephalic nucleus of V. It is likely that other hematogenous macromolecules, including viruses, may select certain peripheral axons and, like peroxidase, be retrogradely transported to the somata of the axons.

In addition to the nucleus of the tractus solitarius, two other cell groups in the brain stem that have axons intrinsic to the CNS were labeled. These nuclei were the A1 and A5 noradrenergic cell groups lying in close proximity to the ambiguus nucleus and the VII nerve respectively. Histochemical fluorescence studies indicate that of the CVO only the ME contains a catecholamine innervation that is thought to originate from the lower brain stem. The peroxidase labeled A1 and A5 neurons may represent the origin of this catecholamine innervation.

The use of the intravascular route for HRP injections avoids the damage to nerve endings that accompanies injections of HRP directly into the innervated tissue and results in labeling of neurohemal, neuromuscular and sensory systems.

1044 EVIDENCE FOR NORADRENERGIC INNERVATION OF INTRACEREBRAL BLOOD VESSELS.
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Rat and Rhesus monkey brains were examined using fluorescence microscopy and local cerebral blood flow to study the association of noradrenergic pre- and terminal axonal fibers with intracerebral blood vessels.

Fluorescent varicosities were observed in the media and adventitial layer of penetrant arteries and arterioles following bilateral superior cervical ganglienectomy (SCG) in monkeys. In addition, large surface vessels such as the medial cerebral artery still maintained on its surface a reticulated mesh of fluorescent varicosities following SCG. The brain regions studied (diencephalon and mesencephalon) seemed to have an equal number of vessels showing the associated fluorescent varicosities but the brain stem appeared to have twice the number of vessels with the same type of terminal contacts. These fluorescent varicosities were determined to be noradrenergic using microspectrofluorimetry and immunofluorescent dopamine-β-hydroxylase microscopic method.

Local cerebral blood flows using hydrogen clearance were determined stereotaxically in discreet rat brain regions following stimulation or ablation of several brain stem nuclei.

A role for the brain stem noradrenergic neurons is postulated as the source for the central synaptic innervation seen associated with the intracerebral arteriolar vessels.

1045 AUTORADIOGRAPHIC STUDY OF PARIETO-PULVINAR CONNECTIONS IN SQUIRREL MONKEY.
J. L. DeVito. Dept. Neurol. Surg. and Reg. Prim. Res. Ctr, Univ. Wash. Sch. Med., Seattle, Washington 98195.

The parietal cortex of squirrel monkeys (Saimiri sciureus) was injected with either tritiated proline or leucine (0.5 µl at 10µC/µl). Survival times were 24 hours or 12 days. Brain sections were processed for autoradiography and given 4-6 weeks exposure.

All regions caudal to the central sulcus were found to project upon the pulvinar. An injection into area 3a, which lies rostral to the central sulcus in squirrel monkeys, gave negative results. Parietal operculum projected heavily to oral pulvinar. The projection from medial parietal cortex was lighter and localized in the caudo-ventral region of oral pulvinar. Posterior parietal cortex projected mainly to lateral pulvinar. These findings confirm the orthograde nature of the parieto-pulvinar connections that I traced previously using Nauta and Fink-Heimer silver stains.

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1046 THALAMIC PROJECTIONS TO PARIETAL CORTEX. Richard T. Robertson. Section of Behavioral Physiology, The Fels Research Institute, Yellow Springs, Ohio 45387.

Projections to parietal regions of cerebral cortex from thalamic nuclei ventralis anterior (VA) and lateralis posterior (LP) were investigated using experimental degeneration and autoradiographic techniques. Small electrolytic lesions or injections of 0.05 - 0.15 μl saline containing ^{3}H leucine (50 μCi/μl) were stereotaxically placed in VA or rostral LP of adult cats. Following survival periods of 8 hr - 5 days (autoradiography) or 4 - 7 days (degeneration), the tissues were processed by standard procedures.

Both techniques indicate projections from VA and LP to areas 5 and 7 of the cat's parietal cortex. In addition to a prominent projection to frontal cortex, the data indicate that VA sends projections to the anterior lateral and middle suprasylvian gyri, as well as the walls of the anterior lateral and middle suprasylvian sulci. Projections from LP extend to the crown of the anterior lateral gyrus, but only very lightly to the crown of the middle suprasylvian gyrus. Heaviest projections from LP are to the medial wall of the middle suprasylvian sulcus.

VA projections terminate relatively heavily in the outer half of cortical layer I, with a poorly focalized projection to layers III and IV, and possibly to deeper layers. LP, however, apparently sends strong projections to cortical layer IV, with only very light termination in layer I.

1047 ORIGIN OF AFFERENT FIBERS TO THE OLFACTORY BULB OF THE RAT. D. M. Jacobowitz and R. D. Broadwell. NIMH and NINCDS, Bethesda, Md. 20014

The origins of the afferent neuronal fibers to the olfactory bulb of the rat were investigated by the retrograde axonal transport of horseradish peroxidase (HRP). Twenty-four hours after a unilateral injection of HRP into the olfactory bulb peroxidase-labelled cells were observed in the anterior olfactory nucleus, horizontal limb of the nucleus of the diagonal band, the rostral median forebrain bundle, anterior amygdaloid area, an area beneath the ansa lenticularis at the level of the globus pallidus, the hypothalamus medial, dorsal and lateral to the fornix, dorsal and median raphe nuclei and the locus coeruleus. A few labelled cell bodies were also present in the contralateral locus coeruleus, raphe nuclei, and anterior olfactory nucleus. The present study suggests that the noradrenergic innervation of the olfactory bulb emanates from catecholaminergic neurons within the locus coeruleus and that the serotonergic innervation is derived mainly from serotonin-containing neurons in the dorsal raphe with some contribution from the median raphe. The localization for HRP-positive perikarya in the hypothalamus correlates with locations given for acetylcholinesterase-containing neurons (JCN 157:13, 1974). It is suggested that these neurons may constitute a cholinergic afferent system to the olfactory bulb.

1048 PROJECTIONS FROM THE NUCLEI OF THE DIAGONAL BANDS: AN AUTORADIOGRAPHIC STUDY IN THE ALBINO RAT. Lily C. A. Conrad and Donald W. Pfaff. Rockefeller University, New York 10021.

Projections from neurons in the nuclei of the diagonal bands of Broca (nDBB) were traced autoradiographically following the injection of 10 to 50 nanoliters of tritiated leucine or proline (200 μCi/μl). The rats were sacrificed 48 hrs. after isotope injection, then perfused and the brains embedded in paraffin, sectioned and mounted. Slides were dipped in Kodak NTB-3 emulsion, exposed for 21 to 30 days and developed. Reduced silver grains were charted systematically. Neurons in the nDBB sent fibers dorsally, through the diagonal bands into the septum, laterally into the lateral and magnocellular preoptic area, substantia inominata, and anterior amygdala, and posteriorly, into the medial forebrain bundle (MFB). From the MFB, labelled fibers turned dorsally into the inferior thalamic peduncle to reach the mediodorsal nucleus of the thalamus. In the hypothalamus, projections were noted to the medial mammillary nucleus and supramammillary nucleus. Label was also noted in the posterior continuation of the MFB in the ventral tegmental area of Tsai. Also, projections were seen to the amygdala, deep layers of the pyriform cortex, and entorhinal cortex. Projections through the stria medullaris to the medial and lateral habenula and a light projection through the precommissural fornix to the hippocampus, dentate gyrus and subiculum were observed. The pattern of projections from the nDBB was significantly different from that observed after injection of isotope immediately posterior, in the medial preoptic area or anterior hypothalamus.

1049 THE EFFERENT CONNECTIONS OF THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS. C. B. Saper*, L. W. Swanson and W. M. Cowan. Depts. of Anat. and Biol., Washington Univ., St. Louis, Missouri, 63110.

The efferent connections of the ventromedial nucleus of the hypothalamus (VMH) have been traced autoradiographically in the rat, cat and squirrel monkey. Small volumes (20-100 nl) of a concentrated solution (20-50 μCi/μl) of either ^{35}S-methionine or ^{3}H-proline, or a mixture of ^{3}H-proline, ^{3}H-leucine and ^{3}H-lysine, were injected stereotaxically into, or around, the VMH. After survival periods of 24 to 96 hours, the brains were fixed by perfusion with formalin and processed in the usual way for autoradiography.

In all three species, fibers from the VMH have been found to ascend ipsilaterally in the medial forebrain bundle (MFB) to reach portions of the medial and lateral preoptic areas, the bed nucleus of the stria terminalis, and the ventral division of the lateral septal nucleus. Fibers from the nucleus also project bilaterally along the route taken by the ventral amygdalofugal bundle, through the substantia innominata, to the basal nuclei of Meynert (in the monkey only), the dorsal portion of the medial amygdaloid nucleus, and the periphery of the central nucleus (in the rat and monkey only). Descending fibers from the VMH follow three pathways: (i) through the MFB into the central tegmental reticular fields; (ii) through the dorsal longitudinal fasciculus into the central gray; and (iii) bilaterally through the ventral supraoptic commissure, where they form a narrow band of fibers closely applied to the medial aspect of the optic tract. As the optic tract winds around the cerebral peduncle, this last group of fibers loops back medially around the caudal border of the peduncle into the central tegmental fields. In the tegmentum, the peripeduncular and peribrachial nuclei receive well circumscribed inputs from the VMH. The pathways to the tegmental fields and central gray are connected by the central gray radiation (of Weisschedel). Some fibers in the central gray extend caudally as far as the locus coeruleus. Intrahypothalamic connections, though difficult to trace because of diffusion of the isotope from the injection site, may involve most of the surrounding hypothalamic nuclei (including the anterior, posterior and lateral hypothalamic areas, and the suprachiasmatic, arcuate, periventricular and dorsomedial nuclei) but, significantly, not the median eminence.

Although there are minor differences in the VMH projections in the three species (for example, we have thus far had difficulty demonstrating the amygdaloid projections in the cat, and the projection to the lateral septum is rather sparse in the monkey), the general pattern of these connections is remarkably constant, no doubt reflecting the rather central rôle played by the VMH in the function of the hypothalamus. Just how it exerts its effect remains to be elucidated; but the widely held view that it does so primarily by a system of short fiber connections to the lateral hypothalamus and the median eminence clearly requires reappraisal.

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1050 ABSENCE OF MONOSYNAPTIC REFLEX IN THORACIC SEGMENTS OF FROG SPINAL CORD. R. Carlsen and L. Mendell. Dept. of Physiology & Pharmacology, Duke Medical Center, Durham, N. C. 27710

Both lumbar dorsal root (DR) afferents and a descening motor pathway, the lateral column (LC), make monosynaptic connections with ipsilateral lumbar motoneurons in the frog spinal cord. Using the isolated Rana pipiens spinal cord, maintained at 15°C ± 1°C, we have determined that a different organization exists in thoracic spinal segments. Stimulation of the lumbar DR evokes a polyphasic discharge from the ipsilateral lumbar ventral root with a latency of 3.4 ± 0.2 msec., which is a measure of central delay in the pathway. In contrast, stimulating a thoracic DR evokes an asynchronous polyphasic discharge from thoracic ventral roots with a latency of 10.2 ± 2.3 msec. . Stimulation of LC evokes a synchronous, spike-like, discharge from ventral roots with a latency of 3.0 ± 0.2 msec. (lumbar) and 2.9 ± 0.3 msec. (thoracic). A condition-test paradigm was used to uncover possible subliminal facilitation of thoracic motoneurons. A supramaximal conditioning stimulus applied to the thoracic DR followed by a submaximal test stimulus applied to the LC produces the first facilitation of the LC response with a latency of 9.0 ± 0.3 msec. . A similar test in lumbar segments produced the first facilitated response with a latency of 2.1 ± 0.2 msec. . On the basis of estimates of conduction time and synaptic delay in the frog spinal cord at this temperature, the latency between thoracic DR stimulation and the thoracic ventral root response suggests that at least 3 synapses are interposed between thoracic afferents and thoracic motoneurons. (Supported by Muscular Dystrophy Assoc., Inc. and NIH).

1051 THE OLFACTORY TUBERCLE PROJECTIONS IN THE RAT. Lennart Heimer, Jose de Olmos, Hay Hardy and M.J. RoBards. Dept. Anat., Univ. of Va., Charlottesville, VA. 22901.

Experimental silver impregnation studies following laminar heat lesions in the olfactory tubercle of the rat have demonstrated three major (olfactory tubercle) projections, i.e., to the medial part of the medio-dorsal thalamic nucleus, the nuclei gemini in the caudolateral part of hypothalamus and the ventral part of globus pallidus. Following iontophoretic injections of 30% horseradish peroxidase into the medio-dorsal thalamic nucleus or into nuclei gemini 24 hours before sacrifice of the animals, labeled cells were found primarily in the s.c. polymorf cell-layer of the olfactory tubercle. The labeled cells were invariably of large size and distributed throughout most parts of the tubercle, even in cases with restricted peroxidase injections. The observation that the olfacto-diencephalic projections originate from large cells corresponds well with previous light and electron microscopic findings, which indicated that the projection fibers were heavily myelinated. Whereas labeled cells in the rostral forebrain were confined to the olfactory tubercle following injections into the nuclei gemini, injections into the medial segment of the medio-dorsal thalamic nucleus usually resulted in the labeling of a few large cells also in the polymorf layer of the prepiriform cortex. Injections into the medial part of medio-dorsal nucleus, furthermore, were always accompanyed by a labeling of many cells in the deep layers of the frontal lobe including the s.c. sulcal cortex.

1052 PREFRONTAL INFLUENCES ON AUDITORY-RESPONSIVE UNITS IN TEMPORAL ASSOCIATION CORTEX. Garrett E. Alexander, John D. Newman and David Symmes. Behavioral Biology Branch, NICHD, NIH, Bethesda, Md. 20014.

The dorsolateral prefrontal cortex has been shown to send fibers to the superior temporal gyrus (STG). Overlapping the termination of this fronto-temporal pathway is an oligosynaptic pathway which emerges from the primary auditory cortex and spreads posteriorly over the STG and into the superior temporal sulcus (STS). A potential functional role for this convergence is suggested by frontal effects on auditory evoked potentials and by the impaired auditory discrimination of prefrontal animals. To investigate the extent and nature of prefrontal influences on auditory processing in the STG, five adult squirrel monkeys were prepared with stainless steel stimulating electrodes over one prefrontal convexity and a chronic single unit recording chamber over the ipsilateral STG. During recording sessions the animals were awake and unanesthetized. One hundred forty-four STG units were tested for responses to clicks, tones and recorded monkey vocalizations, as well as to stimulation of dorsolateral prefrontal cortex. One hundred six of these cells responded to one or more types of auditory stimuli. Twenty-three cells responded to prefrontal stimulation, and the spatial distribution of these units corresponded to the topography of the fronto-temporal pathway: the frontal-responsive STG units were located near STS and the temporal pole. Eighty-seven percent of such units responded to auditory stimuli as well. In all but one case the response to prefrontal stimulation was characterized by inhibition--i.e., reduction or cessation of unit firing during the stimulus train (often followed by rebound excitation). Such inhibition developed with an extremely brief latency and affected both spontaneous activity and acoustic responses of STG neurons. We conclude from this study that the cortex of the prefrontal convexity can modulate the flow of auditory information through the STG.

1053 AFFERENT CORTICAL CONNECTIONS OF THE SUPERIOR TEMPORAL SULCUS IN THE RHESUS MONKEY. Benjamin Seltzer * and Deepak N. Pandya, Department of Neurology, Harvard Medical School, Boston, Mass.

Using silver impregnation techniques, the distribution of terminal degeneration within the cortex of the superior temporal sulcus was studied in rhesus monkeys subject to cortical ablations in various parts of the hemisphere. Several distinct patterns of projections were encountered.

The most rostral portion of the sulcus received input from orbitofrontal cortex and had connectivities similar to those of the temporal pole. A specific region in the posterior part of the sulcus received afferents from primary visual cortex and may represent a "second" visual area. Other sectors of both upper and lower banks were the recipients of projections from auditory, visual, or somatic sensory "association" cortex and may be considered additional modality-specific association areas. In some of these areas, the projections from different modalities overlapped, and here it is possible that sensory convergence is taking place.

In addition, regions of the frontal lobe known to receive projections from certain sensory-related cortical areas, had superior temporal sulcus projections that overlapped those of the corresponding sensory area, thus tieing together related regions in widely dispersed portions of the hemisphere.

This anatomical parcellation, based on afferent cortical connections correlates with cytoarchitectural differences and suggests several specific functional roles for the superior temporal sulcus.

1054 AFFERENT AND EFFERENT CONNECTIONS OF PULVINAR IN RHESUS MONKEY. J. Q. Trojanowski*. (Spon: D. A. Pollen). Anat. Dept., Tufts Univ. Sch. of Med. Boston, 02111

Bilateral and unilateral injections of a combined solution of horseradish peroxidase (HRP) and tritiated amino acids were made in 12 rhesus monkeys. All pulvinar subnuclei receive input from ipsilateral claustrum and thalamic reticular nucleus. Lateral parts of lateral, inferior and medial pulvinar nuclei (PL, PI and PM) receive input from ipsilateral lateral geniculate nucleus and medial parts of PI and PL receive input from laminar II of superior colliculus. Reciprocal connections could be established only in the case of the thalamic reticular nucleus.

In cortex pulvinar distributed mainly to layers iv and adjacent iii. Medial parts of PM project to the rostral third of temporal lobe including the lower bank of lateral fissure and the banks of superior temporal sulcus. Sparser projections were seen in cortex within the confines of arcuate sulcus, in the depths of the caudal third of principal sulcus and the lateral part of caudal orbital frontal cortex. The lateral parts of PM project to more lateral and ventral regions of temporal lobe including the banks of superior temporal sulcus. PI and PL project to posterior parieto-temporal and rostral occipital cortex; PI more inferiorly involving orbital and lunate sulci and PL to more dorsal and medial regions including lunate sulcus and to posterior parietal lobe including the depths of intraparietal sulcus. Oral pulvinar also projects to this posterior parietal region. Preliminary HRP data indicates that these connections are in general reciprocal and that the thalamocortical neurons are restricted to layers v and vi. Pulvinar cortical efferents are directed to sensory association areas rather than to primary sensory areas.

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1055 EPITHALAMIC SUPRA-EPENDYMAL NERVES: THE EFFECTS OF NEUROTOXIC AGENTS AND CNS LESIONS. Jorge L. Ribas, Juan M. Saavedra and Cyril Wingfield*. Dept. Neurophysiology, Walter Reed Army Institute of Research Wash. D. C. 20012 and Lab. Clinical Science, NIMH, Bethesda, Md. 20014.

Ultrastructural studies have established the presence of varicose nerve fibers on the ventricular border of the ependyma. Combined cytochemistry and pharmacology have strongly suggested that an indoleamine, most likely 5OH-tryptamine, is contained in these terminals.

Since dihydroxylated tryptamines (DHT) and 6OH-dopamine (6OH-DA) are selectively taken up by their respective terminals, we undertook a combined morphologic-pharmacologic study to determine the effects of these agents on epithalamic supra-ependymal nerves. The following injections were made bilaterally into the lateral ventricles of adult male rats: 50 ug 5,6-DHT; 50 ug 5,7-DHT (pretreated with 25 mg/kg DMI, I. P.); and 200 mg 6OH-DA. Sham-injected and untreated animals served as controls. Scanning and transmission electron microscopy of the ependymal surface of the habenular region failed to reveal the presence of supra-ependymal nerves after treatment with dihydroxytryptamines. These nerves were present in 6OH-DA-treated, sham-injected and untreated animals. Tractotomy and electrolytic lesions of the habenulo-interpeduncular tract (which includes indoleaminergic fibers from raphé) resulted in the disappearance of the supra-ependymal fibers. On the other hand, lesions of the stria medullaris thalami (which also contains indoleaminergic fibers from raphé) had no effect.

Our findings provide additional support for the serotonergic nature of these fibers and suggest that they originate from rostrally projecting indoleaminergic neurons of the brain stem, probably the raphé nuclei.

1056 DIRECT NEURAL INPUTS TO LOCUS COERULEUS FROM BASAL FOREBRAIN. L. W. Swanson and C. B. Saper*. Depts. Biol. and Anat., Washington Univ., St. Louis, Mo. 63130.

The locus coeruleus is said on the basis of experimental evidence to project widely, though diffusely, to both cerebellar and cerebral cortices; however, little is known of its afferent connections. During the course of a study of the connections of the septum and hypothalamus using the autoradiographic method for tracing connections we have found that a number of basal forebrain areas project to the locus coeruleus. One to four days after the injection of 20-50 nl of 20-50 μCi/μl solutions of ^{3}H-proline, mixtures of ^{3}H-proline, ^{3}H-leucine, and ^{3}H-lysine, or in a few cases ^{35}S-methionine, into the bed nucleus of the stria terminalis, the lateral preoptic area, or the dorsomedial and ventromedial nuclei of the hypothalamus, silver grains indicative of transported label were observed over the fiber capsule and over the adrenergic neurons in the anterior part of the locus coeruleus. In nine rat brains with small injections confined to the bed nucleus of the stria terminalis, only those involving its ventral and lateral portions were found to give rise to a projection to the ipsilateral locus coeruleus, by way of the medial forebrain bundle. This projection enters the central tegmental reticular field between the substantia nigra and the red nucleus and then arches medially to reach the central gray at the level of the trochlear nucleus; the fibers continue caudally through the region of the dorsal tegmental nucleus before terminating in the locus coeruleus. In a further 20 rat brains with injections confined to various portions of the preoptic area it was found that those in which injections involved the dorsal aspect of the lateral preoptic area demonstrated an ipsilateral input to the locus coeruleus. These fibers travel through the medial forebrain bundle and appear to reach the locus coeruleus via two routes; some enter the central tegmental field and, in a course similar to that of the fibers from the bed nucleus of the stria terminalis, course medially at the level of the trochlear nucleus to enter the central gray; the other fibers enter the periventricular gray in the posterior hypothalamus, then continue caudally in the central gray where they are joined by the fibers from the tegmental pathway. Both the bed nucleus of the stria terminalis and the preoptic area project to a number of other sites (Swanson and Cowan, in preparation). Injections into the ventromedial nucleus of the rat, cat, and squirrel monkey demonstrate pathways to the locus coeruleus which travel both through the central tegmental field and posterior hypothalamus-central gray. A number of other projections of the ventromedial nucleus are described elsewhere (Saper, Swanson, and Cowan, this Conference). Finally, a small ^{35}S-methionine injection confined to the dorsomedial nucleus and the immediately adjacent posterior hypothalamic area demonstrated a bilateral input to the locus coeruleus although the contralateral projection was sparse. The fibers course posteriorly almost exclusively in the central gray. Work in other laboratories as well as our own has shown a dense adrenergic input to the ventral aspect of the bed nucleus of the stria terminalis. Thus the projection from the bed nucleus of the stria terminalis to the locus coeruleus described here may provide for a reciprocal input to the central adrenergic system, although probably not to the specific adrenergic cells which innervate the bed nucleus of the stria terminalis (e.g., Ungerstedt, Acta Physiol. Scand., Suppl. 367, '71). Since the amygdala constitutes a major source of afferents to the bed nucleus of the stria terminalis and to the ventromedial nucleus, the amygdala may have a relatively direct input to the locus coeruleus via two routes, as may the ventral aspect of the subiculum which also projects directly to the ventromedial nucleus. On the other hand, the lateral preoptic area and ventromedial nuclei have a very direct input to the locus coeruleus. (Supported in part by grants MH-24604, NS-03777 and NS-10943 from the National Institutes of Health.)

1057 PATTERNS OF DEGENERATION IN THE EXTERNAL CUNEATE NUCLEUS OF THE ALBINO RAT FOLLOWING DORSAL RHIZOTOMIES. J. M. Rosenstein*, R. B. Page*, A. E. Leure-duPree*, (SPON: J. D. Connor). Departments of Anatomy and Neurosurgery, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033.

Ultrastructural alterations in the external cuneate nucleus (ECN) of the albino rat after unilateral division of the dorsal roots (staged between C_3 - T_1), are compared to the normal morphology of the nucleus. The normal characteristic features of the nucleus include synaptic glomeruli, serial synapses and nodal synapses.

Unequivocal signs of degeneration are observed ipsilaterally in the ECN after intradural rhizotomies, while sham operated animals present a morphology identical to that of unoperated controls. Experimental animals are permitted to survive from 3 hours to 12 days. Clumping of synaptic vesicles are observed in axons 3 hours postoperatively. At 14 hours, in addition to vesicle clumping, there is an observable diminution of vesicles in some degenerating terminals. The mitochondria are appreciably more dense than usual; however, cristae are still discernible. "Electron-lucent" and "electron-dense" boutons are more prominent. Glia characteristically contain engulfed debris in their cytoplasm. At subsequent postoperative periods (48 hours to 12 days), extensive morphological alterations are observed in only some affected terminals, suggesting appreciable differences in the rate of degeneration. (Supported in part by Josiah Macy, Jr. Foundation and PHS Research Grant 5S01-RR5680)

1058 ASCENDING PATHWAYS IN THE ANTEROLATERAL FUNICULUS OF THE RAT SPINAL CORD. Lanay J. Land, Beth A. Reese*, and David G. Whitlock. Dept. Anatomy, University of Colorado School of Medicine, Denver, Colorado 80220.

The ascending sensory fiber system in the anterolateral funiculus (ALF) of the spinal cord of the rat was studied using autoradiographic techniques. In eleven adult rats concentrated tritiated proline was injected electrophoretically through a glass micropipette with tip diameter of 4 microns into the lumbar spinal cord gray matter at depths ranging from 250 to 1,000 microns from the dorsal surface of the spinal cord. After post-injection survival intervals of 4 to 19 days the central nervous system was removed, fixed in Bouin's solution, and prepared for autoradiography. The autoradiographs showed that the injection locus containing labeled neuron cell bodies was usually restricted to only a few gray laminae. The injection sites in all cases were restricted in rostro-caudal extent to one spinal segment or less. The number of labeled axons emerging from each injection site which entered the anterolateral pathway were counted with a computerized microscope system.

Labeled axons were found to ascend to brainstem levels in the ALF from the following origins in the spinal gray: the base of the ipsilateral dorsal horn, the ipsilateral anterior horn, the base of the contralateral dorsal horn, and the contralateral anterior horn.

The course of axons entering the ALF from one injection locus was traced in serial 3 micron paraffin transverse sections of the spinal cord. The labeled axons entered both the ipsilateral ALF and the contralateral ALF from this locus. The number of labeled axons at a distance of 3.5 to 4 mm from the center of the injection locus was counted. Essentially equal numbers of labeled axons occupied both the ipsilateral and contralateral ALF, and in the case examined numbered 206 and 214, respectively. As individual labeled axons ascended the anterolateral columns they tended to be widely separated one from another.

The number of labeled axons in the ipsilateral and contralateral ALF in the cervical spinal cord of the same animal was counted and compared with the results above. The number of labeled axons was found to be between 25-40 % less in each anterolateral column. Moreover in the cervical spinal cord the labeled axons tended to form a more compact aggregate, and appeared to lie in the position of the lateral spinothalamic tract both ipsilaterally and contralaterally. The terminal distributions of this bilateral ALF fiber system are under study.

These anatomical data provide further information on the organization of the ascending sensory pathways contributing to the anterolateral fiber system, and give information on the changing composition of fiber pathways in the anterolateral column as they ascend the spinal cord.

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1059 PERMEABILITY OF THE RHESUS MONKEY BLOOD-BRAIN BARRIER (BBB) TO ANTIBODY (Ab) AFTER OPENING BY HYPERTONIC SOLUTIONS. J.T. Hicks*, P. Albrecht* and S.I. Rapoport. Div. Virology, Bur. Biologics, FDA and Lab. Neurophysiol., NIMH, Bethesda, Md. 20014.

SSPE, a fatal measles infection of the brain, occurs without a demonstrable cellular or humoral immune defect. High titers of Ab with or without complement eliminate infection by SSPE virus isolates in vitro (Albrecht et al., in preparation). Since most clinical cases of SSPE lack such high CSF Ab titers, increasing titers of Ab in brain and CSF by therapeutic means might limit the disease in vivo. To achieve this, we reversibly opened the BBB in monkeys immunized against measles by perfusing hypertonic solutions of arabinose or lactamide (1.8 to 2.5 molal) into the left internal carotid artery through a lingual artery catheter (Rapoport & Thompson, Science 188: 971, 1973). The ipsilateral external and common carotids were clamped during the 25 seconds of perfusion. Intravenous Evans blue was used as a visual tracer to determine the extent of BBB opening after an experiment. Monkeys were killed after 1, 4 and 14 days by perfusing normal saline through the brain, and measles-neutralizing Ab titers were measured in the treated left hemisphere with BBB opening and in the untreated, unstained right hemisphere. Under optimal conditions, BBB opening increased brain Ab 20-fold at 4 days and Ab remained in brain even after 14 days. Neurological examinations were normal in the majority of cases, and obvious brain edema was absent. Since the BBB can be osmotically opened to serum Ab, repeated opening might raise brain Ab and complement titers enough to halt the otherwise fatal course of SSPE, and may be of use in treating other viral encephalitides susceptible to Ab and complement.

1060 ISOLATION OF MACROMOLECULES FROM MULTIPLE SCLEROSIS (MS) BRAIN HOMOGENATES BY AFFINITY CHROMATOGRAPHY ON COLUMNS CONTAINING γ-GLOBULINS FROM MS PATIENTS AND CONTROLS. Timothy J.A. Johnson*, Steven E. Kornguth, Ursula Juhl*, David Fuccillo*, and John Sever*. (SPON: F.L. Siegel). Univ. Wisc. Med. Ctr., Madison, Wisconsin 53706 and NIH, Bethesda, Md. 20014.

Affinity columns were prepared from glycophase glass beads activated with cyanogen bromide and then coupled to γ-globulin. γ-globulins were precipitated from spinal fluid or blood serum with 40% saturated $(NH_4)_2SO_4$. The γ-globulin sources were MS spinal fluid, MS blood serum and normal blood serum.

The brain homogenate was prepared from frozen tissues obtained postmortem from MS patients. Pieces of white matter both adjacent to and distant from sclerotic plaques were pooled. The tissues were homogenized in 10 volumes of 0.32 M sucrose, 0.001 M MgCl, 0.0004 M phosphate, pH 7.0.

Equivalent samples of the 10,000 g supernatant were applied to each γ-globulin column. The samples were washed into the columns and eluted with 0.15 M NaCl containing 0.01 M phosphate pH 6.9 until all nonspecifically bound material had eluted. The columns were sequentially eluted with phosphate buffered 1.0 M NaCl and then 2.5% acetic acid containing 1 M NaCl. Although no significant amounts of 254 nm absorbing material were eluted by the 1 M NaCl, the acid eluate from the MS γ-globulin columns contained more than 10 times the 254 nm absorbing material than the acid eluate from the normal γ-globulin column.

The acid eluates from the MS γ-globulin columns were characterized by UV spectra, sedimentation through sucrose, G-25 Sephadex chromatography, and SDS gel electrophoresis. The last three procedures were carried out with material labelled with (^{3}H) $NaBH_4$ in a reductive formylation procedure. The acid eluate material had a UV maximum in the 270-274 nm region and migrated with the excluded front on Sephadex G-25. The UV absorbing macromolecules did not penetrate a 20% sucrose zone when centrifuged at 35,000 rpm in an SW 41 rotor indicating that the components eluted by acid are not particulate. SDS gel electrophoresis of the (^{3}H) labelled isolate, revealed a number of radioactive proteins with different molecular weights. (Supported by NS 42308 and NS 5631).

1061 BEHAVIORAL EFFECTS OF ANTIGEN-ANTIBODY REACTIONS IN RAT BRAIN: IMPLICATION OF ADRENERGIC REGULATION SYSTEMS. Nicole Schupf Smith*, N.Y.U. Med. Cen. New York, NY 10010 and Curtis A. Williams, Rockefeller Univ., New York, NY and SUNY-Purchase, Purchase, NY 10577.

Rabbit antibody made against rat brain tissue antigens introduced via implanted cannulae into the medial septum or the lateral hypothalamus of water deprived rats produced a significant decrease in the drinking response. The maximal effect was always delayed, often as much as 24 hours but reversal was always complete, usually by 72 hours.

Similar tests with immunochemically defined rabbit antibody reactions with antigens unrelated to rat tissues gave the same results. Anti human serum albumin (aHSA) or anti ovalbumin (aOA) reacting in the septum or hypothalamus with the corresponding antigen (HSA or OA) produced criterion ($\geq$ 25%) depression of drinking in a significant number of the animals tested. The fraction of the animals responding increased from one-fifth to four-fifths as the antigen:antibody equivalence ratio in the system was increased from 4:1 to 20:1. Treatment with non cross-reacting systems (aHSA+OA or aOA+HSA) had no effect. These results suggest a mechanism for the effect of antibody in brain involving the release of biogenic amines in a manner similar to passive anaphylaxis.

Histamine introduced into the septum of rats responding to antigen-antibody failed to alter the drinking response while serotonin produced an immediate depression lasting 10 to 30 minutes depending on the dosage. Norepinephrine (NE) in the hypothalamus also suppressed drinking for a comparable period.

The effect of the aHSA+HSA reaction in the hypothalamus on chemically induced eating and drinking was examined to test the possible role of NE as a mediator of the effect. Rats were prepared with cannulae in the perifornical region of the hypothalamus, an area where direct chemical stimulation with NE will induce eating and carbachol (CA) will elicit drinking in sated rats. Baseline eating and drinking with NE, CA and saline were determined. In the experimental injection series antibody and antigen were administered 6 hours prior to drug injections. If the focal antibody effect is mediated by transmitter release, potentiation of the effect due to exogenous transmitter analogue on eating or drinking would be predicted. Pretreatment with aHSA+HSA enhanced the eating response to NE and elicited a significant eating response in saline injected animals, but it had no effect on food intake in CA treated animals. Treatment with aHSA+HSA also suppressed the drinking response to CA, but it had no effect on drinking in NE and saline injected animals.

NE is known to induce eating, to depress natural thirst, and to counteract CA induced drinking. The pattern of our results are consistent with the notion that focal antigen-antibody affects behavior either by causing the release of NE or by activating cellular or synaptic processes involved in the adrenergic control of food and water intake.

The finding that changes in behavior can be caused by immune reactions of exogenous soluble antigens in the brain could greatly expand the scope of investigations into altered behavior of possible immunological origin.

1062 ROLE OF CIRCULATORY ANTIBODIES AND PRIMARY HERPETIC INFECTION IN THE BRAIN OF CATS. Robert S. Pozos*, Richard J. Ziegler*, and Mary A. Hartmann*. Dept. of Physiol., Sch. Med., UMD, Duluth, 55812.

Previous work in our lab has shown that intracortical injection of Herpes Simplex Virus I (HSV) 10^6p.f.u. in adult cats has not been lethal. Using fluorescent antibody & virus isolation techniques, we were unable to locate the virus at any other area of the central nervous system except at the site of injection.

In an attempt to delineate the mechanism(s) behind this apparent isolation of the virus in the central nervous system in cats, we followed the progression of antibody levels in two groups: those inoculated directly in the brain (IC) and the initially inoculated in the footpad (FP) followed by intracortical injection (FP + IC). Intracortical injections were given in the second group when antibody levels began to fall. Antibody levels were monitored previous to inoculation and at weekly intervals. Control groups consisted of cats being injected with sonicated HEP-II cells by the same routes used in the experimental groups. In all control groups antibody levels were nonexistent.

Initially in both experimental groups, the maximum level of antibody peaked at approximately 3 weeks. In the IC group, the level was > 1/4 while those just receiving footpad inoculation, the level was < 1/2. In the FP group when the intracortical injection was given there was an increase in antibody levels (> 1/32).

Since there was an initial low level of circulating antibody for 3 weeks in animals infected with HSV, and the animal did not die during this time, it is tentatively concluded that circulatory antibody levels are not initially responsible for the apparent confinement of the virus to the site of inoculation.

1063 A LABORATORY BAROMETER OF DISEASE ACTIVITY IN MULTIPLE SCLEROSIS (MS) AND GUILLAIN BARRE SYNDROME (GBS) ? Peter Dowling*, and Stuart Cook. Neurology Service, V. A. Hospital, East Orange, N.J. 07019.

No readily measurable parameter for monitoring disease activity or efficacy of therapy exists for demyelinating disorders. Recent evidence suggests that certain components of the serum complement system become transiently elevated during inflammation and tissue destruction. C_3 proactivator (C3PA) appears to be one of the complement proteins which shows the greatest elevation during inflammation.

To determine if C3PA levels might be a useful barometer in demyelinating disorders, a prospective study was done on sera stored at -80°C from 24 consecutive GBS patients and 30 normal subjects. Significantly elevated C3PA levels were found in over 60 percent of the GBS group. With rare exceptions, peak levels occurred in the initial acute phase sample. Sequential studies in many instances revealed a rapid return of C3PA to normal levels with clinical recovery.

A restrospective study of C3PA in aged sera from over 45 cases of MS was also done. Nearly one-third of the MS group had elevated serum C3PA levels; none of the patients had evidence of secondary bacterial infections. Many subjects with elevated C3PA had been hospitalized with recent acute flareups.

Thus elevation in serum C3PA is relatively common in our GBS and MS patients. A larger prospective series of patients is needed to confirm that C3PA may be useful serologic indication of disease activity in MS.

1064 SOME ULTRASTRUCTURAL EFFECTS OF HERPES SIMPLEX VIRUS TYPE 2 REPLICATION ON DORSAL ROOT GANGLION CULTURES. Richard J. Ziegler,*, Robert S. Pozos, Jane B. Hogstrom* and Denise A. Schottler* (SPON: D.J. Forbes). Departments of Microbiology and Physiology, University of Minnesota-Duluth School of Medicine, Duluth, Minnesota, 55812.

Recent studies employing tissue transplantation methods have indicated that herpes simplex virus can persist for months or years in the sensory ganglia of experimentally infected animals and in naturally infected humans. The R-2 strain of herpes simplex virus type 2 (10^6 p.f.u.) was innoculated into organotypic cultures of fetal rat dorsal root ganglia in order to study the interaction of this virus with nervous tissue. At various intervals after infection, ganglia were fixed and sectioned for electron microscopy.

All cell types (neurons, Schwann cells, and glial cells) showed all stages of virus replication. Initially, the most dramatic observation was the membrane reduplication which occurred in these cells. Neurons had a dispersed array of membrane within the nucleus, while some Schwann cells showed intranuclear concentrically whorled membrane and glial cells showed proliferating cytoplasmic membrane. Other observations included the retraction of Schwann cell processes surrounding neuronal somas demyelinization, and polykaryocyte formation. This latter process provides for both intracellular as well as extracellular spread of the virus within the tissue.

1065 ALTERATIONS IN THE AUTONOMIC NERVOUS SYSTEM ASSOCIATED WITH "NEUROGENIC" BLADDER IN THE DIABETIC CHINESE HAMSTER. William G. Dail, Jr., Andrew P. Evan, Jr.*, George C. Gerritsen* and William E. Dulin*. Dept. of Anat., Sch. Med., The Univ. of New Mexico, Albuquerque, NM 87131 and Diabetes and Atherosclerosis Research, The Upjohn Co., Kalamazoo, MI 49001

The genetically diabetic hamster is a valuable animal model since its disease and complications are similar to the human diabetic. It has been suggested that the distended urinary bladder and hydronephrosis of diabetic hamsters may parallel the neurogenic bladder syndrome of human diabetics. The purpose of the present investigation is to determine if pathology of the pelvic autonomic nervous system is associated with urinary bladder dysfunction in diabetic hamsters. Five male diabetic hamsters, matched with appropriate non-diabetic controls, were studied with histochemical and electron microscopic techniques. Diabetics had a 4+ glycosuria (Testape^R) continuously for at least 7 months. None of the diabetics was ketonuric. Four of the 5 diabetic hamsters had greatly distended urinary bladders, ureters and renal pelvises. Acetylcholinesterase activity was markedly reduced in nerves and on smooth muscle fibers in the urinary bladder of diabetics when compared to controls. The occurrence of wide periaxonal spaces and unusual Schwann cell processes in many fibers of the urinary bladder were suggestive of remyelination. Whether these fibers are autonomic nerves or sensory fibers of the urinary bladder could not be determined. Within the pelvic plexus of diabetics large amounts of particulate material of varying size (35-400Å) occurred between expanded myelin lamellae. In addition, cholinergic terminals on some ganglion cells were in various stages of degeneration.

These findings suggest that pathological changes in the autonomic nervous system may be the basis for urinary bladder dysfunction in the diabetic Chinese hamster. (Supported by institutional research funds).

1066 USE OF THE SOMATIC EVOKED RESPONSE TO MONITOR CHRONIC SPINAL CORD COMPRESSION AND SURGICAL DECOMPRESSION

Jack E. McCallum*, Marvin H. Bennett (SPON: J. C. Maroon). Dept. of Neurosurgery, Sch. Med. University of Pittsburgh, Pittsburgh 15213.

Casein plastic is hygroscopic and slowly increases in volume when placed in tissue. Semicircular collars of this plastic were placed in the epidural space posterior to the arch of C1 in six cats. Teflon collars of same size were similarly placed in two control animals. Transcranially recorded averaged somatosensory evoked responses to sciatic nerve stimulation (SER) were serially measured over periods from one to eight weeks. The animals with casein implants gradually lost their SER's and subsequently became profoundly quadriparetic. Decompression laminectomy resulted in recovery of the SER either during the operative procedure or in the early postoperative period. Functional recovery followed in all cases. Animals with Teflon implants remained in tact and had no SER deterioration during the ten weeks for which they were followed.

The SER accurately predicted both loss and recovery of function in this model of gradual spinal cord compression.

1067 CEREBELLAR HYPOPLASIA, PURKINJE CELL DYSPLASIA, AND MOTOR DEFICIT IN THE RAT.

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Injection of the neonate with methylazoxymethanol acetate (MAM Ac) produces cerebellar hypoplasia and readily observable motor symptoms in the dog, ferret, mouse, and hamster. In contrast, the rat shows no motor deficit despite a 20% loss in cerebellar mass and markedly abnormal cerebellar morphology: distorted lobules, reduced and irregular internal granular layer, Purkinje cells scattered amongst the granule cells. Golgi-stained sections show that the Purkinje cells have grossly abnormal and severely reduced aborization of their dendritic branches.

Why, then, does the rat have no motor deficit? Since cerebellar dysplasia, with associated ataxia, can be readily produced in the hamster, we administered graded doses of MAM Ac to neonatal hamsters to determine whether motor deficits are associated with a specific degree of cerebellar pathology. Motor deficits were seen consistently only when the dose was large enough to produce more than a 30% loss of cerebellar mass. These results suggest that a 20% loss of cerebellar mass in the rat, despite marked morphological abnormalities, is not sufficient to produce motor deficit. We therefore attempted to increase the severity of cerebellar hypoplasia in the rat by increasing the dose of MAM Ac given at birth. Graded losses in cerebellar mass were reliably produced up to a 20% loss. Further increases in the dose of MAM Ac from 40 to 80 mg/kg produced no greater cerebellar pathology. Apparently, in the neonatal rat (unlike the neonatal hamster), there is a rate limiting factor - probably the level of available esterases required to hydrolyze the acetate.

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1068 EFFECT OF THE RATE OF LESION DEVELOPMENT ON BEHAVIORAL ADAPTATIONS IN INTRACRANIAL NEOPLASMS. Thomas K. Green and Ralph M. Reitan*, Neuropsychology Laboratory, Dept. of Neurol. Surgery, Sch. Med., Univ. of Washington, Seattle, WA 98195.

A complete battery of neuropsychological tests designed to sample a broad range of behaviors (i.e., sensorimotor, perceptual, cognitive, intellectual, and speech) was administered by trained technicians (unaware of the diagnosis or use to which the data would be put) to a large number of neurological and neurosurgical patients over an extended period of time. The Minnesota Multiphasic Personality Inventory (MMPI) was also administered to evaluate emotional status. From this population patients were selected on the basis of histopathologic verification of the existence of a glioma at the time of surgery; only their preoperative test results were used. They were divided into two groups according to the rate of growth of the neoplasm, being designated Fast (F) or Slow (S). Groups were comprised so that laterality, locus, and size of lesion in the cerebral hemispheres were closely matched. The resulting 25 cases making up the groups were further matched on the bases of age, sex, and education. Controls consisted of non-chronic psychiatric patients and normals (2 groups) also matched on the foregoing bases with the tumor patients. Level of performance (group mean comparisons) did not discriminate the F and S groups on the neuropsychological measures. Level of performance differences were seen in several scales of the MMPI, resulting also in configural differences on that measure of emotional status and personality variables. Emotional correlates of these brain lesions were discriminated features. The results are discussed in terms of the gradual onset (with compensation) of symptoms in slow-growing tumors and the precipitous onset of symptoms in fast-growing tumors. The implications of these results for residual adaptive capacity are stressed.

1069 MYELINATION OF FIBER TRACTS IN QUAKING MICE. Victor L. Friedrich, Jr. Dept. of Biobehavioral Sciences, Univ. of Connecticut, Storrs, 06268.

Quaking is an inherited neurological disorder of mice involving deficient myelination of the central and peripheral nervous systems. Both axons and oligodendrocytes are present in central white matter but most axons are poorly myelinated or are not myelinated at all.

The development of the spinal cord dorsal funiculus was followed between 20 and 150 days after birth. The murine dorsal funiculus consists of the gracile and cuneate fasciculi(GCF) and the corticospinal tract (CST).

In normal mice, the myelin content of the CST increases several fold during the period studied; that of the GCF increases by about 50%. In quaking mice, the myelin content of the CST remains about 10% of normal throughout. By contrast, the myelin content of the GCF improves substantially, from 15% of normal at 20 days to more than 25% of normal in adults. Neuroglial cells in the mutant's GCF are increased in number two to three fold over normal at all ages studied, while those in the CST are about normal in number. The increase in neuroglial cells in the GCF reflects a four fold increase in the number of its oligodendrocytes.

The myelin content of the GCF is considerably greater than that of the CST in adult quaking mice. This difference is minimal at 20 days but increases progressively with age; it reflects enhanced accretion of myelin in the GCF as compared to the CST.

The increase in oligodendrocytes in the GCF may develop in compensation for inadequate accretion of myelin during the early stages of myelination; it is not clear why this increase does not occur in the CST. This phenomenon may bear on the regulation of myelin formation during normal development.

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1070 THE AGING RODENT BRAIN AS A MODEL FOR THE STUDY OF EXPERIMENTAL NEUROPATHOLOGY BY ELECTRON MICROSCOPY. John E. Johnson, Jr. NASA Ames Research Center, Neurosciences Branch 239-18, Moffett Field, California, 94035.

A criticism of the use of animals in studying certain aspects of neuropathology, particularly brain aging, is that structures seen in human patients are not always found in experimental animals. On the other hand, post-mortem changes between death and fixation may limit interpretation of abnormal findings in the human brain. In the course of examining the brains of rats and mice subjected to centrifugation and special dietary treatment, as well as the brains of normal aged animals, numerous examples of structures were seen which have been reported in cases of human neuropathology. Neuroaxonal dystrophy (NAD) was found in the dorsal column nuclei (gracilis and cuneatus) of aging mice and in the lateral vestibular nucleus of centrifuged rats. Dendritic expansions, neurofibrillary tangles, and Lafora bodies also were found in the lateral vestibular nucleus under various conditions, primarily in the older animals.

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1071 PRE- AND POSTSYNAPTIC NEUROCHEMICAL PATHOLOGY OF HUNTINGTON'S CHOREA. J.P. Bennett, Jr., S.J. Enna, D.R. Burt†, I. Creese*, H.I. Yamamura*, E. Bird*, L.L. Iversen*, and S.H. Snyder. Dept. Pharmacology, Johns Hopkins Univ. School of Medicine, Baltimore, Md. 21205

Huntington's Chorea (HC) is an autosomal dominant neurological disorder with onset of symptoms (choreiform movements and dementia) appearing in mid life. The characteristic neuropathology is a diffuse neuronal dropout, most notable in the caudate nuclei and cerebral cortex. We have examined samples of caudate nuclei and frontal cerebral cortex from a total of 16 HC brains and age-matched controls for two presynaptic markers, L-glutamic acid decarboxylase (GAD) and choline acetyltransferase (CAT), and post-synaptic receptor binding for four neurotransmitter candidates, γ-amino-butyric acid (GABA), muscarinic acetylcholine (ACh), dopamine (DA), and serotonin (5HT). The ligands used to study postsynaptic receptor binding to brain membranes are ^{3}H-GABA (GABA receptor), ^{3}H-quinuclidinyl benzilate (^{3}H-QNB, muscarinic ACh), ^{3}H-5HT and ^{3}H-Lysergic acid diethylamide (^{3}H-LSD) (5HT receptor), and an appropriate ^{3}H-ligand for the DA receptor. Our results show the following: 1) Confirming previous observations, we find an 85% reduction in GAD activity and a 53% reduction in CAT activity in HC caudate nuclei. 2) Of the postsynaptic receptors measured, only muscarinic ACh and 5HT showed significant decreases in HC caudate nuclei. 3) There were slight, non-significant decreases in GABA receptor binding and increases in DA receptor binding in HC caudate nuclei.

From such knowledge of changes in pre- and postsynaptic neurotransmitter markers, certain inferences can be made concerning both the neurochemical etiology of extrapyramidal symptoms in HC and the neurotransmitter-specific neuronal connections in normal human caudate nuclei. (Supported by USPHS Fellowships 5T01GM0062414 (JPB), MH01598 (SJE), NS01654 (DRB), RSDA MH33128 (SHS), USPHS Grant DA00266, and the Benevolent Foundation of Scottish Rite Freemasonry, Northern Jurisdiction, USA.

1072 TEMPORARY BILATERAL CAROTID ARTERY OCCLUSION IN THE GERBIL.
Gary Wise, C. Ionnidis*, M.E. Stevens*, E.C. Shuttleworth, and J.N. Allen, Columbus, Ohio 43210

Cerebral ischemia without brain stem-cerebellum ischemia, systemic hypotension or acidosis can be produced by placing microclips on both carotid arteries in the gerbil. The EEG becomes isoelectric within 30 seconds in over 90% of animals. Cerebral lactate is 3 fold elevated with 5 minutes occlusion. Blood lactate remains normal with 30 minutes of occlusion. Cerebral percent water increases significantly ($p < .001$) with 10 minutes occlusion while cerebellum-brain stem water content is normal even with 30 minutes occlusion. Cerebral edema is reversed within 3 hours after 15 minutes of temporary occlusion but not after 20 or 30 minutes of temporary occlusion in untreated animals. Treatment with norepinephrine or glycerol results in reversal of the increased cerebral water content ($p < .01$) 3 hours after 20 minutes of temporary bilateral carotid artery occlusion while low-molecular weight dextran produces a borderline result ($p < .025$). Corticosteroid drugs, glucose, saline, mannitol, papaverine and heparin did not help reverse the cerebral edema in this ischemic model. There was no difference with barbiturate or ether anesthesia in edema reversal after 20 minutes of occlusion. Dextran and saline infusion produced a borderline increase in survivals following bilateral carotid occlusion while corticosteroid drugs, mannitol, glucose or glycerol were not helpful. Survival studies were too dependent upon body fluid balance to accurately assess the degree of ischemic brain insult in our opinion. Reliable survival studies require 24 hour nursing and maintenance of normal body fluid status in this model.

1073 ALPHA BLOCKADE AFTER SPINAL CORD INJURY. Timir Banerjee. Div. of Neurosurgery and Dept. of Oral Surgery, Sch. Med., UNC, Chapel Hill, N.C., 27514, USA.

Catecholamines have been reported to increase 4-fold in an hour after trauma. This may lead to vasospasm, ischemia and massive hemorrhage in the central cord. This study was carried out to determine the efficacy of Phenoxybenzamine, to prevent or favorably modify the traumatic pathology, when injected within the substance of the cord. Phenoxybenzamine was injected, within the substance of the spinal cord of female cats weighing between 1.5-2.5 kg, immediately after a standard 400 Gm cm force was applied on the thoracic cord to produce paralysis. The animals were observed for 6-8 weeks. Control animals were paralyzed using the same force after it was established that intra-axial injection of 0.01-0.03 ml of saline stained with Indigocarmine did not produce any recognizable deficit. The fore limbs and the hind limbs were electrically stimulated to determine thresholds of tolerance and motor movements. A movie will be shown contrasting the control animals with the group treated with Phenoxybenzamine. The treated animals were able to walk and jump better than the control group.

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1074 BEHAVIORAL EFFECTS OF SPINAL CORD TRANSECTION IN THE DEVELOPING RAT. Eric D. Weber* and Dennis J. Stelzner. (SPON: J. A. Horel). Dept. Anat. S. U. N. Y. Upstate Med. Cntr., Syracuse, New York 13210.

Albino rats 0, 4, 9, 12, 15, 18, 21 or >90 days of age were given a mid-thoracic spinal cord transection. Evaluation of responses of the hindlimbs to a variety of behavioral tasks was begun on the day of surgery and at intervals throughout the postoperative survival period (up to 300 days). At the conclusion of the experiment a rating system for the data obtained for each behavioral task was developed. Three investigators, independently and without knowledge of the animals' ages or survival time, rated the response data. Histological study showed all transections to be complete.

Large differences in behavior are observed when animals transected at the neonatal stage (0-4 days of age) are compared with animals transected at the weanling stage (21-26 days of age). (Exp. Neurol. 46:156-177). Results of the present investigation indicate a critical period near 15 days of age; animals lesioned prior to this age (0, 4, 9, 12 days of age) show response development and recovery similar to the neonatally lesioned animal, whereas those animals lesioned at a later age (18, 21, >90 days of age) show little recovery and are behaviorally similar to the weanling transected animal. In animals lesioned prior to the fifteenth postnatal day, postural responses appear depressed for a brief period but recover rapidly while most responses of animals in the older groups are depressed for longer periods and never attain the degree of recovery characteristic of the neonatally transected animal. Finally, like the neonatally transected animal, rats lesioned on the fourth, ninth, and twelfth postnatal day develop certain responses at appropriate times relative to chronological age. If, however, these responses are mature and supraspinal control is present at the time of lesioning, they appear to be permanently shocked and fail to recover to any substantial degree.

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1075 EARLY DECREASE IN CYTOCHROME OXIDASE ACTIVITIES IN EXPERIMENTAL SPINAL CORD INJURY. Terufumi Ito* M.D., Norman Allen, M.D. and David Yashon, M.D., Div. Neurol., Ohio State Univ. Hosp., Columbus, Ohio 43210.

Cytochrome oxidase, an enzyme of the inner mitochondrial membrane, has been measured as an indicator of the distribution and time course of major mitochondrial damage in acute spinal cord injury. Fifteen experimental and four non-traumatized dogs were used. Injury at the level of T 3-4 was produced by a 400 gm. cm. force while control samples were removed from the T 10-13 level. Samples obtained at ¼, ½, 1, 2 and 4 hrs after injury were cut into 2 blocks, the first extending 4 mm. from the apparent center of trauma and the next occupying the adjacent 4 mm. and were frozen at -80°C. The blocks were mounted in a cryostat at -12°C and cut so that a series of 20 micra sections were obtained at 0, 1, 2, 4 and 5 mm. distance from the trauma center. Three sections of each set were dried at -20°C and microdissected for gray matter and white matter samples. Cytochrome oxidase assay was performed by a microspectrophotometric method using reduced cytochrome C. In each set one whole section was also assayed for enzyme and another prepared for histological control. Normal value for dog gray matter was 5.76 ± 0.27 umole/hr/mg dry weight (mean ± S.E.M.), for white matter 0.65 ± 0.03, and for whole undissected cord section 1.14 ± 0.05. In traumatized cords, gray matter enzyme activities were reduced al all time periods at 0 mm, with 3.37 ± 0.25 at 15 min., 4.02 ± 0.95 at 30 min., 3.15 ± 0.41 at 1 hr., 3.56 ± 0.61 at 2 hr. and 4.18 ± 0.38 at 4 hrs. Values at 1 and 2 mm. from center were intermediate and rapidly rose to normal levels at 4 and 5 mm. Values for white matter did not differ from control activities at any time period. Decrease in enzyme activity correlated well with entent of edema and neuronal damage on control slices. Findings are indicative of early mitochondrial damage in gray matter, detectable at 15 min. and maximal by 1 hr. with gradual recovery of enzyme activity at later periods. Supported by NINDS Grant #5P01 NS10165-03.

1076 EXPERIMENTAL SPINAL CORD INJURY: THE CORRELATION BETWEEN THE CLINICAL AND PATHOLOGIC CONDITION. Thomas B. Ducker, John T. Lucas*, and Warren B. Garrison. Dept. of Neurosurgery, Med. Univ., Charleston, South Carolina, 29401. U.S.A.

The question as to just how well post-injury microscopic change translated into clinical function was the objective of this study. The animals of this study were given a clinical rating based upon objective neurologic signs and/or were sacrificed at varying times post-injury and the cord examined grossly and microscopically for pathologic change. Twenty-five Rhesus monkeys were included in the study. Each animal was subjected to a T11/12 laminectomy, followed by trauma inflicted by dropping a known weight for a given number of centimeters upon the cord. One week following injury the animal was graded clinically. A standard grading procedure of 0-4 was used wherein Grade 0 was complete paraplegia and no movement of the joints and Grade 4 was an indication of no noticeable neurologic deficit. The animal was then sacrificed, and in double blind fashion a separate investigator fixed the cord and determined the pathologic grade. Again, Grades 0-4 was used with Grade 0 denoting complete cord disruption and Grade 4 denoting no deficit. The clinical and pathologic grades were then compared. Of the grades, 64% were identical while 28% were within one digit. Eight percent had a discrepancy of three digits. It is apparent that the overall correlation between clinical and pathologic grading systems for spinal cord injury are high 92%. This finding would indicate that the extrapolation from either such scale to the others a logical procedure.

1077 THE EFFECT OF DORSAL SPINAL CORD INFARCTION ON FUNCTION AND THE SOMATOSENSORY EVOKED RESPONSE IN CATS. M. H. Bennett, J.E.McCallum*, T.S.Stasiak*. Dept. of Neurosurgery, Sch. Med., University of Pittsburgh, Pittsburgh, 15213

The dorsal and dorsolateral columns of the spinal cord are said to be responsible for conduction of the cortical evoked response to peripheral nerve stimulation in cats. In order to test this hypothesis the pia mater and the vessels which it invests were removed over 1 segment of the dorsal half of the lower cervical spinal cord in six cats. Infarction of the entire posterior cord resulted. Averaged evoked responses to sciatic nerve stimulation (SER) were recorded from scalp electrodes before surgery and sequentially for one to five days after.

In each case the SER amplitude was immediately decreased and the latency was increased by pial stripping. Beginning forty-eight hours postoperatively, a new SER component with a latency of 42-48 msec appeared. At this time, the animal reacted to peripheral stimulation by howling, thrashing with all four limbs, and attacking. Unilateral removal of the dorsal pia and occlusion of the anterior spinal artery failed to reproduce either the functional or electrical changes described above.

Infarction of the dorsal spinal cord in cats caused a transient loss of the SER. Secondarily, a new SER component appeared and was accompanied by development of inappropriate functional responses to cutaneous stimuli.

1078 SUBARACHNOID HEMORRHAGE OR TRAUMATIC LUMBAR PUNCTURE? THE PROBLEM OF BLOOD IN THE SPINAL FLUID. Edwin C. Shuttleworth, James M. Parker, Gary R. Wise, and Mary E. Stevens*. Dept. of Neurol., Ohio State University, Columbus, Ohio 43210

Two possible methods of distinguishing early subarachnoid hemorrhage from traumatic lumbar puncture were investigated, utilizing intracisternal injections of autologous blood in the rabbit as a model.

The percent hemolysis in simulated subarachnoid hemorrhage of from one to 24 hours duration varied from 0.3 to 7%, and was independent both of time after onset and of total red blood cell count in the spinal fluid; hence differentiation cannot be accomplished by this method. On the other hand, a statistically significant ($p<.01$) increase in lactate concentration of approximately 1 mEq/L occurred over the first four hours following onset of subarachnoid hemorrhage, which then returned almost to normal by 24 hours. Other potential sources of lactate (derived ultimately from cerebral hypoxia) were excluded by assuring that the CSF lactate/pyruvate ratio remained normal during the experiments.

The detection of a significant elevation of CSF lactate in hemorrhagic fluid with a normal lactate/pyruvate ratio is strongly suggestive of early subarachnoid hemorrhage.

1079 EXPERIMENTAL SPINAL CORD INJURY BIOMECHANICS. Maurice S. Albin, Tin-Kan Hung*, Thomas D. Brown*, Peter J. Jannetta, Leonid Bunegin* and Roger L. Albin*. Dept. Neurosurg. & Neuroanes. Research Lab., Univ. Pittsburgh Sch. Med. and Biomed. Eng. Prog., Univ. Pittsburgh Sch. Engineering, Pittsburgh, Pa. 15261

This study evaluates the dynamic biomechanical responses of the spinal cord to experimental in vivo impact injury; calculates energy relationships between spinal cord, investing membranes and CSF when subjected to mechanical stresses; and plots some of the deformation characteristics of this neural tissue in order to ascertain the possibility of predicting irreversible mechanical damage. Temporal deformation of the spinal cord of 10 anesthetized cats subjected to prior laminectomy were recorded by an electronically synchronized high speed camera (1500-3000 frames/sec) and the associated biomechanical parameters evaluated.

Peak impact force produced by a 20 gm mass falling from 15 cm height (300 gram centimeters of force/energy) averaged about 1.2 lbs and corresponding to this force, the stress acting on the dural surface reached 40 lb/in^2, equivalent to 2100 mmHg. This high speed photography revealed large deformations, the cord being rapidly compressed to half its posterior-anterior diameter and then returning to its original shape. Peak compression occurred approximately 3 milliseconds after impact force reached its maximal value. The generation and propagation of pressure waves in the CSF was measured and recorded reaching more than 150 mmHg 1.5 cm upstream and downstream from center of impact injury site. Shock absorbing characteristics of the CSF was estimated by CSF removal immediately prior to impact injury resulting in even more marked spinal cord deformation when compared to the cord without CSF removal.

The biomechanical characteristics of the spinal cord can be further understood when the dynamic response of the cord is correlated with various magnitudes of impact forces. It was found that the peak impact force is nonlinearly related to the initial potential energy of the falling weight. The correlation of these biomechanical findings with the physiopathological responses to spinal cord impact injury will be discussed.

1080 HISTOPATHOLOGY OF EXPERIMENTAL SPINAL CORD COMPRESSION INJURY. Eugene D. Means and Douglas K. Anderson. Research Service, V.A. Hosp., and Depts. of Neurol. and Physiol. Sch. Med., Univ. of So. Fl., Tampa, 33612.

Reversible (16 animals) paraplegia was produced in mongrel cats by applying an 170 gm wt transdurally for 5 min to the upper lumbar spinal cord. Two animals each were sacrificed at 15 min, 30 min, 1 hr, 4 hrs, 8 hrs, and 24 hrs and 1 animal each at 5 days, 7 days, 22 days, and 58 days post trauma. Petechial hemorrhages were evident at all post-compression intervals through 24 hours. The hemorrhages occurred predominately in gray matter and appeared to encompass both small veins and arteries. Hemorrhage consistently occurred in propinquity to and into the central canal as well as along the anterior median fissure. Other changes occurring in gray matter were: (1) macrovacuolation and ischemic nerve cell change in anterior horn cells beginning at 30 min after injury; (2) a sweeping loss of most neuronal elements in anterior and posterior horns by 24 hrs in both hemorrhagic and contiguous otherwise normal appearing tissue; (3) central chromatolyses in a few anterior horn cells; (4) diffuse invasion of gray matter by polymorphonuclear leucocytes, and; (5) neuronal inclusions believed to represent leucocytes occurred in anterior horn cells at 24 hrs post injury. "Edematous" changes appeared in white matter as early as 30 min after trauma. At 24 hours axonal swellings were evident in the anterior and lateral funiculi. At 3 days post compression severe necrosis and cavitation involved both gray and white matter. At all subsequent survival periods, a single large cavity or cavities obliterated the gray matter and large areas of white matter with relative sparing of the dorsal columns. Astrogliosis was present throughout necrotic gray and white matter in survivals greater than 3 days. Also in these later survivals lipid-laden macrophages occurred diffusely in involved tissue. The histopathology is similar to that observed in impact injury and suggests a common pathogenic mechanism.

1081 LEAD: THE PERSPECTIVES OF CHILDHOOD ENCEPHALOPATHY , PERSISTENT NEUROLOGICAL SEQUELAE, AND ALZHEIMER'S DISEASE

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Although the deleterious effects of acute as well as chronic exposures to Pb have been recognized since antiquity, little is known in regard to the basic cellular mechanisms of Pb poisoning, especially in the CNS. Above all, our knowledge on the effects and mechanisms of long-term intake of seemingly insignificant doses of Pb on the CNS is woefully deficient.

Our experimental studies during the past 6 years dealing with the subcellular aspects of Pb toxicity in the CNS revealed a few conclusive data relevant to the above problems. Matched sets of young adult rabbits were used in this multidisciplinary approach. Acute Pb poisoning was produced by a single dose injection of tetraethyllead-$Pb(C_2H_5)_4$-(TEL).

TRACE METALS: Levels of Cu and Zn are fairly constant in control animals, as measured in 6 different areas of the brain during the course of the year. Surprisingly, Fe levels show considerable seasonal fluctuation, different for cortical and subcortical tissues.

After TEL intoxication, Cu levels are increased. Zn levels are depressed. The Fe level in winter is the same or somewhat less than in the controls, but, remarkably, is now constant and no longer season-dependent. In some areas, particularly in the cerebral cortex, summer Fe levels then may be much higher than normal, since no decline has occurred. Observe that lead encephalopathy occurs mostly in children, who have lower Fe levels in the brain than adults, and then in the summer, when there normally is a seasonal drop, at least of the cortical Fe. It is hypothesized, furthermore, that the excess of Cu initiates a change of the cell membrane properties, as further indicated by the loss of Zn and alkaline phosphatase, primarily by interfering with the ATP-ADP system.

CYTOPATHOLOGY: Electron microscopy revealed that after exposure of rabbits to TEL, nerve cells of the CNS undergo either a hydropic or pyknotic degeneration which depends on the brain area and antedates the development of major neurological disorders. Hydropic degenerating pyramidal cells revealed in light and electron microscopy the presence of neurofibrillary tangles, consisting of 200 Å tubules. In few animals short segments of 800 Å twisted tubules were discerned. This is quite significant, in view of a recently reported case with histopathological manifestation of Alzheimer's disease 42 years following childhood lead encephalopathy. Since brain tissue retains Pb, it is highly possible that its presence induces: (1) severe defects of the cell membranes, and (2) neurofibrillary transformation of nerve cells, responsible for persistent neurological sequelae.

ENZYMES: Biochemical assays of alkaline phosphatase (AlPh) and acid phosphatase (AcPh) in subcellular fractions of the same brain areas revealed a general loss of AlPh activity, whereas AcPh activity was either increased or decreased dependent on the brain area and mode of cell alterations. The elevated AcPh activity in brain tissues with preferentially hydropic-changed and neurofibrillary-transformed pyramidal cells indicates an interference of Pb with AcPh. This could explain why even in severe cases of Pb poisoning no major lesions occur in the CNS. Additionally, these findings indicate a possible mechanism of neurofibrillary degenerations as seen in experimental Pb poisoning and in human cases with Alzheimer's disease, particularly those with a clinical history of Pb exposure.

These preliminary multidisciplinary data and observations would seem to shed light on the mystery of lead poisoning - the silent epidemic -.

In conclusion, we wish to advance the hypothesis that Pb, ingested or inhaled chronically by the present population as a result of an increasing environmental pollution and reflected by rising blood Pb levels in children and adults, could be one important factor in certain persistent neurological disorders and particularly in the pathogenesis of Alzheimer's disease, senile dementia, and certain other diseases.

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1082 ANTECEDENT EVENTS IN AMYOTROPHIC LATERAL SCLEROSIS.

Mark T. Felmus*, Bernard M. Patten and Linda Swanke*. Dept of Neurol, Baylor College of Medicine, Houston, Texas 77025.

It has been suggested that amyotrophic lateral sclerosis (ALS) is an accelerated aging process in motor systems in the CNS with environmental factors possibly influencing its development and course in predisposed individuals. Metallic toxins (especially lead, mercury and manganese) and participation in athletics are considered to be such predisposing factors with the metals possibly injuring the neuron directly and the athletics possibly causing excessive fatigue of motor neurons leading to subsequent exhaustion and death.

A prospective study of 25 ALS and 25 age and sex matched diseased control patients was done to identify possible antecedent events contributing to the development of ALS. The incidence of factors previously associated with motor neuron disease were tabulated and many biographical items were recorded as an historical screening test. In each patient diagnosis was established in the usual clinical manner by integration of all clinical and laboratory data. Patients and controls were interviewed in an identical manner using a standardized questionnaire which emphasized exposure to metals (lead and mercury), athletic participation, milk ingestion, fractures of bone, previous infections, malabsorption, neoplasms, trauma, electric shock, hypoglycemia, previous surgeries and anesthetics, number and types of dental fillings, periodontal disease, travel to Guam and Kii, Japan, educational achievement, socioeconomic status and time of clinical onset.

Five antecedent events had a significantly different distribution between the 2 populations. The ALS group had a significantly higher degree of exposure to lead and mercury than the control group. Before statistical analysis, exposure was subdivided into 4 categories: heavy, moderate, light and none based on duration and amount of contact with the metallic toxin. A history of heavy lead exposure was found in 24% of the ALS group compared to 8% of the controls ($p < 0.005$). Of the ALS group 28% had no history of exposure to lead whereas 48% of the control group had no exposure to lead ($p < 0.05$); the ALS group had a higher exposure to mercury as well ($p < 0.05$). Patients with ALS were more active in sports: 36% earned varsity letters in high school or college compared to 12% of the controls ($p < 0.025$). Including those who received major athletic awards other than letters, the difference is still significant ($p < 0.05$). In the ALS group 16% gave no history of athletic participation compared to 36% of the control group ($p < 0.05$). Milk ingestion was significantly increased in the ALS population. In the ALS group 76% said they consumed at least one quart of milk per day at age 18 and 36% continued this intake throughout adult life. In contrast, 40% of the control group consumed this quantity at age 18 ($p < 0.05$) and 8% continued this intake throughout adulthood ($p < 0.025$). Significantly more ALS patients sustained bone fractures within 5 years of clinical onset of the disease than the control group (32% vs 12%, $p < 0.005$) but had fewer accidents and major traumas in the same period. Finally, the ALS group had a significantly higher annual income than the control group with 88% of the ALS group earning more than $10,000 per year compared to 64% of the controls ($p < 0.025$).

The data suggest that patients with ALS have a greater exposure to lead and mercury, participate more often in athletics and consume more milk. It is concluded that these antecedent events may be contributing to the pathogenesis of ALS and may constitute "risk" factors which predispose to the development of ALS.

1083 EFFECTS OF LEAD SALTS ON THE C.N.S. MICROCIRCULATION OF RECENTLY HATCHED CHICKS. James S. Nelson, Robert Dahlgren*, and Vernon W. Fischer. Depts. Path. and Pediat., Sch. Med., Wash. Univ., St. Louis, 63110.

Newly hatched chicks were given drinking water containing 1% lead acetate (PbAc) and a commercial chick starter ration containing 4% lead carbonate ($PbCO_3$). After 24 hours, administration of $PbCO_3$ was stopped; PbAc was administered continuously during the experiment. Controls received no lead salts. Control and experimental animals were killed at daily intervals. Sections of brain, heart, lung, liver, and kidney from all animals were examined by light microscopy. Sections of cerebrum and cerebellum from selected animals were examined by electron microscopy. Changes in cerebrovascular permeability were identified by injection of trypan blue. Brain water and lead concentrations were measured during the acute phase of the lead intoxication.

Chicks receiving lead develop progressive ataxia within 3-5 days. The earliest pathological changes affect the C.N.S. microcirculation, and consist of increases in the size and number of capillary endothelial cells. These alterations, which are demonstrable by light microscopy, and breakdown of the blood brain barrier to trypan blue are seen within 3-4 days after initiation of the lead regimen. Parenchymal edema and perivascular hemorrhage occur after the microcirculatory lesions. The lesions are found in cerebellum, cerebrum, and optic lobes, but develop earliest and most extensively in the cerebellum. No lesions are found outside the C.N.S. During the acute phase of the intoxication brain lead concentration is increased 15-30 fold and the percent dry weight of the cerebellum is decreased by one fourth. Death occurs in all animals within 10-12 days.

Our observations demonstrate that an experimental model of selective, C.N.S. microcirculatory injury characterized by increased vascular permeability, may be rapidly and conveniently produced by feeding lead salts to recently hatched chicks. The parenchymal changes in the hatched chick differ from those produced by Hirano and Kochen with lead salts in the embryonic chick (Lab. Invest. 29:659, 1973). (This study was supported by U.S.P.H.S. Grant N.S. 11277).

1084 TRANSFERENCE OF DYSTROPHIC MURINE MYOTONIA BY SCIATIC CROSS-REINNERVATION OF DYSTROPHIC/NORMAL PARABIOTIC MICE ($129B6F_1Jdy/dy$ and $C57BL/6Jdy^{2J}/dy^{2J}$). W. B. Douglas. Dept. of Neurosciences, McMaster University Medical Centre, 1200 Main Street West, Hamilton, Ontario, Canada L8S 4J9.

In addition to atrophy and weakness, one of the chief characteristics of dystrophic mouse muscle is persistant myotonic activity. Among the three inbred strains and two hybrid crosses in which the disease is expressed, the myotonic activity is of increasing severity in the following order: C57BL/6J dy/dy; 129/ReJ dy/dy; $129B6F_1J$ dy/dy; $129B6F_1J$ dy/dy^{2J}; and C57BL/6J dy^{2J}/dy^{2J}. Isometric myographic measurements indicate that these progressive differences are related to the maximal, indirectly evoked, twitch and tetanic tensions of triceps surae (TS) and tibialis anterior (TA) of each type of mutant mouse. Therefore, outward expression of the myotonia is dependent on the contractility of the striated muscle affected by the dystrophic process.

Sciatic cross-reinnervation of 3-week mice united in parabiosis, as N→dy and dy→N pairs, has shown that normal muscle structure, strength, and histochemical properties are not initiated and maintained when dystrophic leg muscles (TS, SOL, TA, EDL) are reinnervated by normal sciatic nerves. Conversely, normal muscles reinnervated by sciatic nerves from spinal cords of parabiotic dystrophic mice retain their normal structure, contractility, and chemical composition (Exp. Neurol. 48: - , 1975 in press). Parabiotic union of normal and dystrophic mice reveal, however, that the presence of myotonia is modified by the direction of cross-reinnervation: myotonia is absent in dystrophic muscles of the N→dy sciatic cross; leg muscles which are structurally and mechanically normal gain myotonic properties when reinnervated by dystrophic sciatic nerves in dy→N parabionts. A spinal locus which generates persistant muscular contraction in dy/dy mice is strongly suggested by these observations.

Morphologic (Bradley and Jenkison, J. Neurol. Sci. 18: 227, 1973) and electrophysiologic (Huizar, Kuno and Miyata, J. Physiol. 247: -, 1975 in press) studies indicate that interaxonal "cross-talk" is present in the spinal roots of dystrophic mice. In single dystrophic mice and in dystrophic donors of dy→N crosses, the myographically detected myotonia of TS and TA is abolished by procaine anesthetisation of sensory roots or by dorsal rhizotomy at segments L_4, L_5, and L_6, which supply afferent fibers to murine sciatic nerves. Mid-thoracic spinal cord transection enhances the myotonic activity, although this effect is not always clearly observable. Single-unit recording of muscle spindle and Golgi tendon organ activity, by both dorsal rootlet dissections and teased sciatic nerve preparations reveal that muscle afferents are active in dystrophic mouse leg muscles. The dynamic and static responses of the muscle stretch receptors, related to isometric extrafusal muscle contractility, are not normal. The direction of change, i.e. enhancement or damping, and its relation to the severity of extrafusal dystrophic atrophy, is currently being evaluated. (This research is supported by a grant-in-aid from the Muscular Dystrophy Associations of America, Inc., and a Special Fellowship from the Muscular Dystrophy Association of Canada.)

1085 NEUROFILAMENTOUS AXONAL DEGENERATION IN VIVO AND IN VITRO PRODUCED BY 2,5-HEXANEDIONE. Peter S. Spencer*, Herbert H. Schaumburg* and Edith R. Peterson* (SPON: Cedric S. Raine). Depts. of Pathology (Neuropath.) and Neurology, Albert Einstein College of Medicine, The Bronx, New York 10461.

In 1973, a group of factory workers engaged in the color-printing of polyvinyl fabrics, at a plant in Ohio, developed clinical symptoms of peripheral nerve disease. Experimental animal studies with the printing-ink solvents utilized in the plant, revealed that prolonged exposure to one solvent, methyl n-butyl ketone or MBK ($CH_3.CO.CH_2.CH_2.CH_2.CH_3$), consistently produced signs of peripheral nerve disease, characterized first by symmetrical weakness of the hindlimbs and, subsequently, by weakness of all four limbs. Examination of the nervous system of these rats showed nerve fiber changes consisting of multifocal axonal swellings containing a core composed of neurotubules and mitochondria, surrounded by a proliferation of 10nm neurofilaments. These giant axonal swellings were spatially related to paranodal retraction of myelin sheaths and, subsequently, to remyelination of denuded axons. Early in the disease, the degenerative changes were largely confined to the distal regions of certain long peripheral and central nerve fiber tracts but, with time, these changes spread proximally toward the perikaryon, while the distal regions which were earlier affected underwent complete fiber breakdown. Such a time-related retrograde spread of axonal degeneration is generally termed "dying-back".

2,5-hexanedione or 2,5-H ($CH_3.CO.CH_2.CH_2.CO.CH_3$) was found to be a major, water-soluble metabolite of MBK. In the present study, we report the results of testing this compound both in experimental animals and in an organotypic tissue culture model of the peripheral nervous system.

For the study in vivo, six rats were injected subcutaneously 5 days a week with up to 340 mg/kg/day of 2,5-H for periods of 19 to 23 weeks. Animals were perfused with fixatives, and selected distal and proximal levels of peripheral nerves and central nerve fiber tracts in the spinal cord were examined by light and electron microscopy. The results showed that the animals developed a dying-back axonal disease indistinguishable from that produced by MBK. Large nerve fibers supplying muscles in the calf appeared to be most vulnerable although, when clinical impairment was apparent, giant axonal swelling and secondary demyelination were found throughout the peripheral nerve supply of the hindlimb and in the distal regions of certain long tracts within the spinal cord.

The in vitro study utilized mature and well-myelinated organotypic cultures composed of structurally and functionally coupled explants of spinal cord, dorsal root ganglia and striated muscle. These cultures were exposed continuously to a drop of nutrient fluid containing 300-400 μg of 2,5-H. Nutrient fluid containing the toxin was replaced twice a week and exposed cultures were studied by bright-field microscopy for periods up to three months. Beginning change was noted after 2 weeks. The distal regions of large myelinated peripheral nerve fibers developed multifocal axonal enlargements sited both paranodally and internodally. This was followed by demyelination of the affected regions. After a more prolonged exposure, distal regions of axons underwent breakdown while more proximal sites displayed axonal swelling and demyelination. These degenerative changes were most marked in motor fibers continuous with the ventral root components. This was accompanied by atrophy of the striated muscle. Axonal swelling was also detected in broad fibers within the spinal cord portion.

In summary, prolonged exposure to 2,5-H produced dying-back axonal disease of the giant axonal type both in vivo and in vitro. These studies show that 2,5-H is responsible for the neurotoxic effects of MBK. Together with n-hexane ($CH_3.CH_2.CH_2.CH_2.CH_2.CH_3$), these chemicals form a class of compounds capable of inducing non-specific 10nm neurofilamentous accumulation within axons and subsequent axonal breakdown.

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1086 QUANTITATIVE CYTOCHEMISTRY OF RNA IN RUBRAL NEURONS DURING AXON REACTION. S. Schreiber* and K. D. Barron. Dept. Neurology, Albany Medical College, Albany, New York 12208.

Severance of axons of neurons intrinsic to the mammalian CNS seldom is accompanied by anatomic or functional regeneration. In contrast extrinsic neurons, projecting outside the CNS, react to axonic interruption by constituting new efferent processes. Since the nerve cell body is essential to axon growth and maintenance it seems reasonable to seek the key to failure of CNS regeneration by study of the somal response to axonic injury.

We report RNA content and concentration in feline red nucleus (rn) neurons after one-sided (left) rubrospinal tractotomy performed at the C-2 segment by lateral funiculotomy. The cervical rubrospinal tract is crossed completely and the rn ipsilateral to operation serves as a control. The operation cannot directly traumatize the rn or the brain. Spinal afferents to rn are non-existent or inconsequential so that direct transneuronal effects are not a consideration. In contrast corticectomy performed in investigations of retrograde thalamic atrophy introduces potential complications such as indirect trauma to the thalamus and deafferentation of thalamic nuclei (destruction of corticothalamic fibers).

Adult cats survived lateral funiculotomy 2, 6, 9, 14, 28 and 60 days. Sacrifice was by intra-aortic perfusion of 2 liters of 10% neutral phosphate-buffered formalin and 4 liters of Clarke's solution (3:1, ethanol: acetic acid) at room temperature. The midbrain was immerse-fixed in Clarke's solution for 4-6 hours. Following paraffin embedment 10 u sections through the rn were stained for RNA with azure B (Shea, 1970) after DNAse pre-treatment. Quantitative microspectrophotometry at 590 nm utilized a Zeiss cytoscan system and microscope and NPM-05 photometer head and an Apamos II program operated "on line" with a Digital Equipment Corporation PDP-12 computer. Most measurements, except in the 60 day animal were made from the caudal 1/2 of the nucleus. Rubral neurons were divided into large and small classes based on the total number of absorbing points. Mean extinctions for cytoplasm and nucleolus were measured separately. At least 50 neurons were assayed on each side.

In the reacting red nucleus concentration of cytoplasmic RNA was increased in large neurons 2 days postoperatively but thereafter decreased progressively. By 28 days neuronal atrophy was apparent and mean extinctions for cytoplasmic RNA of large neurons showed an approximate 30% decrease as compared to the control (non-reacting) side. Small nerve cells also were depleted of cytoplasmic RNA at this time. By 60 days postoperatively loss of cytoplasmic RNA was apparent in large and small neurons through the rostro-caudal extent of the rn.

Nucleolar size determined by filar micrometry was not significantly different for large reacting neurons 2 days postoperatively and ME values likewise were similar to controls. Qualitative changes in nucleoli, especially vacuolation, were sometimes encountered, however. Nucleolar ME values for large neurons at 28 days were significantly less (20-25%) than on the control side. A similar depletion of nucleolar RNA occurred in small neurons in some animals.

The results will be compared with morphometric and RNA data reported by investigators who have studied axon reaction in peripherally-projecting nerve cells.

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1087 PSYCHOVIROLOGY OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS INFECTION OF RATS. Andrew A. Monjan, Lee S. Bohl*, and David S. Olton*. Depts. of Epidemiology and Psychology, Johns Hopkins Univ., Baltimore, Md. 21205.

In the rat, neonatal infection with lymphocytic choriomeningitis (LCM) virus can result in an acute destructive lesion of the retina or cerebellum. Aside from these early necrotic pathologies, which are immune-mediated, there is a marked retardation of general somatic as well as of brain growth, with no apparent associated pathology, as well as a slowly developing lesion within the dentate gyrus of the hippocampus. It is these latter observations which led us to study the long-term behavioral consequences of LCM virus infection of the neonatal rat. Rats were infected with LCM virus at different ages during the first 3 postnatal weeks. As adults, they were subjected to a variety of behavioral tests and then the brains were examined histologically or analyzed for DNA, RNA, and protein levels. Animals infected during the first postnatal week differed from controls only in behaviors associated with a component of emotionality; infected animals were less emotionally reactive than controls. Rats inoculated during the second and third postnatal weeks did not differ from controls on any measures. Morphologically, these behavioral alterations were associated with the loss of the hippocampal dentate gyrus. Biochemical analyses indicated that the non-necrotic loss of brain mass was due to decreased cell number rather than cell size. This effect was again limited to early postnatal inoculation with LCM virus, and possibly due to an interference with neuronal replication. Thus, viral infection of the CNS can have effects on the organism far outlasting the acute phase of the disease. Alterations in limbic system reactivity are possible sequelae, as are somatic effects such as growth retardation. Age at infection appears to be a critical variable in determining the severity of these residua.

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1088 STRUCTURAL CHANGES OF NEURONS IN AGING MICE: A GOLGI STUDY. Jesus Machado-Salas*, M. E. Scheibel*, and A. B. Scheibel. Dept. Anat., Sch. Med., UCLA, Los Angeles, 90024.

Golgi studies of the aged cerebral cortex in patients who showed behavioral evidence of senile deterioration have revealed a sequence of changes in the dendrite domain of pyramidal neurons. These alterations progressed from irregular swelling, spine loss and nodulation through increasing loss of dendrite mass to eventual disappearance of entire dendrite shafts. Basilar dendrite systems were lost preferentially, with later appearing loss of apical shafts immediately preceding cell death.

We have now extended our studies to the spinal cord and brain stem, using very old mice (26 and 30 months of age) as subject matter, controlled with young adult mouse material (3 & 9 months). Modifications of the Golgi method were used on all tissue, and controlled with usual histological technique.

Changes observed in various types of spinal neurons and in virtually all types of brain stem cells included: 1) swelling and lumpiness of soma-dendrite silhouettes, bizarre distortion of cell bodies, nodulation and fragmentation of dendrite systems, loss of dendrite domains and eventual loss of neurons.

We suggest that prior to cell loss, the widespread impoverishment of available dendritic surface in aging neural systems progressively restricts information input, thereby degrading appropriateness of response to the environmental challenge.

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1089 FURTHER OBSERVATIONS ON CHANGES IN NORADRENERGIC INNERVATION FOLLOWING CEREBRAL CORTEX LESIONS. Florry P. Bowen and Jana Kosarova. Dept. of Neurology, Mt. Sinai School of Medicine, New York, N.Y. 10029 and Bronx, V.A. Bronx, N.Y. 10468.

Using the histofluorescent technique (Falck Hillarp, 1962), it has been found that bordering freeze lesions of the rabbit cerebral cortex were many abnormal noradrenergic nerve terminals as well as sprouting terminals (Bowen, et al, 1975). In the initial studies changes in noradrenergic nerve terminals were observed up to 3-1/2 months. In additional studies, the following has been observed: 1. abnormal noradrenergic nerve terminals were still visible in the area of the lesion 8 months after freeze injury, 2. when freeze lesions (epileptogenic) are contrasted with stab wound lesions (non-epileptogenic), noradrenergic changes in stab wound lesions are minimal compared to those seen in freeze lesions, 3. examination of the area homotopic to the freeze lesion, revealed some green pyknotic cells (similar to those in the area of the freeze lesion) and apparent changes in noradrenergic innervation of blood vessels, 4. topical application of 0.1 cc. of 10^{-3}M solution of 6-OHDA to either rabbit or rat sensorimotor cortex produced focal spiking, with secondary foci or seizure activity over the entire cortex. The possible role of extracallosal pathways in the potentiation of cortical foci will be considered.

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1090 A SERIAL ELECTRON MICROSCOPY ANALYSES OF CEREBRAL CORTEX ADJACENT TO CHRONIC EPILEPTOGENIC FOCUS IN CATS. Marcos Velasco, Francisco Velasco and Alfredo Feria-Velasco. Divisions of Neurophysiology and Pathology, Scientific Research Department, IMSS, México, D. F.

Present study analyzes the ultrastructural changes of alumina cream (AC) epileptogenic lesions in animals sacrificed during latent, convulsive and remission stages. Analyses include the histopathological elements found at the lesion core and edge as well as the perilesional cortical tissue. Data were compared with those observed both from animals with silicon (S) non epileptogenic lesions and from control animals operated but no lesion produced.

1- At the central core all AC epileptogenic lesions and S non epileptogenic lesions were similar, showing macrophages with dense spherical bodies and vacuoles filled with electron dense amorphous material. Rest of destroyed cells and amorphous material were also seen at the interstitial space.

2- At the edge all AC epileptogenic lesions showed neither fibrotic capsule nor other inflammatory changes shile S non epileptogenic lesions showed morphological signs of a chronic granulomatous inflammatory reaction. No peculiar features of these inflammatory elements were observed under the electron microscope.

3- Changes in number of microglial processes (fibrous astrocytes) and morphological signs of progressive degeneration of neuronal elements at the perilesional cortical tissue were found through the latent, convulsive and remission stages of AC lesions. By contrast, cortical tissue adjacent to S non epileptogenic lesions showed no astrocytic proliferation whereas degeneration signs of neuronal elements were similar although less pronounced than those found in the convulsive stage of AC lesions. No gross ultrastructural alterations were found at the cortical tissue obtained from intact cortices, in spite of scarce microglial processes at the perivascular regions and in the vecinity of myelinated fibers.

Present findings at the lesion edge support the idea that presence of clinical and EEG seizures in AC lesioned animals is independent of the scar formation and other inflammatory chronic elements. On the other hand, further studies should be done to clarify the significance of increase astrocytic processes and progressive degeneration signs of the perilesional cortical tissue in the physiopathogenesis of convulsive activity.

1091 RELATIONSHIPS BETWEEN SELF-STIMULATION BEHAVIOR, BRAIN ETHANOL AND ACETALDEHYDE AFTER ETHANOL VAPOR WITHDRAWAL IN RATS. T.D. Tyler* and C.K. Erickson. Dept. Pharmacol. Toxicol., Sch. Pharmacy, Univ. of Kansas, Lawrence, KS, 66045.

Female albino rats with bipolar stainless steel electrodes in the medial forebrain bundle were trained to self-stimulate in a lever-press situation, and a lever-pressing profile at various current intensities was obtained for each rat. The rats were then exposed to gradually increasing concentrations of ethanol vapor in air in a sealed chamber maintained at 37°C for 4-5 days. Blood samples were obtained from the tail at daily intervals during exposure. Upon withdrawal of ethanol vapor, 15-min self-stimulation samples were obtained every hour. The brains of vapor-exposed rats without electrodes were removed at hourly intervals after ethanol withdrawal began and analyzed along with blood samples for ethanol and acetaldehyde. Analyses of brain and blood ethanol and acetaldehyde were performed using a gas chromatography micromethod. It was found that self-stimulation behavior was depressed while brain ethanol and acetaldehyde levels were high. Self-stimulation behavior resumed 2-3 hrs after withdrawal began, while ethanol and acetaldehyde disappeared from the brain over a longer period of time. Visual observations of withdrawal hyperexcitability correlated with the resumption of self-stimulation behavior. It is concluded that declining brain ethanol and acetaldehyde levels are temporally related to resumption of self-stimulation behavior in rats. (Supported by USPHS Research Grant No. AA-01417 from NIAAA.)

1092 MORPHOLOGICAL EVIDENCE OF SECRETORY ACTIVITY IN THE RAT ORGANUM VASCULOSUM OF THE LAMINA TERMINALIS. Nabil A. Azzam, Heather M. Murray*, Abdallah V. Kubbeh*, and Ching Y. Shih*. Dept. Anat. and Scanning EM Lab., The Univ. of Iowa, Iowa City, Iowa 52242.

The organum vasculosum of the lamina terminalis (OVLT) has been studied by light and electron microscopy in a wide variety of vertebrates and, in all species investigated, is located in the rostral wall of the supraoptic recess of the third ventricle.

In the rat and the rabbit this area of the third ventricle is separated from the main ventricular cavity by an approximation of the anterolateral walls. This approximation is caused by the anterior hypothalamic areas which bring the ependymal surfaces into apposition such that their cilia interdigitate. However two channels of communication remain open. The inferior one leads suprachiasmatically towards the median eminence, while the superior one leads retrocommissurally towards the subfornical organ. Both communicate with the ventricular cavity proper.

The function of the OVLT has not been determined yet. However, it is considered one of the circumventricular organs, in which the blood-brain barrier is either lacking or shifted to the ependymal surface. Based on its ultrastructure, LeVeque and colleagues (1967) suggested a secretory function of the rat OVLT, whereas Weindl and Joynt (1972) proposed that the organ might function as a central receptor. The present study was undertaken in an attempt to shed light on this question by means of SEM, LM, TEM, and Fluorescence Microscopy. Our preliminary results suggest that the ependymal cells covering the ventricular surface of the rat OVLT are themselves specialized secretory cells. Our evidence is gained through observation of the formation of membrane-bound secretory material on the surface of these ependymal cells. (Supported by funds provided by the Graduate College for the utilization of SEM in this project.)

1093 EFFECTS OF CLONIDINE AND APOMORPHINE ON THE RESERPINE-INDUCED ENHANCEMENT OF ELECTROSHOCK SEIZURE IN RATS AND MICE. P. C. Jobe, P. F. Geiger*, and P. K. Staab*. L.S.U. School of Medicine, Shreveport, Louisiana 71130 and Northeast Louisiana University, Monroe, Louisiana 71201.

We have previously reported that central dopamine (DA) and norepinephrine (NE) receptor stimulation in rats antagonizes the electroshock (ES) enhancing effects of the benzoquinolizine Ro 4-1284, a drug which severely depletes brain catecholamine (CA) stores. In addition, DA and NE receptor blocking agents selectively disallow the anticonvulsant effects of the corresponding receptor stimulants. The purpose of the present study was to determine whether or not stimulation of CA receptors also antagonizes reserpine-induced ES enhancement. It is known that, compared to Ro 4-1284, reserpine causes a more prolonged depletion of brain CA stores. We found that the electrical current strength required to produce tonic extensor convulsions in 50% of rats and mice [tonic convulsive current$_{50}$; (TCC$_{50}$)] was decreased by administration of reserpine (5 mg/kg given 24 hr prior to electroshock) and that this decrease in the TCC$_{50}$ in both species was antagonized by administration of clonidine (10 mg/kg) a NE receptor stimulant, but not by apomorphine (10 mg/kg), a DA receptor stimulant. Administration of both clonidine and apomorphine did not produce a greater antagonizing effect than did clonidine alone.

These data support the concept that NE acts as an endogenous attenuator of ES intensity in both rats and mice. They do not, however, support the concept that DA plays a significant role in this activity. The possibility that DA depletion contributes to Ro 4-1284-induced ES enhancement but not to the enhancement produced by reserpine will be the subject of additional dose response studies with the receptor stimulants.

1094 SUPPRESSION OF AUDIOGENIC SEIZURE IN RATS BY TAURINE, GAMMA-AMINOBUTYRIC ACID, GLYCINE AND AMINO-ISOBUTYRIC ACID. H.E. Laird II, R. Huxtable,* B.C. Jones* and L. Chin. Department of Pharmacology and Toxicology, College of Pharmacy and Department of Pharmacology, College of Medicine, The University of Arizona, Tucson, Arizona 85721.

Taurine (TA), gamma-aminobutyric acid (GABA), glycine (GLY) and amino-isobutyric acid (AIB) were evaluated in male audiogenic rats for anticonvulsant activity. Each amino acid was injected into the right lateral ventricle via implanted cannulae, in doses of 4.8, 9.6 and 19.2 μmoles in a volume of 20 μl. Five minutes following treatment the rats were exposed to a sound level of 110 decibels produced by electric bells. Audiogenic seizure was rated according to ranked scores (i.e., 0 = no convulsion to 9 = maximal convulsion) and anticonvulsant activity was calculated as % reduction of audiogenic seizure response score (ARS), relative to control values obtained following intraventricular injection of 20 μl of saline. The 3 graded doses of TA, as listed above, respectively reduced ARS 31% ($p<0.05$), 46% ($p<0.01$) and 80% ($p<0.01$). The low dose of GABA did not significantly affect ARS but the intermediate and high doses reduced it 37% ($p<0.05$) and 46% ($p<0.01$), respectively. Similarly, GLY in the low dose failed to reduce ARS, whereas the intermediate and high doses reduced it 39% ($p<0.05$) and 49% ($p<0.01$), respectively. In comparison, low and intermediate doses of AIB did not reduce ARS, whereas the high dose reduced it by 39% ($p<0.05$). All 4 amino acids appear to have anticonvulsant activity following intraventricular injection, with TA being the most potent. However, it is noteworthy that all doses of amino acids which caused decreases in ARS also produced varying degrees of behavioral depression.

1095 MODULATION OF AUDIOGENIC SEIZURES BY CORTICAL NOREPINEPHRINE IN THE RAT. William M. Bourn* (SPON: J. Manno). College of Pharmacy and Allied Health Professions, Northeast Louisiana University, Monroe, Louisiana 71201.

Newborn rats from the University of Arizona colony of audiogenic seizure-susceptible rats were given three daily subcutaneous injections of 6-hydroxydopamine (100 mg/Kg). At 60 days of age the animals demonstrated a significant increase in seizure intensity compared to vehicle-treated controls. This was accompanied by depletion of norepinephrine in the cerebral cortex and spinal cord and an increase of norepinephrine in the pons-medulla region. In addition, audiogenic rats subjected to radiofrequency destruction of the locus coeruleus exhibited increased seizure intensity and depletion of norepinephrine only in the cortex. Dopamine concentration was unaffected in both experiments. This work indicates that the noradrenergic modulation of convulsive seizures which has been demonstrated by numerous other investigators involves the locus coeruleus-cortical noradrenergic neuron system.

1096 IS PENTYLENETETRAZOL (PTZ) A "HIT AND RUN" DRUG? Brian A. McMillen* and Lawrence Isaac. Dept. Pharmacol., Univ. Ill. Med. Ctr., Chicago, Ill. 60612.

We have reported that a single injection of PTZ increased 5-hydroxyindoleacetic acid levels in feline cerebrospinal fluid (CSF) for more than 24 hr (Biochem. Pharm. 23: 1223, 1974). It is possible that this persisting effect is related to drug level; is due to an increase in plasma tryptophan which is thought to increase brain 5-hydroxytryptamine metabolism; or is caused by a modification of central neuronal activity which outlasts the presence of drug. We obtained serial samples of CSF and plasma from cats for the determination of PTZ concentrations and the total plasma tryptophan concentrations. The animals given 20 mg/kg i.p. of PTZ had an initial plasma concentration of 28.5 μg/ml, while the value in CSF was 48.7 μg/ml. The half-life of PTZ in both fluids was about 45 min. However, a dose of 40 mg/kg i.p. of PTZ had a half-life of 150 min and the initial plasma level was 59.2 μg/ml. Twenty-four hours after drug injection, the concentration of PTZ in these fluids was below the sensitivity (less than 50 ng/ml) of the gas-liquid chromatographic method used to measure this drug. Total plasma tryptophan was unchanged after 20 mg/kg of PTZ, but 40 mg/kg reduced the tryptophan concentrations 6 to 24 hr after injection. These findings indicate that the brain rapidly accumulates PTZ; that the increase in CSF concentrations of 5-hydroxyindoleacetic acid following PTZ administration is central in origin; and that the modification of central neuronal activity by PTZ far outlasts the presence of drug.

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1097 PRESUMED INHIBITORY SYNAPSES PRODUCED BY ELECTRICAL STIMULATION OF UNDERCUT NEOCORTEX. L. T. Rutledge. Dept. Physiol., Univ. Mich. Med. Sch., Ann Arbor, Mich. 48104

Long-term electrical stimulation of undercut cortex can prevent certain electrical, chemical and morphological changes seen in this model of experimental epilepsy. Such stimulation also alters the excitability of spontaneously firing neurons to test electrical stimulation of the cortical surface. Neuronal elements in the upper layers of cortex from adult cats were measured in an electron microscopic study. Tissues were from: Intact Cortex (IC), Undercut Cortex (UC) and Undercut Stimulated Cortex (USC). After a systematic sampling of areas in cortical sections and then working from electron micrographs, counts were made of terminals with round vesicles (RV), flat vesicles (FV), mixed RV and FV, and of synaptic types (Gray's 1 and 2). RV terminals were significantly fewer in UC and USC as compared with IC, but only UC differed from IC in having reduced numbers of Type 1 contacts. The most important finding concerned Type 2 contacts and FV terminals. USC had significantly more (55%), Type 2 contacts than did IC and UC. There was no difference in this measure between IC and UC. When comparisons were made of FV terminals, including those with mixed RV and FV, there was nearly twice as many such terminals in USC than there was in either IC or UC. With the mixed terminals eliminated, the difference, though smaller (84%), was in the same direction. Long-term stimulation apparently produced new terminals with flat vesicles and more Type 2 contacts. If FV terminals and Type 2 synaptic contacts function as inhibitory elements then these studies indicate that long-term electrical stimulation of undercut neocortex produced new inhibitory synapses. The presence of an increased amount of functional inhibition in USC is a likely basis for some of our previous observations. The results also support the "Use Theory" of synaptic plasticity. (Supported by NIH Grant NS-04119).

1098 EEG SPATIAL PATTERNS OVER PALEOCORTEX IN WAKING ANIMALS. Walter J. Freeman. Dept. Physiol.-Anat., Univ. California, Berkeley, 94720.

Stainless steel wires (250 microns diam.) were pre-assembled and cemented into rectangular arrays of 6 x 10 electrodes (4 x 7 mm) and were attached to miniature connectors. Each array was placed on the dura over the bulb, anterior olfactory nucleus or prepyriform cortex following appropriate surgical exposure in cats and rabbits. The array and connectors were attached to the skull. Following recovery from surgery, the EEG patterns were observed over several weeks with minimal restraint of the animals and with controlled olfactory stimulation.

The signal from each electrode was amplified on one of 60 preamplifiers (fixed gain = 10^4; filters 3 db fall-off at 0.1 Hz/3KHz or 6 Hz/200 Hz), multiplex, and digitized (6 bits) at intervals of 1.0 msec. Data were taken into core (Interdata Model 7) in blocks lasting up to 900 msec (54,000 samples), displayed on an oscilloscope for editing, and written onto magnetic tape. Sets of 60 EEG traces were drawn in Cal-Comp plots.

Data were recorded during the occurrence of single bursts (100-500msec) associated with inspiration in the presence of filtered air or selected odors (e.g., food, amyl acetate, etc.). In most sets the wave form of each burst was almost constant over the surface except in respect to amplitude and time of onset. Because the bursts were nearly sinusoidal the time relations were expressed as the phase at the common frequency (from the fast Fourier transform). An ensemble average was taken of the 60 waveforms, and by cross correlation at appropriate lag times, the phase of each wave form was determined with respect to the ensemble average. Contour plots were made of the root mean square amplitude and phase for each burst. The time-amplitude distributions in relation to specific stimuli are to be discussed. MH 06686.

1099 THE EFFECTS OF FIGURE-GROUND ON EVOKED POTENTIALS.

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Dept. Psychiatry. Univ. Pgh. Sch. Med., Pgh., PA 15261, and +Dept. Elec. Eng. Penn State Univ., Univ. Park, PA 15802.

As part of a conditioning experiment 9 chronically implanted cats, while Flaxidelized, were habituated to light (L) and click (C) stimuli. Preliminary results have indicated that in habituated animals the waveform of averaged evoked potentials (EPs) elicited by identical stimuli can vary substantially depending on the "pattern" in which the stimuli are delivered. EPs elicited by both L and C were recorded from several loci along the primary sensory pathways and subcortical polysensory structures, during the following three "patterns" of delivering these two stimuli.

a. Ls and Cs alternated 1/sec. for a total duration of 30 min. This phase served as an initial desensitization period.

For example, ... L_1-C_2-L_3-C_4-L_5-C_6-L_7-C_8-L_9-C_{10}-L_{11} ...

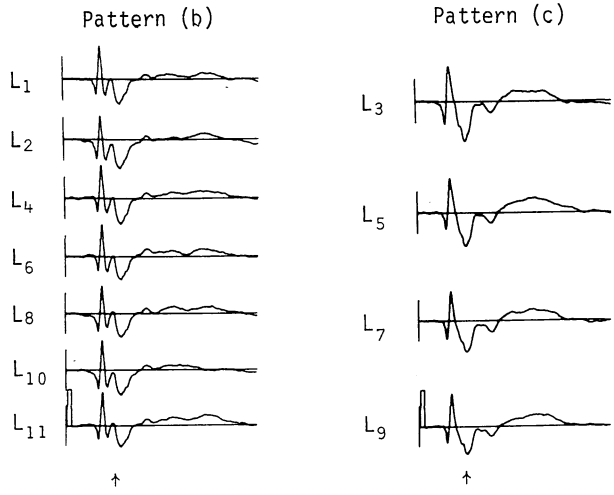
b. Ls were delivered continuously at the rate of 1 every 2 sec. Cs were presented in trains of 4 at the same rate as Ls but were always out of phase by 1 sec. There were 75 trials presented at randomized intervals. Each train of 4 Cs constituted a trial.

For example, ... L_1-L_2-C_3-L_4-C_5-L_6-C_7-L_8-C_9-L_{10}-L_{11} ...

c. This pattern was the same as in (b) except the relationship between Ls and Cs was reversed.

For example, ... C_1-C_2-L_3-C_4-L_5-C_6-L_7-C_8-L_9-C_{10}-C_{11} ...

In order to control for cross modality interactions C_3, C_5, C_7 and C_9 EPs of pattern (b) were compared with C_4, C_6, C_8 and C_{10} EPs of pattern (c); similarly, L_4, L_6, L_8 and L_{10} were compared with L_3, L_5, L_7 and L_9. In most of the structures examined the averaged EPs elicited by the same stimulus were surprisingly different in amplitude, latency or components. Illustrated below, the L EPs recorded from the posterior nucleus show a component which had a latency of 100 msec. in pattern (b); however, this component is absent in the EPs obtained from pattern (c). Startle, orienting reflex or distraction cannot account for these differences because the EPs within each set were invariant. The changes were observed only between the two sets. These results indicate that EPs are not simple responses to stimuli but reflect the total context or the figure-ground relationship of that stimulus. Furthermore, they suggest that caution must be exercised in attempting to relate EP changes to complex psychological processes.



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1100 SECONDARY EPILEPTOGENESIS IN FROG FOREBRAIN: EFFECT OF INHIBITION OF PROTEIN SYNTHESIS. F. Morrell, N. Tsuru*, T.J. Hoeppner, D. Morgan* and W.H. Harrison. Dept. Neurol. Sci., Rush Medical College, Chicago, Il. 60612

Intermittent, low level electrical stimulation of the hippocampal cortex of the encephale isole bullfrog at current densities just above threshold for eliciting a brief after-discharge (AD) leads to progressive augmentation of AD duration (the "kindling" phenomenon described by G.V. Goddard, 1967). Stimuli (balanced biphasic pulse pairs, of 1 msec pulse dur., 62.5 pps and approximately 100μA per pulse) were delivered for 2 sec once per hour. Recordings were obtained from the stimulated hemisphere and from the contralateral homotopic cortex. The AD arising in the 1°, ie. the stimulated, hemisphere was rapidly propagated to the opposite (2°) hemisphere and as the AD duration at the 1° site increased, a similar lengthening was seen in the 2°. Eventually the AD in the 2° region developed considerable independence of wave-shape and sometimes outlasted the AD in 1° cortex. In addition to the stimulus-bound AD, spontaneous epileptiform potentials (SEP) began to appear in the inter-stimulus intervals, first in the 1° hemisphere, then in the 2° hemisphere, eventually becoming independent in the 2° region. The frequency of SEP's increased as a function of number of stimulations. Thus, the measures available included the duration of AD at 1° and 2° sites, frequency of SEP's at the 1° site and evidence of independent epileptiform activity (SEP's) in the unstimulated cortex.

To examine the role of protein synthesis in secondary epileptogenesis incorporation of systemically administered ^{14}C leucine into brain protein was assayed using standard scintillation counting. The normal curves thus established for the frog were comparable to those for goldfish published by Brink et.al. 1966. Intraperitoneal injection of cycloheximide (CXM) (50 mg/kg) resulted in profound inhibition of protein synthesis (over 80% in all animals and over 90% in most) lasting well over 24 h. Animals treated with cycloheximide and then subjected to the kindling procedure revealed virtually no evidence of the progressive prolongation of AD duration characteristic of the untreated frog ($p=0.1205 \times 10^{-7}$). The frequency of SEP's was markedly reduced in the CXM-treated animals and SEP's were confined to the 1° hemisphere ($p=0.6 \times 10^{-10}$). Most important was the fact that there were no independent spikes in the 2° hemisphere.

Since the CXM did not disturb normal electrogenesis and did not interfere with the paroxysmal response to direct electrical stimulation, ie: the AD, this experiment distinguishes between processes dependent upon purely electrical events and those requiring macromolecular synthesis. It suggests that protein synthesis is critical to the emergence of spontaneous and autonomous epileptiform behavior of neural aggregates.

1101 Cerebellar Inhibition of Intramuscular Penicillin Epilepsy in the Cat. Peter Kellaway and John J. Hablitz, Department of Neurophysiology, The Methodist Hospital and Baylor College of Medicine, Houston, Texas

Adult cats were prepared with chronically implanted electrodes for recording of brain electrical activity and with transcortical stimulating electrodes near the midline of the cerebellum. Following recovery from surgical procedures (7-10 days) these animals were given intramuscular injections of 300,000 units of sodium penicillin G per kilogram of body weight. As reported by previous investigators, this led to the development of diffuse, bilaterally symmetrical spike-wave discharges approximately 1 hr subsequent to penicillin administration. When fully developed, these discharges were quite stable and regular, and persisted for up to 6 hr. This procedure gave consistent results when repeated at 3-day intervals.

Cerebellar stimulation (0.1 msec pulses, 0.25-0.50 mA) was applied for 10-sec periods alternating with 10-sec off periods. The stimulus current levels employed did not evoke any observable movement and stimulus onset at 10-sec intervals did not visibly alert the animal. Stimulation resulted in a prompt and dramatic decrease in the number and amplitude of paroxysmal events. Computer techniques developed in this laboratory for the detection and quantification of paroxysmal events in human EEG recordings were adapted to the study of this model of epilepsy. These methods allow more thorough and precise analysis of long epochs of data and fully document the extent of cerebellar suppression. This analysis showed that several 10-sec periods of stimulation were necessary before the cerebellar inhibition was obtained. Subsequently, the abnormal discharges were abolished for periods of 3-5 min and significantly suppressed below baseline levels for up to 30 min. Cessation of stimulation resulted in a gradual return of paroxysmal activity to prestimulus levels which was complete after a few minutes. Stimulation at frequencies of 10 and 100 Hz reduced the number of abnormal discharges but 10 Hz was more effective.

It has been previously reported (Gloor & Testa, 1974) that intravertebral injection of pentylenetetrazol diminishes spontaneous epileptiform discharges in this model and this effect was attributed to brainstem activation. The present results suggest that the cerebellum, which also receives irrigation from the vertebral circulation, may have played a significant role in the inhibition demonstrated in that study.

These findings are in contradistinction to the activating effects of cerebellar stimulation we reported in a chronic focal model of epilepsy in primates (Hablitz, et al, 1975). To date, we have been unable to compare data from primates with the current results since massive doses of intramuscular or intravenous penicillin (up to 2,000,000 units/Kg) have failed to induce paroxysmal discharges in the primates. If this model can be produced in primates, it will provide a unique means of assessing species differences in cerebellar function.

1102 CEREBRAL CORTEX NEURONS WITH EXTRA SPIKES: A NORMAL SUBSTRATE FOR EPILEPTIC BURSTING NEURONS? William H. Calvin and George W. Sypert, Dept. of Neurological Surgery, University of Washington, Seattle, Wash. 98195.

Like other CNS neurons, pyramidal tract neurons (PTNs) in the barbiturate-anesthetized cat will discharge rhythmically to a sustained depolarizing current injected through the recording microelectrode. In 25% of the fast PTNs (see Sypert & Calvin abstract, this volume), this rhythmic discharge is interrupted by a double spike: an extra spike perhaps 2 msec after a rhythmic spike. They arise from the peak of hump-like depolarizing afterpotentials, which are very prominent in the neurons with the doublet phenomenon. Currents can be found where one threshold-straddles the phenomenon: the sustained rhythmic firing will be interrupted by extra spikes after only perhaps 20% of the rhythmic spikes. Further increases in the size of the current steps (400 msec long, repeating every 1200 msec) will cause extra spikes to appear after every rhythmic spike, so that the neuron appears to fire rhythmically in double spikes. The double spike separation is typically about 1.5-3.5 msec in PTNs and can be quite fixed for a given neuron. A scatter plot of interspike interval vs. the following interspike interval is particularly instructive: it shows the usual rhythmic discharge as points along the diagonal, progressing towards the origin as current increases shorten the interspike intervals. When extra spikes appear, they form a group of points parallel to the axes. The "arrow pointing to the origin" shape of this scatter plot is characteristic of the extra spike properties of fast PTNs. In some PTNs, a triple spike will be seen at higher currents, where the hump following the extra spike has itself elicited another extra spike in a regenerative cycle. A few neurons will progress to bursts of many extra spikes, each at the 2 msec interval. The stereotyped nature of these extra spike bursts is quite reminiscent of the stereotyped bursts which have been extracellularly observed in both PTNs of epileptogenic cortex (Calvin, Sypert & Ward 1968; Fetz & Wyler 1973) and in normal and deafferented neurons of the external cuneate nucleus (Kjerulf et al 1973; Calvin & Loeser 1975). If the "epileptic" burst is quite stereotyped (one can be superimposed upon the next), extra spikes may be a more parsimonious explanation than massive synaptic inputs driving the rhythmic firing mechanism to high rates. There is, however, another phenomenon which aids in identification: some "epileptic" bursts are stereotyped from the second spike onwards, with the first spike standing out in front of the stereotyped afterburst by fluctuating intervals (3-15 msec). Some spinal motoneurons (Calvin 1974) and PTNs tend to develop large hump-like depolarizing afterpotentials only after the second spike of a rhythmic train; therefore, in these neurons, extra spikes are more likely to start with the second rhythmic spike rather than the first. Thus an extracellularly observed "long-first-interval stereotyped burst" probably represents the extra spike phenomenon. Fetz & Wyler (1973) noted that long-first-interval bursts were very common after a long silence in the spontaneous activity; in a few cases, we conditioned our long-first-interval burst response to a step of current by placing a single spike (elicited by a brief current pulse) beforehand. The stereotyped burst could be made to occur after the first, rather than the second, rhythmic spike by conditioning within 200 msec; between 200-300 msec the interval between the first and second spikes could be graded. It would thus seem as if the extra spike mechanism can be primed, ordinarily by the first rhythmic spike, but also by a conditioning spike in the preceding 200-300 msec. This is a time scale quite unlike the afterpotentials and other membrane properties of PTNs. The postspike humps are thought to be associated with the antidromic invasion of the soma and dendrites (see Hartline & Calvin abstract, this volume), so the 200-300 msec time scale may reflect changes in dendritic excitability.

Supported by NIH grants NS09677, NS04053, and NS05211. Dr. Sypert's present address is Neurological Surgery, University of Florida, Gainesville.

1103 REPETITIVE FIRING PROPERTIES OF FAST AND SLOW PYRAMIDAL TRACT NEURONS. George W. Sypert and William H. Calvin, Dept. of Neurological Surgery, University of Washington School of Medicine, Seattle, Wash. 98195.

From approximately 500 intracellular recordings in the sensorimotor cortex of barbiturate-anesthetized cats, we have examined a population of 70 neurons which 1) could be antidromically identified from the medullary pyramids and 2) which fired repetitively to long pulses of current injected through the recording micropipette. While this population is more biased towards fast conduction velocities than are extracellularly obtained populations, our histogram of antidromic latencies is approximately identical to that of Takahashi (1965) who did not select for the ability to sustain a repetitive discharge (which is considered to be an important indicator of cell "health"). As did Takahashi, we found a direct correlation between the spike duration and the antidromic conduction time; cells with spike durations less than 1.0 msec are invariably fast PTNs (those with conduction velocities greater than 20 m/sec) and vice versa. Extrapolating this linear relation to the slowest PTNs which one would expect on the basis of the axon diameter spectrum would yield 3.8 msec spike durations; the widest observed antidromic spikes were 1.7 msec (2.1 in Takahashi's study) because intracellular recording samples only the fastest 42% of the cells. A prominent property of our slow PTNs was the inconstancy of their frequency-current curves; often we could observe a hysteresis effect where, following high firing rates, the slope of the f-I curve was substantially reduced. One could see an AB (IS-SD?) subdivision of the differentiated antidromic spikes in 77% of the slow PTNs but in only about half of the fast PTNs. The fast PTN population subdivided into two major groups: those exhibiting double spikes during sustained rhythmic firing to injected currents, and those which had only the usual rhythmic firing at a rate proportional to current strength. About 25% of the fast PTNs exhibited these double spikes (see Calvin and Sypert abstract, this volume) and these cells tended to have antidromic spikes which, when differentiated, did not exhibit an AB subdivision. These cells had large hump-like depolarizing afterpotentials during repetitive firing; usually there was a deep (e.g., 4mv) notch between the falling phase of the spike and the peak of the hump, about 2 msec later. Unlike cat spinal motoneurons, extra spikes arising from the peak of this hump occur primarily at firing rates above about 75/sec (in both human and cat motoneurons, this phenomenon is seen at low firing rates and disappears as the synaptic drive increases the rhythmic firing rate). We doubt that PTNs exhibiting double spikes were injured: we were often able to maintain satisfactory recordings from them for up to several hours. Also, fast PTNs exhibiting double spikes during sustained rhythmic firing had the same distribution of antidromic spike heights as did nondoublet PTNs; while there were some double spike PTNs with 60 mv spikes, there were also many with 90 mv spikes. Double spike PTNs were always fast PTNs but they were distributed throughout the range of conduction velocities above 20 m/sec. We would conclude that, while there are some PTN properties which grade uniformly across the entire observed range of conduction velocities (e.g., spike durations), there are other properties (such as extra spikes in fast PTNs) which distribute evenly throughout a more limited range of conduction velocities. Thus, "fast" and "slow" PTNs are not only two ends of a spectrum but may represent some major subtypes. One interesting feature which we have noticed in both fast and slow PTNs is the presence of large (e.g., 2 mv) exponential events in the synaptic noise; they often occur in bursts. If these are unitary PSPs, they are an order of magnitude larger than those seen in spinal motoneurons; the possibility must be entertained that they could be of postsynaptic origin, e.g., repetitive firing in a dendritic spike generator, whose spikes spread passively into the soma.

Supported by NIH grants NS09677, NS04053 and NS05211. Dr. Sypert's present address is: Neurological Surgery, University of Florida, Gainesville.

1104 THE EFFECTS OF CEREBELLECTOMY ON EXPERIMENTAL SEIZURES AND THE ANTICONVULSANT EFFICACY OF DIAZEPAM IN THE RAT. Arthur Raines and Rebecca J. Anderson, Dept. of Pharmacology, Georgetown University, Schools of Med. and Dentistry, 3900 Reservoir Road, Washington, D.C. 20007

Work from several laboratories indicates that cerebellar activation can inhibit convulsive seizure activity. The present experiments were performed to assess the influence of cerebellectomy on convulsions produced by electroshock (corneal electrodes 60 Hz, 200 msec duration) and the convulsant drug, pentylenetetrazol.

Rats were cerebellectomized 72-96 hours prior to evaluation in the maximum electroshock seizure test and for their capacity to respond to pentylenetetrazol-induced clonic seizures. Cerebellectomized rats failed to exhibit tonic hindlimb extension, an endpoint characteristic of maximal electroshock seizures, indicating that the cerebellum is necessary for the full expression of a maximal seizure. The dose of pentylenetetrazol required to produce clonic seizures or death was not different in cerebellectomized and sham-operated controls. The anticonvulsant efficacy of diazepam, when assessed as a pentylenetetrazol antagonist, was not influenced by removal of the cerebellum. These latter data indicate that whereas cerebellar influences may suppress seizure activity which is largely focal, seizures of more diffuse origin are not markedly influenced by cerebellar activity. It is therefore essential that the role of the cerebellum in suppressing seizures be characterized for each kind of experimentally-induced seizure process.

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1105 EFFECTS OF DIPHENYLHYDANTOIN ON VOLTAGE CLAMPED BURSTING NEURONS IN APLYSIA. D. Johnston and G. F. Ayala, Neurol. Dept., Univ. of Minn. Mpls., Mn. 55455

Several groups have investigated the effects of convulsant agents on invertebrate nervous systems. One of the findings has been that a normally silent neuron develops slow membrane potential oscillations when exposed to a convulsant agent such as pentylenetetrazol (PTZ). The purpose of this study was to investigate the effects, if any, of the commonly used anticonvulsant Dilantin (DPH) on neurons which exhibit this slow wave bursting activity. Identified neurons in the abdominal ganglion of Aplysia were impaled with two microelectrodes and routinely voltage clamped. The current-voltage (I-V) characteristics of bursting neurons (L_2-L_6) were obtained before, during and after exposure to .02 to .1 mM DPH by means of voltage step commands from a holding potential which resulted in minimum holding current. Before applying DPH, there was a characteristic region of negative resistence in the I-V curves of these bursting pacemaker neurons. During DPH perfusion, however, the membrane potential oscillations were stablized and bursting activity stopped. The I-V curves of the cells became nearly linear and the region of negative resistance had disappeared. Upon rinsing, the negative resistance returned and the cell resumed bursting. This action of DPH on decreasing the bursting pacemaker response occurred regardless of whether the bursting behavior was endogenous to the cell or induced by PTZ. When depolarizing voltage commands are given to these bursting cells, a slow potassium current is activated which is not a part of the sodium dependent negative resistance. This slow K current was also decreased in the presence of DPH and/or low external calcium. Neuronal bursting activity or negative resistance in cortical cells has been suggested as a contributing factor in seizure initiation. With the results of our experiments the possibility exists that the anticonvulsant effects of Dilantin are due, at least in part, to a suppression of this bursting behavior. (Supported by NIH Grant NS09784 and Minnesota Medical Foundation.)

1106 DIPHENYLHYDANTOIN AND CALCIUM ON APLYSIA NEURONS. J. W. Whisler* and D. Johnston, Biomed. Engrg. Prog. and Neurol. Dept., Univ. of Minn., Mpls.Mn. 55455 (Spon.: W. Raabe).

The common anticonvulsant Dilantin (DPH) has recently been studied quite extensively on invertebrate preparations. One theory for the action of this drug is that it decreases the calcium influx to a neuron. This investigation explored the effects of DPH on the inward calcium current during the action potential (A.P.) in Aplysia neurons. Identified neurons (BL_1 and BR_1) of the buccal ganglion were routinely impaled with double barrelled (Theta glass) microelectrodes. Second order coupling compensation was employed in order to pass current and record membrane potential from adjacent barrels with a minimum of coupling artefact. When the ganglion was perfused with .2 mM DPH, the height of the A.P. was unchanged, but its width (40 mV below the peak) increased by as much as 40%. The duration of the undershoot (from onset to minimum) increased by about 60%. In observing the first derivative of the A.P., we found the positive phase unchanged, but the negative phase was notched and reduced about 50% in amplitude. The notch in the first derivative represented the "hump" in the A.P. which was quite evident with DPH. To explore the ionic basis of these effects the external calcium ion concentration was reduced to zero in the presence of DPH. The prolongation of the falling phase was reversed under these conditions, while the duration of the undershoot remained significantly increased. With 70 μM TTX added to the normal sea water medium, an impulse could be elicited which was predominately due to an inward calcium current. DPH again delayed and prolonged the undershoot of these calcium spikes, but had no other noticeable effects. Although DPH is exerting no apparent effect on the inward calcium current, it is likely that the prolongation of the A.P. is due to an action on the outward potassium current, in addition to the known decrease in the inward sodium current. (Supported by NIH Grant NS09784 and Minnesota Medical Foundation.)

1107 DPH-PHENOBARBITAL INTERACTIONS DURING CHRONIC DOSING IN MONKEY. Joan S. Lockard, Rene H. Levy*, Vladimir Uhlir*, and John A. Farquhar*, Depts. Neurol. Surg. and Pharmaceut. Sci., Univ. Wash., Seattle, Wash., 98195.

In the treatment of focal motor and secondarily generalized epilepsy, DPH and phenobarbital are often prescribed together. The present study investigated the drug interactions in terms of plasma levels during ten-day periods of multiple dosing in four monkeys. DPH at a dose of 30 mg/kg and phenobarbital at a dose of 3 mg/kg were administered separately to different animals by nasogastric intubation daily for ten days. In three subsequent ten-day periods the drugs were administered together in the same animals at different times of the day (immediately following one another, a half hour apart and six hours apart) and in a different order of administration (either phenobarbital first and DPH later, or DPH first and phenobarbital later). Blood samples were obtained on the third, fifth, eighth and tenth day of each ten-day period at 2, 6, 8, 14, 18, 20 and 24 hours from the time of daily administration of the drugs. The plasma drug-level data indicate self-induction of DPH, DPH and phenobarbital absorption and systemic interactions and a decrease in the biological half-life of DPH when administered with phenobarbital.

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1108 INTERCELLULAR SPACE IN EPILEPTIC BRAIN. A. Basil Harris and Donald P. Jenkins*, Dept. Neurol. Surg., Sch. Med., Univ. Wash., Seattle, Wash., 98195.

The extracellular marker horseradish peroxidase (HRP) has been shown to move through the intercellular spaces (ICS) of the brain in a predictable manner. Around epileptic lesions there are general electrical abnormalities with asymmetrical maximal electrical foci. Gliosis spreads in cortex about lesions to relatively great distances and often eccentrically. Using HRP, this gliotic cortex containing increased numbers of glial junctions was studied for alterations of the ICS. Under halothane inhalation anesthesia, 2-3 ml HRP-Ringer's solution was injected at a rate of 0.079 ml/min through a polyethylene catheter inserted through a 30 gauge needle hole in the arachnoid into the central fissure subarachnoid space of control and epileptic hemispheres in Rhesus monkeys. Delivery rate of HRP was 1.3 µl/sec. One hour later, intracardiac aldehyde perfusion fixation occurred. Parasagittal cut serial frozen sections every 300 µm from interhemispheric fissure through temporal lobe, 60 sections per hemisphere, were treated to show HRP with 3, 3'-diaminobenzidine and mounted. Depth of penetration measurements were made in a standard manner at 1.0 mm intervals over the cortical surface, 50/section, or 3000 measurements/hemisphere. Even though the injection sites varied slightly, the pattern of HRP penetration was similar. Maximum penetration occurred in the central fissure, 0.95 ± 0.08 mm, at a rate of ∿ 0.26 µm/sec. Over other parts of control cortex the penetration was ∿ 0.12 µm/sec. Thus central fissure penetration was 53% greater than other sites. In the hemispheres containing experimental epileptic alumina cortical lesions, central fissure HRP at the level of lesions was not significantly different from controls. Within 700 µm peripheral to the lesion sites, the adjacent central fissure cortex penetration was reduced to 67% of that of controls and at 4.3 mm distant was only 42% of that of controls. The data indicate that the nature of the extracellular space adjacent to epileptic lesions, in epileptic cortex, has been modified. Penetration of HRP was more rapid into the extracortical alumina granuloma than any other site, but HRP was impeded from movement out of the granuloma into surrounding brain. Abutting the lesion, HRP moved through the ICS in gliotic cortex more slowly than elsewhere.

Because the HRP molecule is large, it has been used to magnify alterations in the ICS which may affect the movement and communication of small molecules and ions between the intercellular spaces, subarachnoid fluid and serum. The significance of these studies is that for the extracellular marker, HRP, molecular wt. ∿ 40,000, one or two conditions appear operant. Either there is impeded movement through scarred epileptic cortex, diminished intercellular space, or both. The microenvironment of neurons and their processes being thus affected may be the greatest factor contributing to epilepsy.

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1109 KINETICS OF POTASSIUM MOVEMENT IN BRAIN: A COMPARISON OF NORMAL AND GLIOTIC CORTEX. Robert S. Fisher*, Timothy A. Pedley and David A. Prince. Dept. of Neurology, Stanford University Sch. of Med., Stanford, CA 94305.

It has been proposed that abnormalities of resting extracellular potassium concentration ($[K^+]_o$) or of regulation of $[K^+]_o$ levels in injured cortex might be a common factor underlying epileptogenesis associated with focal glial scars. To test this hypothesis we performed a series of experiments comparing the kinetics of K^+ movement in normal and gliotic cortex. Areas of focal cortical damage were produced by freeze lesions made 3 to 12 months before K^+ measurements. $[K^+]_o$ was measured as a function of time and depth during superfusion with solutions containing 12 mM K^+. Baseline $[K^+]_o$ in the scarred areas was normal (3.21 ± 0.21 mEq/L). In both normal and gliotic cortex, analysis of the $[K^+]_o$ versus depth and $[K^+]_o$ versus time profiles showed that distribution of extracellular K^+ could be best modelled by a process of diffusion corrected for a surface barrier and a small amount of uptake into cells and blood vessels. The intracortical diffusion coefficient for K^+ in gliotic cortex (0.93 ± 0.15 mm^2/m) did not differ significantly from that in normal cortex (1.03 ± 0.16 mm^2/m) but the barrier constant (0.6 ± 0.2 mm^{-1}) was significantly greater (0.8 ± 0.2 mm^{-1}). Deviation from a model of pure diffusion, interpreted as being consistent with cellular uptake and/or movement into blood vessels, was approximately the same in normal and chronically injured cortex. These data demonstrate that gliotic cortex is able to maintain a normal $[K^+]_o$ and that the functional capacity to regulate increases in $[K^+]_o$ is not altered.

1110 METABOLIC STUDY OF FOCAL PENICILLIN EPILEPSY IN THE RAT. Robert C. Collins* and Fred Plum. Dept. Neurol., Cornell, New York City, 10021.

A model for studying the neurochemical changes of focal epilepsy has been established in paralyzed ventilated rats. Ten units of penicillin dissolved in 0.13 ul of CSF was injected into the superior lateral aspect of the motor cortex at a depth of 0.5 mm. Electrocorticogram (ECoG) showed interictal spike discharges within one minute. ECoG spike amplitude (2.0 mv) and frequency (30 min^{-1}) reached peak at 10 min. and maintained a steady rate of discharge for 60 to 90 min. before cessation. At 60 min. the animals' heads were frozen in situ with liquid nitrogen. The epileptic focus (3.5 mg), as well as comparable tissue from homotypic contralateral cortex and non-epileptic occipital lobe was dissected at -20°C and assayed for energy substrates by enzymatic cycling. Compared to electrically uninvolved cortex or CSF injected controls there was a slight decrease in the epileptic focus for high energy compounds (Phosphocreatine + ATP). There was no change in tissue glucose, glu-6-PO_4, pyruvate, or lactate. Homotypic cortex contralateral to the focus showed synchronous spike discharge and identical metabolic changes. The results suggest that small changes in energy substrates reflect increased metabolic activity within the epileptic focus and transynaptic tissue. No evidence of metabolic exhaustion accompanied diminution of the discharge and energy reserves appear to be well maintained during the focal seizure.

1111 ELECTROENCEPHALOGRAPHIC CORRELATES OF AUDIOGENIC SEIZURES IN GENETICALLY SUSCEPTIBLE AND ACOUSTICALLY PRIMED INBRED MICE.

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The cortical EEG of five inbred strains of mice susceptible to audiogenic seizures (DBA/1/Bg, DBA/2/Bg, C57BL/6/GAD-1/Bg, HS-B/Bg, and HS-G/Bg) and three acoustically primed inbred strains (DBA/lasr/Bg, C57BL/6/Bg and LS-B/Bg) were recorded during audiogenic seizures and either picrotoxin (4 mg/kg, i.p.) or thiosemicarbazide (50 mg/kg, i.p.) seizures. The mice were between 28 and 33 days of age. During the audiogenic seizure, there was no evidence of spike waves or paroxysmal activity in the trace from the bipolar cortical electrodes. Rather, these might be a slight amplification and acceleration of the trace during wild circling activity, but with a diminution of the trace during clonic and clonic-tonic convulsions. However, during chemoconvulsive seizures, these same mice showed spike wave and paroxysmal activity in the trace from the cortical electrodes. Similar results have been obtained in mice with audiogenic seizures induced by ethanol-withdrawal by us as well as by others in genetically susceptible rabbits and pharmacologically susceptible cats. It is suggested on the basis of these findings that the neural mechanisms for the expression of audiogenic seizures and chemoconvulsive seizures are different, that all audiogenic seizure responses have a common neural mechanism, and that the audiogenic seizure is a type of subcortical epilepsy.

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1112 POSTSYNAPTIC EFFECTS OF DIVALENT CATIONS. Wilkie A. Wilson. Epilepsy Center, V. A. Hospital and Dept. of Physiology, Duke Univ., Durham, North Carolina.

Divalent cations are known to play an important role in the regulation of neuronal excitability. Previous studies have shown that invertebrate neurons perfused with solutions containing low concentrations of divalent cations develop bursting firing patterns (reminiscent of the Paroxysmal Depolarizing Shift (PDS) seen in neurons from epileptogenic foci) independent of synaptic inputs (Barker, J. L. and H. Gainer, Brain Res. 84:479-500). The convulsants strychnine and pentylenetetrazol have also been shown to produce similar PDS-like behavior in these cells (Klee, M.R., et. al., Science 179:1133-1136; David, R. J., et. al, Brain Res. 67:549-554) but they also have profound postsynaptic effects (Faber, D. S. and M. R. Klee, Brain Res. 65:109-126; Wilson, W. A. and A. V. Escueta, Brain Res. 72:168-171). The present study was undertaken to determine if solutions containing lowered concentrations of divalent cations might similarly alter postsynaptic mechanisms.

A neural network from the abdominal ganglion of Aplysia californica was used. The network consisted of interneuron L_{10} with its follower cells L_2-L_5 and R_{15}. L_2-L_5 receive dual cholinergic inhibitory inputs from L_{10} (a short chloride IPSP and a long potassium IPSP) and R_{15} receives a sodium dependent EPSP. EPSPs can be recorded in L_2-L_5 when the right connective nerve is shocked. (The neurotransmitter involved is not known.)

Postsynaptic responses were evaluated in two ways: (1) by stimulating L_{10} and observing the responses in L_2-L_5 and (2) by iontophoresis of acetylcholine into L_2-L_5 and R_{15}. Since reducing only the Ca++ concentration in the external solution blocks transmitter release, both Mg++ and Ca++ were reduced maintaining the normal concentration ratios. Voltage clamping and signal averaging techniques were used to quantitate the synaptic currents and allow time separation of the long and short inhibitory events.

Experiments using the L_{10} to L_2-L_5 connections showed that reducing the divalent cation concentration to 1/5 of normal markedly reduced the short (chloride) inhibitory current (with no significant change in reversed potential) but only marginally reduced the long component. The excitatory current, produced by shocking the right connective nerve, was unchanged, or became larger. All effects could be rapidly reversed by washing with normal seawater.

Iontophoresis of acetylcholine onto cells L_2-L_5 produced both short and long inhibitory currents. The short inhibition was again strongly reduced in low divalent cation solutions but a lesser effect was seen on the long IPSC. Similar studies of R_{15} showed that the cholinergic excitation of R_{15} was also markedly reduced in the presence of low divalent cation seawater.

These results, together with previous studies, suggest that seawater containing low concentrations of divalent cations can have both synaptic and non-synaptic effects similar to those of convulsants.

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1113 FRONTAL CORTICAL LESIONS RETARD AMYGDALOID KINDLING IN RATS

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A pattern of independent spike discharge appeared in the motor cortex of rats during the development of clinical convulsions evoked by repeated electrical stimulation of amygdala (kindling), suggesting that anterior neocortex participates in amygdaloid kindling. To test this hypothesis bilateral aspiration lesions were made in various cortical areas in rats prior to kindling. Lesions of the orbital cortex (i.e., on the dorsal lip of the rhinal sulcus) or prefrontal cortex (area 10) significantly retarded the rate of amygdaloid kindling; lesions of motor cortex, anterior cingulate cortex, or visual cortex were without significant effect. Detailed analysis of the pattern of seizure development indicated that the orbital-lesioned and frontal-lesioned rats kindled relatively normally up to the second-last stage of amygdaloid kindling, in which stage they perseverated significantly longer than the controls and the other lesioned rats.

These results suggest that areas of the frontal lobe participate in the elaboration and generalization of amygdaloid seizures in rats. Surprisingly, motor cortex does not play a similar role. Although frontal and orbital lesions retarded the rate of kindling, the lesioned rats nonetheless eventually developed fullblown kindled convulsions, indicating that these cortical areas are not absolutely essential for the development of amygdaloid seizures.

1114 THE EFFECT OF ANTICONVULSANTS ON SEIZURES INDUCED BY PENICILLIN, PICROTOXIN AND BICUCULLINE. Harvey L. Edmonds, Jr. College of Pharmacy, Washington State University, Pullman, Washington 99163.

Diazepam (DZP), diphenylhydantoin sodium (DPH) and sodium phenobarbital (PHB) were shown to be ineffective in blocking penicillin-induced cortical afterdischarges (AD) in freely moving adult male Sprague-Dawley rats bearing chronically implanted cortical electrodes (Edmonds, et al., Exper. Neurol. 45:377, 1974). However, a new anticonvulsant, SC-13504 [1-benzhydryl-4(6-methyl-2-pyridylemethyleneimino)-piperazine] has been shown to decrease the number of AD, the total amount of AD activity and the average length of AD at a 20 mg/kg dose (10 times the protective dose [PD_{50}] against maximal electroshock seizures). Since penicillin may exert its convulsant action by antagonizing gamma-aminobutyric acid (GABA)(Curtis, et al., Brain Res. 43: 242, 1972), the effects of these 4 anticonvulsants were evaluated in seizures produced by 2 putative GABA antagonists, bicuculline and picrotoxin. The convulsant dose (CD_{99}) of bicuculline HCL was 5 mg/kg i.p. The PD_{50} of each anticonvulsant was determined graphically and found to be: DZP 6 mg/kg p.o.; DPH>60 mg/kg i.p.; PHB>60 mg/kg p.o.; SC-13504>320 mg/kg p.o. The CD_{99} of picrotoxin was 10 mg/kg i.p. The PD_{50} of each anticonvulsant was: DZP 13 mg/kg: DPH>60 mg/kg; PHB>60 mg/kg. The dose-response relationship for SC-13504 was not linear, but a 160 mg/kg dose protected 40% of the rats. These data are consistent with the interpretation that penicillin, picrotoxin and bicuculline do not each produce convulsions in the rat by identical mechanisms because: 1) SC-13504 partially blocked penicillin seizures at moderate doses and picrotoxin seizures at high doses but did not block bicuculline seizures; 2) DZP blocked bicuculline and picrotoxin seizures at moderate doses but did not block penicillin seizures at high doses. (This work was supported by grants from the Epilepsy Foundation of America, G. D. Searle & Co., Chicago, Ill., and Washington State University.)

1115 SENSORY STIMULI ARE IDENTIFIED ACCURATELY IN SINGLE EPOCHS OF VISUAL EVOKED RESPONSES RECORDED ON SCALP. Jacques J. Vidal, Marshall D. Buck*, Robert J. Hickman*, Ronald H. Olch* and Tulsi D. Ramchandani*. Dept. Computer Science and Brain Research Institute, UCLA, L.A., Calif. 90024.

Extant research on visual evoked responses has been almost exclusively based on averages. The visual stimulus (typically a flash presentation) is repeated a number of times. Corresponding sequential samples of EEG amplitude, indexed from the stimulus instant, are averaged. Averages obtained for different stimulus conditions (i.e. intensity, color, pattern, etc.) are then compared visually or with a computer according to some heuristic rule (i.e. peak-to-peak amplitudes, component latencies, etc.) Results are then taken to represent an EEG sign or code for the sensory event triggered by the stimulus.

The potential inadequacy of the average as a descriptor of evoked events has been often discussed. Yet it is generally believed that EEG is too "noisy" and the evoked event of too small amplitude with respect to the "on-going" activity, to be detected in any other way. Two years of experimentation at the Brain Computer Interface Laboratory at UCLA have shown this not to be the case for a large class of discriminable visual stimuli consisting of color flashes with or without patterns. A computer procedure has been developed that identifies the stimulus condition on individual epochs with near certainty. For instance a mutual information measure $I = 1.87$ bits in a 4 stimulus class experiment ($I_{max} = 2$ bits) has been obtained, providing a better than 90% classification accuracy. In these experiments multi-electrode data is taken (5 in most experiments) and data acquisition includes some analog preprocessing. The procedure first uses a stepwise discrimination routine over the set of EEG samples, based on individual F ratios, to reduce dimensionality and remove irrelevant data. The routine selects a subset of the time samples with the most information, across electrode channels. Next, a discriminant function is calculated for each stimulus condition or class using estimates of the covariances of the selected samples within each class. This calculation is done iteratively in a training-testing sequence.

The remarkable recognition rate is attributed to the following: 1) Artifact generating events such as eyeblinks are monitored, on-line, and potentially corrupt data is ignored. 2) Stepwise discrimination zeroes in on the time windows that contain the information, and discards the other samples. 3) The iterative (testing-training) approach refines the decision rule by rejection of the outliers in the training sets, i.e. those epochs where, presumably, activity unrelated to the stimulus overshadowed the response. 4) In real-time application of the decision rule to unknown responses, outlier rejection is retained in the form of an extra "don't know" class (allowing the computer to decline identification in dubious cases). 5) Subjects are their own control and thus decision rules can be subject specific. The approach promises a significant improvement in the usefulness of evoked response techniques in clinical and research applications. The coefficients of the discriminant function provide information on the expected amplitude of the response (as would the average value) but embedded in the decision rule and as such attached to selected samples they provide a descriptor of the evoked event that has much more predictive value than the usual average response. These early results support the views that there may be no "noise" in EEG (in the sense of stochastic, fundamentally unpredictable fluctuations) and that at least in laboratory conditions, procedures can be devised in which cortical events remain simple enough to elicit scalp EEG signatures of demonstrable deterministic causality.

1116 THE EFFECTS OF HYPOXIA ON THE EXTRACELLULAR POTASSIUM ACTIVITY OF THE CAT CEREBRAL CORTEX. William F. Blank, Jr. and Howard S. Kirshner*. NIH, NINCDS-LPP, Bethesda, Maryland 20014.

The effects of hypoxia on the extracellular potassium activity were studied in the cat cerebral cortex using potassium specific microelectrodes. Twenty-eight cats were anesthesized with pentobarbital (35-40 mg/kg), paralyzed with gallamine (2-4 mg/kg) and artificially respired. The extracellular potassium activity (Ko), electrocorticogram (ECoG), cortical DC potential, and blood pressure (B/P) were monitored continuously The cats were exposed to varying concentrations of oxygen (0 to 8%) by changing the composition of the breathing air with nitrogen. A control Ko of 3.4 $\pm$ .2 s.e.m. meq/l was found in the anterior suprasylvian gyrus. With a pO_2 threshold of 20mmHg, Ko increased during hypoxia and followed two general patterns: 1) Slow gradual rises with maximum rates of 0.02 to 0.20 meq/l/s, and 2) fast rises with maximum rates of 0.78 to 7.0 meq/l/s. Rises with slopes of intermediate magnitudes were occasionally seen. The slow rises occurred at higher concentrations of oxygen (15-20 mmHg) and tended to be linearly correlated with both time and B/P. The maximum Ko generally did not exceed 20 meq/l. The fast rise resembled spreading depression in time course and magnitude of maximum Ko (more than 40 meq/l). This pattern was a complex exponential function of time and was not correlated with B/P. The fast rise tended to occur during insults in which the pO_2 fell rapidly to very low levels (less than 10 mmHg). In both patterns the ECoG became isoelectric when the Ko was between 2.8 and 9.1 meq/l. No consistent level was associated with the loss or recovery of the ECoG. Elevations of the B/P with epinephrine injections to some extent reversed both the increases in Ko and the ECoG flattening. The DC potential of the cortex after an initial positive shift showed a negative shift (5 to 20 mv) as the potassium activity increased.

The hypoxic insults were reversed by changing the breathing air to 100% oxygen. As the B/P began to rise towards control values, the Ko began to fall. The Ko then fell over several minutes towards an initial Ko level 0 to 3.4 meq/l above control values. It then fell slowly to the control value over a time which varied from several minutes to several hours. Undershoots of Ko below the control value were not seen. The rate of the initial fall of Ko could be described by a simple exponential function once the B/P was re-established. Rate constants were determined by finding the slope of $\ln \Delta Ko$ versus time using linear regression methods (where Δ Ko is the Ko in question minus the initial Ko to which it is falling). The "r" values for the fit of the data to the regression lines was greater than 0.95. The rate constants for the fall varied from 0.01 to 0.09 s^{-1}. Rate constant magnitude correlated inversely with the duration of the peak Ko reached during the insult, but was not correlated with other indices of the insult severity. The results will be discussed in terms of the possible mechanisms of accumulation and clearance of potassium from the extracellular space of the cat cortex.

1117 CEREBRAL TRAINING AS A RESTORATIVE AGENT. E. Schmidhofer, M.D. Clinical Neurophysiologist, Department of Psychiatry Cook County Hospital. Chicago, Ill. 60612

Cerebral Training had its beginnings in 1944. It is a very highly structured, massively standardized regimen, that makes use of neurophysiologic principles instead of psychiatric concepts--cerebral mechanisms rather than mental dynamisms. It is a nonmedicinal, controlled, methodic, organized, sustained training program which concerns itself with the systematic and orderly cultivation of continuing and progressive self-potentiated, self-development.

It is estimated that upward of 8000 persons have been exposed to this method. Heterogeneous diseases and disorders may be worked with concomitantly. Large groups of 100 to 150 may be worked with concurrently. Nonprofessionals may be trained to become effective instructors. A lengthy history is not required. Insight is unnecessary. Ventilation is excluded.

Each trainee participates actively in his own recovery by studying the 30 training manuals he receives, and by practicing the various principles between class instruction periods. The method may be used indefinitely after the formal course of training has ended. Perhaps the greatest asset is the prevention of recurrence of medical afflictions and of offending acts. In many cases symptom relief began to appear in 1 to 3 weeks. In one study of a group of 100 alcoholics, 64% were reported as being abstinate 12 to 18 months later. Hard core heroin addicts have also responded favorably.

This method has been made up literally into the form of a package program and can be delivered as such.

1118 EEG-Somatosensory Evoked Response Relationships

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Somatosensory evoked responses (SER) in a modified recovery function paradigm were related to a 10-minute resting EEG. Both sets of records were obtained from the left parietal scalp in 43 nonpatients. Computer analysis of the SER's produced 18 variables. These variables and the EEG mean amplitude, mean frequency and their respective variability measures were subjected to canonical correlational analysis. Significant correlations exist between the mean amplitude of the EEG and SER measures in the 15 to 99 millesecond poststimulus time period. Mean EEG frequency significantly correlated with the 31 to 99 millesecond poststimulus SER. Similar analyses with schizophrenic patients indicate that different EEG-ER relationships exist.

1119 PROPAGATION PATTERNS OF SEIZURE ACTIVITY INITIATED IN THE LIMBIC SYSTEM IN PATIENTS WITH TEMPORAL LOBE EPILEPSY. Jeffrey P. Lieb, Thomas L. Babb, and Paul H. Crandall*. Div. Neurosurg., Sch. Med., UCLA, Los Angeles, 90024.

The surface and depth ictal signal was compared in 30 patients with intractable temporal lobe epilepsy in whom depth EEG electrodes had been chronically implanted in order to localize epileptogenic sites with a view to therapeutic surgery. Both sub-clinical and clinical seizure records were judged with respect to the manner in which seizure activity originating unilaterally in the depths of one of the temporal lobes spread to the surface. For each seizure, surface activity was judged as: 1) not spreading to the cortex; 2)spreading bilaterally to the cortex; 3) spreading initially or more noticeably to the cortex ipsilateral to the depth site(s) from which the seizure was triggered; or 4) spreading initially or more noticeably to the cortex contralateral to the depth site(s) from which the seizure was triggered. Sub-clinical seizure activity was found to spread only rarely to the surface. Clinical seizure activity usually, but not always spread to the surface; such activity spread ipsilaterally far more often than contralaterally. These data indicate the usefulness of the surface ictal signal in the lateralization of temporal lobe epilepsy.

1120 ELECTROENCEPHALOGRAPHIC (EEG) EFFECTS OF THYROTROPIN RELEASING HORMONE ON RABBITS. Richard P. White, and J. Stephen Beale*. Dept. of Pharmacology, Univ. Tennessee Ctr. Hlth. Sci., Memphis, TN. 38163.

Many reports have shown that thyrotropin releasing hormone (TRH) is an analeptic, induces hyperthermia, and causes behavioral excitation via central mechanisms. However, others show that TRH inhibits single neurons and enhances anticonvulsant effects of barbiturates. Since some drugs cause "dissociation" between EEG and behavior, the present study was performed on curarized rabbits to ascertain whether 200 μg of TRH given into the cisterna magna produces EEG changes resembling normal activation. The results indicate that TRH acts as a physiological excitant, producing an EEG activation pattern lasting at least 30 min. Moreover, TRH antagonized the EEG synchrony induced by 5 mg/kg of pentobarbital. Initial studies suggest, however, that there are no residual action of TRH since once the EEG effects have terminated, responses from electrostimulation of the reticular formation are not altered. Blood pressure rose an average of 21 mm Hg for at least 9 min. and Mayer-like (but never Traube-Hering) waves were evident with this rise. However, these changes in blood pressure sometimes occurred with saline injections or electrostimulation of the reticular formation and were independent of the EEG pattern. Also, hyperthermia was not produced by TRH in these curarized animals.

1121 SOMATOSENSORY EVOKED RESPONSES AND SLOW POTENTIAL OSCILLATIONS IN HUMANS J.A. Kusske, J.L. Rush*, R.W. Porter*, R.C. Dill* and M. Verzeano. Neurosurgery Section, VA Hospital, Long Beach, Div. Neurological Surgery and Dept. Psychobiology, Univ. of Calif., Irvine 92664

Previous investigations conducted by the authors have demonstrated that responses evoked, at the vertex and in the parietal and frontal regions, by a brief electrical pulse delivered to the median nerve or to the common peroneal nerve, contain time locked components which occur as late as 500 to 3500 msec., after the stimulus. Averaging and spectral analysis showed these components to be distributed in the 1.5 to 2 and 2.6 to 4 Hz frequency bands. It was postulated that their presence in the evoked response might be due to the driving by the stimuli, of oscillations of similar frequencies which can be identified in the spontaneous activity recorded from the same sites. Subsequent investigations showed that these low frequency oscillations can be elicited with periodic as well as random sequences of stimuli. However, when the frequency of stimulation was varied, in small steps, over the range of 0.25 to 5 pulses per second, the responses of maximum amplitude occurred when the stimuli were given at the rates of 1.5, 2 and 3 to 3.6 pulses per second or at harmonics thereof, indicating that the best "driving" occurs at these frequencies. In order to determine whether the motor response of the stimulated extremity was implicated in the development of these slow processes, experiments were conducted in which evoked potentials were recorded from the same regions, in the same subjects, with and without the occurrence of a twitch in the muscles innervated by the median nerve. The same results were obtained under either condition, indicating that the motor response does not play a significant role in the generation of these evoked potentials.

1122 NEUROSCIENTISTS AND THE CEREBRAL DEATH ISSUE. R. J. Grimm. Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, Oregon, 97209.

There is growing public and legal acceptance that an individual's death is characterized by irreversible and irreparable changes in brain with or without cessation of respiration and heartbeat, the older conception of death. A neuroscientist's contribution to this sober and many-sided discussion should be informed, reflective, technically competent and critically addressed. While neuroscientists may not be expected to share directly the burden of cerebral death determination, it is clearly a province of interest. Discussions with colleagues can proceed on various points: (1) What is the nature and the quality of the evidence for cerebral death; what does this term mean? (2) What devices are used in the determination; are they measuring what they say they are measuring, and are they sensitive enough to preclude error (Am. Neurol. Assoc., 96: 271, 1971)? (3) Do changing cerebral death criteria reflect current basic science concepts e.g., spinal reflexes, cerebral blood flow, etc.? And, (4) are clinicians aware of new views about the vulnerability of neurons to anoxia *per se*, rapid endothelial changes (J. Neurol., Neurosurg., Psych., 36: 497, 1973), and other experimental observations of potential therapeutic usefulness to coma management. With contributions from neuroscientists, such discussion sharpens the issues and provides physicians, their patients and family with another level of service.

1123 NEUROPHYSIOLOGICAL CHANGES FOLLOWING SPINAL CORD TRANSECTION IN MAN. Peter Ashby* (SPON: J. W. Scott) Univ. Toronto, Dept. Med., Toronto, Ontario.

A study has been made of the neurophysiological changes that follow spinal transection in man. The Achilles tendon reflex (ATR) is used to estimate transmission in the Ia monosynaptic pathway, and the tonic vibration reflex (TVR) to estimate transmission in the Ia polysynaptic pathway to motoneurons. The inhibition of the H reflex by vibration is used as an estimate of presynaptic inhibition of the Ia monosynaptic pathway. Immediately following a complete lesion of the spinal cord presynaptic inhibition of the Ia monosynaptic pathway appears to be greatly increased. This enhanced inhibition may last several months but it eventually declines and in some instances becomes less than normal. Transmission in the Ia polysynaptic pathway is permanently abolished by a complete spinal lesion. A hypothesis is developed from these findings to explain the evolution of some of the clinical features that follow complete spinal lesions in man.

1124 PARYLENE-C AS A CHRONICALLY STABLE, RELIABLE DIELECTRIC COATING FOR IMPLANTED ELECTRODES. M.J. Bak, G.E. Loeb, E.M. Schmidt, and M. Salcman. Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD 20014.

Recent research in neuroprosthetic and clinical monitoring devices has suggested a variety of designs for chronically implantable stimulating and recording electrodes. One of the major obstacles is the difficulty of providing a long term barrier between electronic components and body fluids, particularly where the insulator must be thin, flexible, and non-reactive. Parylene-C (Union Carbide), a unique vapor-deposited linear polymer, has extremely low water vapor permeability, is non-toxic, and conformally coats complex objects with thin, pin-hole free, unstressed layers. In these studies, chronic intracortical "thumbtack" microelectrodes (Salcman and Bak, IEEE-BME-20: 253, 1973) with 2 mm long etched iridium shafts and 25 μ dia. gold lead wires were coated with 3 μ of Parylene-C. The submicron tips were exposed by an electrostatic field in air, which produces highly uniform and clean tips (13.8 $\pm$ 3.7 μ^2, 0.82 $\pm$ .26 Megohms @ 1 kHz for 4 electrodes at one set of parameters).

12 such microelectrodes implanted in monkey motor cortex recorded single units (100-500 μV) and had stable impedances for over four months in vivo. Post-mortem SEM examination of the microelectrodes showed the insulation to be intact with a clean edge at the exposed tip. Parylene-C was also used on tungsten microelectrodes for acute penetrations of the dural sheath of the dorsal root ganglion, where it was found to be highly mechanically and electrically reliable.

1125 GRAPHIC REPRESENTATION OF PATHOLOGICAL TREMOR USING COMPRESSED SPECTRA TECHNIQUES. F.R. Freemon, J.R. Bourne, J. Berman, and M. Sur. Neurology Service, VA Hospital, and Schools of Medicine and Engineering, Vanderbilt U., Nashville, Tennessee.

The involuntary and abnormal oscillatory movements of human pathological tremor, although easily recorded using a variety of techniques, have been infrequently quantified. An analysis technique, termed the Compressed Spectral Array (CSA), widely used in EEG analysis, has been adapted for use in the study of pathological tremor. A patient suffering from tremor has a Grass accelerometer attached to the index finger of his most tremulous hand. Computer analysis of this accelerometer output produces a CSA consisting of fast Fourier transforms of 5 second epochs. These transforms, plotted and stacked vertically with hidden lines suppressed, yield the percept of a three-dimensional time-frequency plane. The CSA methodology converts one form of diagnostic pattern recognition to another; that is, the clinician observes the displacement of a tremulous limb in space over time while this technique yields a power spectral analysis of acceleration over successive 5 second periods. Although the first method is closer to clinical reality it suffers from the lack of permanency characteristic of all clinical observations. Future development of this technique may permit characterization of tremor types and aid in the diagnosis of underlying pathological conditions.

1126 VISUAL EVOKED RESPONSES IN NORMAL AND DISABLED ADULT READERS. Malcolm S. Preston, Barton Childs*, Irwin Kirsch* and David Gertman*. Dept. Peds., Sch. Med., Johns Hopkins Univ., Baltimore, Md., 21205.

Visual evoked responses, recorded from occipital and parietal placements (O1,O2,P3, and P4) all referenced to linked mastoids, were obtained from two groups of normal and one group of disabled adult readers under two test conditions: a series of non-patterned flashes of light and a series of three-letter words. Each of the nine subjects in each group was tested twice under both conditions with fifty 100 msec presentations per run. The disabled group contained readers who were mothers or fathers of at least one reading disabled child. One of the normal groups consisted of mothers or fathers of at least one disabled child, while no reading disabilities were present in the families of the other normal group. All subjects viewed the flashing lights passively, while in the word runs they were required to count the number of times a target word occurred. The amplitude of the positive wave (P200) peaking within a latency band of 150 to 250 msec was measured from baseline. Comparisons were then made between evoked responses to light flashes and words in each group. The results for the two normal groups showed P200 amplitude greater for words than light flashes on the parietal electrodes, while the reverse was true for the occipital electrodes. For the disabled readers, P200 amplitude was greater for flashes than words on both parietal and occipital placements. This pattern was most apparent on the left parietal electrode. The results suggest that VER procedures with further refinement may eventually be useful in the clinical assessment of reading disability.

1127 SLEEP PATTERNS IN MATERNAL FEMALE RATS. Stephen Zoloth*, Robert Stiller*, and Norman T. Adler. Department of Psychology, University of Pennsylvania Philadelphia, Pa. 19174.

Continuously recorded EEG's from chronically implanted female rats were used to monitor arousal, slow wave sleep (SWS), and paradoxical sleep (PS). Sleep patterns were studied in maternal female rats (maternal), in the same individuals at least two weeks following weaning of their litters (post-maternal), and in estrous cycling virgin rats (virgin).

Maternal female rats showed significant increases in the amount of PS and arousal, with a reduced amount of SWS when compared with post-maternal or virgin females. Post-maternal females and virgins (sampled on diestrus) were not significantly different in their pattern of sleep and arousal.

Even though the total amount of sleep time in maternal female rats decreased, the amount of time spent in PS was elevated. This is an unusual result; in most studies, an increase in PS is accompanied by an increase in SWS. The increase in the amount of PS is due to a longer duration of each PS episode rather than to an increase in the frequency of episodes. Furthermore, these long PS episodes occur primarily during the lights-on segment of the photoperiod, the period when the female spends the most time with her offspring (Grota and Ader, *Hormones and Behavior*, 5:272, 1974).

1128 DEVELOPMENT OF SLEEP DURING THE PERIPUBERTAL PERIOD IN THE RAT. G.C. Sieck*, J.A. Ramaley, R.M. Harper* and A.N. Taylor. Dept. of Physiol. & Biophys., U. Nebraska Med. Center, Omaha, NE 68105 and Dept. of Anat. & Brain Research Institute, UCLA, Los Angeles, CA 90024.

Sleep-related changes in neuroendocrine function have been shown to be associated with puberty in humans. It was therefore of interest to determine whether puberty is associated with changes in sleep. Female Sprague-Dawley rats were implanted with cortical and neck-muscle electrodes at 26 days of age and maintained on a 12:12 light-dark cycle. Continuous 24-hr polygraphic and analog tape recordings were begun 5 days later and repeated on selected days through the time of vaginal opening (day 38-40). Sleep-wakefulness states were classified by visual analysis of the polygraphic recordings based on established ECG, EMG and somatic activity criteria. A circadian distribution of sleep and wakefulness was observed in the earliest recording session with significant differences for values of quiet sleep, total sleep and waking times between light and dark periods. Paradoxical sleep time was evenly distributed between both periods. The same patterns were observed on the day of vaginal opening However, significant quantitative changes occurred in all parameters between the pre- and pubertal recording sessions in both light and dark periods: quiet sleep, paradoxical sleep and total sleep times were reduced while waking time was increased. Changes occurring most closely associated with the pubertal event were a significant reduction in paradoxical sleep time in both light and dark periods and an apparent reduction in quiet sleep and total sleep times during the dark period. These observed changes in sleep and wakefulness related to the pubertal event may reflect either a developmental process or hormonal actions specific to puberty. (Supported by NIH grants HD-08703 and NS-09122.)

1129 ENTRAINMENT OF A SLEEP RHYTHM BY SHORT LIGHT-DARK CYCLES IN THE RAT. Alexander A. Borbély. Institute of Pharmacology, University of Zürich, 8006 Zürich, Switzerland.

The vigilance state of the rat can be controlled to a large extent by 2-hr light-dark (LD 1:1) cycles (Borbély et al.,Brain Res.1975, in press). However, under LD 12:12 and LD 1:1 schedules, a sleep cycle with a period length of approximately 10 min is present, and may be analogous to the 90 min cycle in man. I investigated to what extent this sleep rhythm can be entrained or synchronized by short LD cycles. EEG, EMG and motor activity were recorded by telemetry during 23.5 hrs per day. The records were used to score the predominant vigilance state for successive periods of 24 sec. The rats were subjected first to the LD 12:12 schedule, then to schedules of 2.5:2.5 min, 5:5 min, 7.5:7.5 min and 10:10 min, which were maintained each for one day. The interval histogram of paradoxical sleep (PS) periods was computed for each condition. During LD 12:12 the intervals showed a rather flat distribution with a peak at approximately 10 min. This sleep rhythm could be entrained to some extent by the LD 2.5:2.5 min and 7.5:7.5 min cycles, but not by the LD 10:10 min cycle. Under the LD 5:5 min cycles, the PS intervals exhibited a prominent peak at 10 min. Thus an optimal synchronization is achieved if the period length of the external LD cycle and the intrinsic sleep cycle are in close correspondence.

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1130 GLUCOSE METABOLISM DURING SLEEP AND WAKEFULNESS IN HAMSTER. James R. Macho* and Arabinda K. Sinha. Dept. Physiol., CMDNJ - Rutgers Med. Sch., Piscataway, N.J. 08854.

We have used a radiorespirometric technique to test the hypothesis that there is a relative change in the rate of glucose metabolism via pentose phosphate pathway between sleep and wakefulness. Adult male hamsters, implanted for EEG-EMG recording, were kept awake for 3 hours and were then injected with 2.5 μCi of either [^{14}C-1] or [^{14}C-6]-D-glucose. During the fourth hour, one group was allowed to sleep and the other group was kept awake. Ten minute serial samples of expired CO_2 were collected during the experimental hour. By EEG-EMG criteria the sleeping group slept 88% of the time and the awake group was awake 94% of the time. The mean CO_2 output decreased from 6.48±.16 (s.e.m.) m.moles/hr during wakefulness to 5.61±.10 m.moles/hr during sleep. The breath specific activity of $^{14}CO_2$ from [^{14}C-6] glucose was 97% higher ($p<.001$) in the sleeping group. The mean ^{14}C-1/^{14}C-6 ratio in the expired CO_2 in sleeping hamsters was 0.71±.34. The ^{14}C-1/^{14}C-6 in the awake hamsters was initially 3.30 and gradually fell to 1.33 by the end of the hour. The mean ^{14}C-1/^{14}C-6 ratio in this group was 1.55±.19 suggesting increased operation of the pentose phosphate pathway relative to glycolysis. An awake experiment in which the hamsters were not sleep deprived prior to the experimental hour resulted in a ^{14}C-1/^{14}C-6 ratio of 1.62±.42. This differential rate of metabolism of the first and sixth carbons of glucose suggests a re-orientation of glucose metabolism between sleep and wakefulness.

1131 CHRONIC CANNULATION AND PERFUSION OF CEREBRAL VENTRICLES IN CATS FOR THE STUDY OF CEREBRO SPINAL FLUID DURING SLEEP AND WAKEFULNESS. Xavier Lozoya and Xavier Velázquez*, Sci. Res. Dept., Natl. Med. Ctr., I.M.S.S., México, D. F.

Preliminary studies based on ventriculo-cisternal perfusion during acute experimental conditions in cats have suggested the presence of neurohumoral factors in cerebro spinal fluid (csf), which might be related to different states of cortical excitability. Animals chronically implanted with cannulae, allow the study of effects of ventricular perfusions, upon spontaneous sleep and wakefulness. Herein such animals behavior during ventriculo-cisternal perfusions of artificial csf, saline solution, and distilled water was studied. Simultaneously, various physiological parameters (EEG, frequency bands analysis, EOG, EMG, rectal temperature) were registered. It is suggested that this technic permits neurochemical analysis of csf from cerebral ventricles, during different states of sleep and wakefulness.

1132 REGIONAL DISTRIBUTION OF PIPERIDINE IN THE BRAIN OF WAKING MICE. H. Dolezalova* and M. Stepita-Klauco. Department of Biobehavioral Sciences, University of Connecticut, Storrs, Connecticut 06268.

It has been recently demonstrated that the concentration of piperidine in the central nervous system increased during hibernation in molluscs (Brain Res. 72:115, 1974), and during behavioral sleep in mammals (Science 183:536, 1974). In the mouse brain, the observed increase in the mesencephalon was larger than in the prosencephalon or in cerebellum (Brain res. 74:182, 1974). In order to establish whether there is a preferential occurrence of piperidine in regions of the brain which are known to be connected with generation of the sleep-wakefulness cycle, we have studied the regional distribution of piperidine in the waking mouse brain. Three-month-old C57Bl/6 mice were decapitated, the brain was dissected on a cold stage, and the samples of the brain tissue and the whole blood were homogenized in perchloric acid. The homogenate was dansylated and separated by thin-layer chromatography on silica gel. DANS-piperidine was identified mass spectrometrically, and was quantified by comparing the integrated ion current of its molecular ion (m/e 318.140) with that of an added internal standard of DANS-pyrrolidine (m/e 304.125) in AEI MS902 mass spectrometer. The piperidine concentrations were as follows (in pmole/mg wet tissue, S.E., the number of measurements in parentheses): bulbus olfactorius, 24.4±11.6 (10); prosencephalon, 1.16±0.28 (10); mesencephalon, 5.86±2.01 (10); cerebellum, 3.70±0.49 (10); pons, 8.19±3.02 (10); medulla oblongata, 6.37±1.25 (10); medulla spinalis, 86.7±26.6 (10); blood, 5.61±0.96 (10). The higher concentration of piperidine in the brain stem than in the prosencephalon (without the bulbus olfactorius) might reflect a participation of piperidine in sleep generating structures. For the unexpectedly high concentrations in the bulbus olfactorius and in the cervical part of the spinal cord, however, a rational interpretation is still lacking. (Supported by grants from the University of Connecticut Research Foundation and from NIH).

1133 EFFECT OF CHRONIC BARBITURATE TREATMENT ON THE SLEEP PATTERNS OF SQUIRREL MONKEYS.[1] Perrie M. Adams and Ernest S. Barratt. Dept. of Psychiatry, Behavioral Science Laboratory, University of Texas Medical Branch, Galveston, Texas 77550.

The effects of daily treatment with 5mg/kg of sodium pentobarbital on the sleep-wakefulness patterns of the squirrel monkey were studied. Following a 30-day baseline, 60 days of pentobarbital treatment were given 2 hours prior to the start of a 12-hour recording of EEG and EOG. Following drug treatment, a 30-day period was used to determine the rate of recovery. The EEG and EOG were used to determine the percent of time spent in stages of awake, drowsy, light sleep, slow wave sleep and rapid eye movement sleep. Repeated pentobarbital treatment significantly decreased the amount of slow wave sleep while increasing the amount of time spent in drowsy and light sleep. Awake time decreased significantly after 10 days of chronic treatment. Rapid eye movement sleep was significantly depressed initially then recovered and subsequently decreased by 20 days of chronic treatment. This decrease in rapid eye movement sleep continued throughout the remaining pentobarbital treatment but showed a marked increase in recovery. This increase was not found for slow wave sleep and the other stages of sleep failed to return to initial baseline levels during the recovery period.

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1134 THE EFFECTS OF BILATERAL LOCUS COERULEUS LESIONS ON THE SLEEP/WAKING CYCLE IN THE CAT. Barbara E. Jones, Stanley Harper*, and Angelos E. Halaris. Dept. Psychiatry, Univ. Chicago, Chicago, Ill., 60637.

Previous studies have inferred that central noradrenergic neurons are involved in the maintenance of tonic electrocortical activation of waking and in the generation of tonic and phasic components of paradoxical sleep (PS). In the present study bilateral lesions of the locus coeruleus nuc., which includes all the noradrenaline-containing cell bodies in the pons, were performed in 7 adult cats which were chronically implanted for EEG recording of the sleep/waking cycle. Continuous recording was performed 23 hours per day for 5 days prior to and 21 days following the operation, after which the animals were sacrificed for biochemical assay of the monoamines and histological examination of the lesion.

Following an initial period of coma characterized by continual EEG slow wave activity and associated with respiratory apneusis and absence of spinal reflexes, electrocortical activation returns to normal levels (40% of total recording time) by the third day following the operation and remains at this level for the duration of survival. Both tonic and phasic components of PS are altered immediately and permanently. Muscular atonia is absent. Phases of PS persist (12% of total recording time as compared to 14% in controls) with electrocortical activation in association with body and limb movements, rapid eye movements and PGO spikes which are reduced in frequency by 50% of control.

The lesions are associated with 85% depletion of endogenous noradrenaline in the neocortex and hippocampus, 60% depletion in the thalamus and midbrain and 15% depletion in the hypothalamus, with no changes in levels of serotonin, 5HIAA or dopamine. (Supported by NIMH grant DA-00250).

1135 MESENCEPHALIC RETICULAR INFLUENCES ON OUTPUT NEURONS OF LATERALIS POSTERIOR THALAMUS. A. Diallo* and M. Steriade (SPON: J. Tremblay). Lab.Neurophysiol., Dept. Physiol., Fac. Med., Univ. Laval, Québec, Canada.

Extracellular unit recordings were performed in encéphale isolé cats to investigate the effects of high-frequency stimulation of the mesencephalic reticular formation (RF) on excitatory-inhibitory sequences in electrophysiologically identified output cells of the lateralis intermedius-lateralis posterior (LI-LP) thalamic nuclei. Thalamo-cortical neurons were recognized by their antidromic invasion following stimulation of anterior suprasylvian areas 5 and 7. Some of these cells received in turn a monosynaptic projection from the same sites of cortical stimulation, thus suggesting a thalamo-cortico-thalamic loop.

A monosynaptic, high-security reticulo-thalamic pathway was disclosed, considering that LI-LP cells were able to follow fast (100-150/s) RF stimuli, at latencies of 1.5-2 ms. Short trains of high-frequency shocks to the RF preceding testing cortical stimuli had the following excitatory and disinhibitory effects: (1) Increased probability of cortically evoked synaptic discharges; (2) Transformation of partial antidromic (initial segment) discharges elicited by juxtathreshold cortical stimuli into full spikes and increased responsiveness to antidromic volleys; (3) Reduced duration of the subsequent inhibitory phase, as judged from the period of suppressed spontaneous discharges and from responsiveness to testing shocks (recovery cycle).

It is assumed that ascending projections of reticular origin subserve maintenance of a higher excitability level in thalamic association nuclei during the waking state. The withdrawal of reticulofugal excitatory impulses at sleep onset results in disfacilitation of output thalamic neurons, with consequent deafferentation in cortical areas, thus contributing to further development of sleep.

1136 CORTICO-THALAMIC CELLS AND LOCAL INTERNEURONS IN AREAS 5 and 7 DURING THE SLEEP-WAKING CYCLE. A. Kitsikis* and M. Steriade. Lab. Neurophysiol., Dept. Physiol., Fac. Med., Univ. Laval, Québec, Canada.

Two cellular classes were differentiated in anterior suprasylvian areas 5 and 7 according to their distinct changes in spontaneous firing during the sleep-waking cycle in the behaving cat. (1) Long-axoned cortico-thalamic neurons were identified by antidromic invasion from lateralis intermedius (LI), lateralis posterior (LP) and center median (CM) thalamic nuclei. Direct synaptic inputs were found in some units to arise from the LI or LP target nuclei; cortico-CM neurons received a monosynaptic excitatory projection from the LI. As a rule, thalamically elicited synaptic excitation of cortical output cells consisted of a single spike discharge. (2) Short-axoned interneurons were inferred from the same criteria as used to recognize precentral motor cortical interneurons (Steriade et al., J. Neurophysiol. 37: 1065, 1974), namely short or long barrages of high-frequency (300-800/s) spikes occurring spontaneously and in response to synaptic volleys, as well as lack of antidromic invasion from all stimulated sites.

(1) Identified corticofugal neurons exhibited decreased firing during synchronized sleep (S) compared to waking (W), while during desynchronized sleep (D) discharge rates reached a maximum level. The analysis of transitional periods revealed: (a) progressive or sudden decreased firing, preceding by 20-40 s the first EEG signs of behavioral drowsiness; (b) dramatic rise in firing rate associated with phasic periods of arousal from relaxed W or drowsiness; (c) increased firing rate during the shift from S to D, preceding the EMG suppression by 30 to 120 s; (d) no consistent changes related to REMs; (e) regularization of discharge patterns 10-20 s before behavioral arousal from D.

(2) Interneurons showed differential changes when compared to output cells, with respect to both temporal patterns and mean-rates of spontaneous firing. (a) They discharged arhythmic high-frequency spike bursts interspersed with long periods of silence, regardless of the vigilance state; bursting firing during W is a distinctive feature of interneurons, since output cells do not exhibit such a pattern during this state. (b) Their highest firing rates occurred during S. Lower discharge frequency was observed during D; often, an arrest of firing appeared abruptly at D onset, followed by phasically increased discharge during the first saccadic REM. Mean-rates of discharges during steady W was much lower than those seen in both S and D. (c) *Interneurons stopped firing on arousal from S or D.* This was a remarkably constant finding. It could be observed during arousal reactions occurring spontaneously, elicited by sensory stimulation (noise or light in the experimental room) or induced by brief shock-trains to the mesencephalic reticular formation at liminal intensities required for EEG activation and slight behavioral alertness without detectable movements.

The above findings indicate that the commonly observed changes in spontaneous firing of cortical cells during the sleep-waking cycle, as described by other studies in literature dealing with unidentified neurons (highest mean-rates during D, lowest during S), actually concern only Golgi I type cells. This is presumably due to the bias of unit recordings for larger size neurons. The powerful spontaneous firing of interneurons during S, and their still present activity during D, as disclosed in the present experiments, does not support recent hypotheses claiming that such elements, likely underlying complex integrative processes during W, benefit from sleep recovery. Instead, interneurons constantly stopped firing during phasic periods of arousal, when their synaptically elicited discharges were also inhibited. The mechanisms underlying the reduction of high-frequency interneuronal bursts by ascending arousing systems, contrasting with facilitatory effects on output cells, is a matter of future investigation.

1137 Ponto-Geniculo-Occipital (PGO) Spikes: A Sleep Epiphenomenon? Robert M. Bowker* and Adrian R. Morrison. Labs. Anat., Sch. Vet. Med. Univ. Pa., Phila. 19174.

The functional significance of PGO spikes has puzzled sleep researchers since their discovery. Our experiments suggest that PGO spikes are merely a sign of spontaneous activation of a "startle network" just prior to the onset and during paradoxical sleep (PS). Nine cats were implanted with standard sleep recording electrodes. Sounds (1500 Hz pure tone bursts of 100msec. duration or other environmental noises) produced PGO spikes during quiet wakefulness, drowsiness, synchronized sleep (SS) and PS. PGO spikes identical in amplitude, shape and duration to those occurring spontaneously just prior to and during PS could be produced at the same stimulous intensities in the lateral geniculate body (Fig. 1). PGO spikes almost always followed stimuli spaced 10-20 sec. apart and presented for 3-4 minute periods. Stimulation at less than 5 sec. intervals resulted in habituation after a couple of presentations. At low stimulus intensity only PGO spikes appeared; but with graded increases of intensity during SS, spikes would follow increased neck muscle activity and would occur in conjunction with EEG activation. At greater intensities, first orientation movements of the ears, and then, behavioral arousal were observed. Amplitudes of potentials associated with eye movements during wakefulness were 50% of those of spontaneous and sound-induced PGO spikes. The latter appeared simultaneously with eye movements, if these occurred; but waking eye movement potentials followed spontaneous eye movements. In further support of our hypothesis, cats with paravermal cerebellar cortical lesions abruptly flex the ipsilateral forelimb if startled when awake and exhibit a flexion jerk with each sound-induced and spontaneous PGO spike in SS. We conclude that PGO spikes are a neural sign of a startle response to either external stimulation or the internal stimuli provided by the increased neuronal activity which begins prior to PS onset. This conception is a novel one, which bestows far less importance on PGO spikes than is common in the sleep literature.

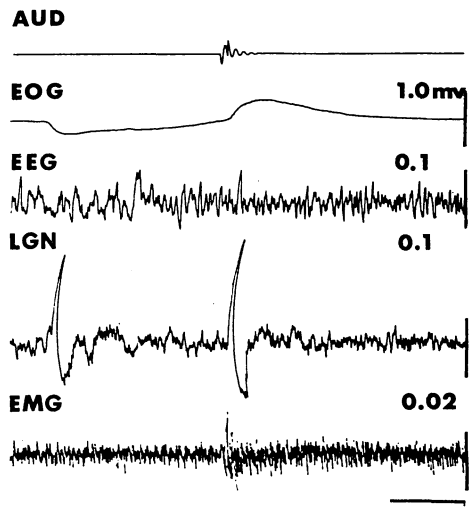

Fig. 1 Spontaneous and sound-induced (AUD) PGO spikes in lateral geniculate body (LGN) in SS. Note similar relation of PGO spikes to eye movements. Slight changes in EEG and neck EMG records can also be seen although no arousal occurred. Time-1sec.

1138 RELATIONSHIP OF PONTINE FTG UNIT ACTIVITY TO PGO SPIKES. Jerome M. Siegel and Dennis J. McGinty. VA Hospital, Sepulveda, California 91343 and Dept. of Anatomy, UCLA, Los Angeles, California 90024.

Studies by Hobson, McCarley and their co-workers have led them to suggest that neurons in the gigantocellular tegmental field (FTG) of the pontine reticular formation may have a role in generating REM sleep. The principal evidence supporting this conclusion is the finding that these cells fire at high rates during the ponto-geniculo-occipital (PGO) waves preceding REM sleep and during REM sleep itself. However, many brain units increase their firing during PGO waves. Therefore, it is of interest to know whether the FTG cells have any unique relationship to the PGO wave. In this study we have calculated the temporal relationships between FTG unit firing and PGO waves recorded in the lateral geniculate nucleus (LGN). We find that the peak FTG firing rate precedes the peak of the LGN PGO wave by 15 msec. Since the ascending phase of the LGN PGO wave lasts about 30 msec and since pontine PGO activity precedes LGN PGO waves, we conclude that FTG unit firing does not precede the initiation of the pontine PGO wave. Thus, it is unlikely that FTG unit firing initiates the PGO waves of REM sleep.

1139 A COMPARISON OF THE RECORDING QUALITIES OF MICROWIRES AND MICROELECTRODES

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Until recently, single-cell recording in sleeping animals has been performed using microelectrodes that require lengthy and involved processes for their production. Reports of results using fine wires as electrodes claim satisfactory recording qualities and have the advantage of simplicity of construction since the wires are available commercially. The wires can also be multiplexed allowing two or more units to be simultaneously sampled and they can be left in place for very long term recordings. Initial skepticism about microwires was based upon the obvious difficulty of conceptualizing comparable resolving power by electrode tips differing radically in shape and in surface area. We have, therefore, compared the recording capacities of 25 u and 75 u wires with those of traditional microelectrodes.

Multiple-point probes were made by bonding one or two microwires to a microelectrode shaft with lacquer. The wire tips were trimmed to equal length under a dissecting microscope and the probes were hydraulically advanced through the cerebellum and pontine brain stem of unanaesthetized cats, stopping every 0.5 mm to collect data. Every tip was monitored at each stop point and each probe tip was alternately monitored en route; if a cell field was entered, the probe was stopped and resolution made optimal by fine adjustment of the advancer. At every stop point the output of the tips was tape-recorded; later signal-to-noise ratios (S:N) were determined for spike height to background noise level or to the level of background spikes (a more stringent test).

Table of Quantitative Data

Probe	Surface Area	Resistance	# Sites Sampled	% Sites $\bar{c}$ U/MUA > 3	% Sites $\bar{c}$ U/N > 4
uE	100 u^2	1.0M	123	18.7	40.7
25 u	485 u^2	100K	105	3.7	5.7
75 u	4415 u^2	50K	56	10.7	16.1

U=Unit Amplitude; MUA=Multiple Unit Amplitude; N=Noise Amplitude

The table shows that, depending on the comparison made, the microelectrodes were about 6-8 times more capable of resolving units than were 25 u wires. Paradoxically, the 75 u wire was about 3 times more successful than the smaller wire. Thus the microelectrode had only twice the resolving power of the relatively gross wire probe. Histograms for each class of probe revealed the expected step-wise decline in number of cells with increasing S:N (from 2:1 to 10:1) for the microelectrode and 25 u wires but the 75 u wires had an approximately equal number of instances of 2:1, 3:1, 4:1 and 5:1 ratios and few that were higher. This seemed to indicate that the large tip was capable of discriminating large (and only large) cells but that it resolves large cells as well as much smaller probes.

The results suggest that the microelectrode has a higher resolving power than 25 u or 75 u microwires. It is thus the probe of choice for sampling purposes since it probably resolves many more small cells than the 25 u wire, and the 75 u wire appears to be incapable of discriminating some units resolvable with the microelectrode. However, 75 u wires have a resolving capacity for large neurons which is considerably greater than smaller wires and which compares favorably with microelectrodes. They may thus be useful in recording from large cell zones, especially where multiple tips could compensate the loss in resolving power. This modification might also allow such wires to be fixed in place for long term observations in a large-cell field.

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1140 A SIGNIFICANT DIFFERENCE BETWEEN THE AMOUNT OF SLOW-WAVE SLEEP AND WAKEFULNESS OR SLOW-WAVE SLEEP AND DROWSINESS IN CATS AFTER ADMINISTRATION OF 10 MG/KG OF L-TRYPTOPHAN. M. Radulovački and R. L. Buckingham. Dept. Pharmacol., Univ. Ill. Med. Ctr., Chicago, Ill. 60612.

L-tryptophan was administered orally to cats implanted with a cannula to the cisterna magna and electrodes for EEG, EMG and EOG recording. Control experiments were conducted with animals which were kept awake for 1 hr, after which period 1 ml of cerebrospinal fluid (CSF) was taken. After maintaining one additional hour of wakefulness, a placebo capsule was administered orally. Subsequently, the animals' behavior was observed for 1 hr and the EEG monitored for 5 hr. CSF samples were withdrawn 1, 3 and 5 hr after placebo administration. The same procedure was used when a capsule containing 10 mg/kg of L-tryptophan was administered. CSF 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of serotonin, and homovanillic acid (HVA), a metabolite of dopamine, were determined by the method of Korf and Valkenburgh-Sikkema.

One hour after administration of the placebo there was no significant difference between the amount of slow-wave sleep (SWS), wakefulness (W) or SWS and drowsiness (D). However, 1 hr after tryptophan administration, there was a significant difference between the amount of SWS and W ($P < 0.01$) or SWS and D ($P < 0.01$). Also, there was a tendency for the amount of SWS after tryptophan to be increased and for SWS latency to decrease. The significant increase in SWS from W after 10 mg/kg L-tryptophan was related to a significant increase of 5-HIAA from HVA after 1 hr ($P < 0.005$) and a significant increase of 5-HIAA from 5-HIAA concentration ($P < 0.05$) 1 hr after placebo. The significant decrease of 5-HIAA concentration from HVA 1 hr after placebo ($P < 0.025$) coincided with no significant differences between SWS, W or D. (Supported by USPHS Grant NS 10921)

1141 DECREASED DOPAMINE IN THALAMUS AND INCREASED 5-HYDROXYINDOLEACETIC ACID AND HOMOVANILLIC ACID IN HIPPOCAMPUS DURING SLOW-WAVE SLEEP IN THE CAT. Ružica Kovačević and Miodrag Radulovački. Dept. Pharmacol., Univ. Ill. Med. Ctr., Chicago, Ill. 60612.

5-Hydroxytryptamine (5-HT), dopamine (DA), norepinephrine (NE), 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-HT, and homovanillic acid (HVA), a metabolite of DA, were determined in cerebral cortex, caudate nucleus, hippocampus and thalamus in sleeping and waking cats. To extract these brain structures in different states of vigilance we have developed a new surgical technique for ablating one brain hemisphere of a cat during slow-wave sleep (SWS) and the other one during wakefulness. Monoamines and their metabolites in the identical structures in both hemispheres were determined spectrofluorometrically by described methods.

Under pentobarbital anaesthesia, left and right parieto-temporal bones of the skull and underlying dura mater were removed. After screw electrodes for EEG recording were implanted into the frontal cortex and frontal air sinus, exposed hemispheres were covered by skin. The incision was made airtight by suturing. Two days following surgery, cats were restrained in bags with EEG cables attached to their electrode plugs and conditioned to sleep. Five to six days after surgery, stitches were removed under local anaesthesia, and hemispheres exposed. EEG was monitored and animals observed. After 20 min of spontaneous SWS, one hemisphere was ablated and specific brain sites were dissected on ice. Another hemisphere was removed after 30 min of wakefulness, and the same procedure performed as before. During SWS, DA content was decreased in thalamus ($P < 0.05$), and 5-HIAA and HVA concentrations increased in hippocampus ($P < 0.01$ and $P < 0.05$). No change in 5-HT and DA was found in any other structure during SWS. (Supported by USPHS Grant 10921)

1142 SLEEP IN THE AGED CAT. Michael H. Chase, Stephen Rich* and Margaret I. Babb. Depts. Physiol. and Anat., Sch. Med., UCLA, Los Angeles, 90024.

Standard values for the daily time spent in sleep and waking states have been documented in kittens and adult cats. However, no comparable information is available in aged cats. Consequently, we examined the sleep and waking patterns in four cats, varying in age from 9 to 15 years. The age of these cats was verified by obtaining their date of birth from veterinary records. For the "15+ year-old cat" the date of birth was unknown, but it was an adult when first placed under a veterinarian's supervision 14 years prior to this experiment. Each animal was given a complete physiological and neurological examination by a licensed veterinarian in order to preclude pathological alterations of sleep/waking patterns.

Following a one-week acclimatization period, recordings were obtained from each cat during consecutive 24-hour periods. Electroencephalographic, electroocular and electromyographic indices were used to differentiate the states of wakefulness (which included the drowsy state), quiet sleep and active sleep. The following data was obtained:

Age of Cat	Consecutive hours run	% Wakefulness and Drowsy	% Quiet sleep	% Active sleep
9 years	72	52.2	37.9	9.9
12 years	48	72.9	20.0	7.1
15 years	72	67.7	23.8	8.5
15 years +	72	79.3	15.4	5.3

In the adult cat, standard values for these states are: wakefulness/drowsy, 43%; quiet sleep 42%; and active sleep 15%. Our results therefore indicate that the percentage of time spent in the combined states of wakefulness and drowsy increases as a function of age, while the time spent in both quiet and active sleep decreases. Supported by NIH grant NS-09999.

1143 STRUCTURAL EVIDENCE OF FUNCTIONAL UNITS IN THE OLFACTORY MUCOSA OF MICE.
P. P. C. Graziadei, Dept. Biological Science, Florida State University, Tallahassee, Florida, 32306.

Evidence has been provided in lower vertebrates that the olfactory neurons are orderly organized, and that the supporting cells act as focal points in their regular arrangement (Graziadei, JCB 59: 523, 1973 and AAA (Abst.), 1975). The purpose of this communication is to describe the organization of the olfactory receptor neurons in mouse where it has reached a higher degree of sophistication. Frontal (i.e. cornal) sections of the olfactory region of the nose in mouse show that in the concave surface of the turbinates the neuroepithelium is thinner than in the convex surface and that the receptors are arranged almost constantly in a characteristic columnar fashion if the section is cut accurately perpendicular to the surface of the epithelium and not slanted to this plane. The neurons are placed one above the other, forming columns shaped as chandeliers that appear to be one, two or three neurons thick. This arrangement is particularly evident on the convex surface of the turbinates, where the epithelium is thicker and we have encountered columns made up of as many as seven neurons. The analysis of these columns with the electron microscope shows notable ultrastructural differences between the cellular components. At the base of the columns the cells have the characteristics of young or immature elements (neuroblasts), while toward the surface of the epithelium they acquire the cytological make up of mature receptor neurons. Sections of the epithelium conducted parallel to its surface show that the dendrites of the mature neurons arrange themselves in groups, each one of these groups surrounding a supporting cell and thus establishing rings of receptor dendrites which have often obvious interconnections. Autoradiographic studies give evidence that the cellular organization above described does not represent a simple morphological peculiarity, but it is the result of a characteristic modality of neurons differentiation from staminal cell groups located at the very base of the epithelium. The possibility that these columns actually represent isogenetic clones which develop during the entire life of the animal is, at present, an interesting working hypothesis in our laboratory. With pulse labeling we have noticed that H3 thymidine is readily incorporated in the basal cells 24 hours after administration, and after a period of 15 days the labeled cells progress upwards, towards the surface of the epithelium, orderly maintaining the columnar arrangement above indicated. However, at 15 days the labeled nuclei at the base of the epithelium have a grain count inferior to the mature neurons. We believe that the basal cells divide along two main lines, one which may be called a "maturation division" and the other "duplication division". In the latter the progenitor neuroblasts duplicate several times providing a persistent supply of staminal cells. We are presently working on a quantitative analysis of this phenomenon. We have also noticed that, even when repeated pulses of labeled material are administered over a period of up to 10 days, only discrete small clusters of cells are labeled, providing the indication that microcenters of proliferation do exist, each microcenter giving origin to a structural unit. We believe that this orderly pattern of neurons, as observed in the olfactory mucosa of mice, as well as the seemingly isogenetic formation of columns cannot be casual and should be furtherly investigated in order to clarify its true functional value.

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1144 BRAIN HISTOGENESIS IN VITRO: RECONSTRUCTION OF SUBSTANTIA NIGRA STRUCTURES IN MIDBRAIN CELL AGGREGATES. Pat Levitt*, Robert Y. Moore, and Beatrice B. Garber. Depts. of Biology and Anatomy, Univ. of Chicago, Chicago, Ill. 60637 and Dept. of Neurosciences, UCSD, La Jolla, Calif. 92037.

Falck-Hillarp histofluorescence procedures were adapted successsfully to visualize and identify CNS dopaminergic (DA) neurons in developing brain cell aggregates. These miniature 3-dimensional tissues were reconstructed in vitro from dissociated cell suspensions derived from 13-18 day embryonic mouse midbrain stem regions and allowed to differentiate in culture for 1-7 days. It was found that DA neurons retained histofluorescence immediately after dissociation and could be followed throughout their developmental course in culture. Randomly dispersed DA neurons in suspension were observed to associate selectively, first grouping into several small clusters within each aggregate by 48 hrs., and then forming a single thick and elongated band across one side of the aggregate by 4-5 days in culture. This reorganization within an aggregate of a structure closely resembling the morphology of the substantia nigra during its migratory phase in situ was accompanied by increasing levels of dopamine, as determined by the radioisotopic assay of Coyle and Henry. The normal developmental sequence of cytodifferentiation observed in vitro in these brain cell aggregates and the implications for histogenetic analyses of monoamine neuron systems will be discussed. (Supported by NIH grants HD-04583, NS-12080, NS-10714, and CA-14599.)

1145 MYELINATION IN THE REGENERATING OPTIC NERVE OF THE NEWT (TRITURUS VIRIDESCENS). James E. Turner, Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina 27103.

The adult newt (Triturus viridescens) optic nerve maintains many embryonic characteristics, one of which is the presence of a single type of glial population which was characterized as consisting of primitive ependymoglial cells (Turner and Singer, J. Comp. Neurol. 156: 1-18, 1974). Earlier reports have implicated this cell in the myelinating events during optic nerve regeneration (Turner and Singer, J. Exp. Zool. 190: 249-268, 1974). In order to further characterize these observations, optic nerves of the newt were transected and the early stages of myelination of regenerating axons by ependymoglial cells were observed.

By 117 days after transection a few of the large diameter fibers were undergoing myelination. Myelin formation began with the engulfment of regenerating axons by ependymoglial processes forming a mesaxon in much the same manner as Schwann cells. Subsequently, there was initiated a succession of spiral elongations of the mesaxon eventually resulting in the formation of the first dense line which was formed by the apposition of the cytoplasmic surfaces of the previously noncompacted spiral. After two or more dense lines of compact myelin were generated the first intraperiod lines were formed. There appeared to be no morphological differences between the myelin sheaths of normal and regenerating optic nerves except that the regenerating myelin contained fewer lamallae during these early stages of myelination.

There appeared to be a definite relationship between axon diameter and the initiation of myelination, for no axons below 1.0μ were seen to be myelinated. (Supported by General Research Support Grant RR-5405 and NS 12070 from NIH and by the National Society for the Prevention of Blindness)

1146 CONTINUING GROWTH IN THE AVIAN CILIARY GANGLION. Enrico Mugnaini. Lab. of Neuromorphology, Dept. of Biobehavioral Sciences, Univ. Connecticut, Storrs 06268

In the avian ciliary ganglion each ciliary neuron receives its innervation from a single preganglionic fiber. At the last embryonic stages the preganglionic fiber presents a calyciform nerve ending which embraces one pole of the egg-shaped ciliary neuron. This relationship changes progressively with age. At hatching one often sees filopodial extensions of the calyciform ending invaginating into the neuronal perikaryon. During the first months of life the nerve cells emanate short evaginations, some of which may bear simple or crestlike synaptic junctions, and the calyciform ending changes into a beaded terminal. The preganglionic fiber sprouts a progressively increasing number of collaterals which form a bouton cap covering almost half of the perikaryal surface. In 1-year-old chickens many ciliary neurons present a few short dendrites which intrude between the boutons and receive numerous synapses. The number of these dendrites increases with age. In 18-month-old chickens the number of preterminal branches of individual preganglionic fibers has increased enormously and the ciliary neurons present one or more deep invaginations of the synaptic pole which are filled by synaptic endings of the preganglionic fiber. Thus the area of apposition between preganglionic fiber and ciliary neuron continues to increase with age and new axodendritic and axosomatic synapses are formed during adulthood. (Supported by NIH Grant NS-09904.)

1147 MUSCARINIC CHOLINERGIC BINDING SITES IN THE DEVELOPING AVIAN HEART. Antonio Sastre, D. Bruce Gray and Mary-Ann Lane*. Section of Neurobiology and Behavior, Cornell U., Ithaca, N.Y. 14853.

A study of the muscarinic cholinergic binding sites during development of the chick heart using an affinity label has been made.

Detailed understanding of the heart's cholinergic system at the molecular level has been hampered by the absence of ligands with high affinity and specificity for the muscarinic acetylcholine receptor. Yamamura and Snyder (PNAS 71:1725-1729, 1974) have shown that [^{3}H]-Quinuclidinyl Benzilate (^{3}H-QNB) binds with high affinity and specificity to muscarinic sites in rat brain. We have confirmed their findings in rat brain, and established that ^{3}H-QNB binding to heart exhibits a similar pharmacologic profile. In particular, specific ^{3}H-QNB binding to heart tissue is saturable. Binding at 10^{-10} to 10^{-11} M is not affected by the presence of 10^{-5} M d-tubocurare or nicotine, nor by 6 X 10^{-6} M α-bungarotoxin. ^{3}H-QNB binding is inhibited by micromolar concentrations of each of the following agonists: acetylcholine, carbamylcholine or oxotremorine, and by nanomolar concentrations of atropine, scopolamine or isopropamide.

Specific ^{3}H-QNB binding to "microsomal" pellets of Gallus domesticus hearts was found to increase throughout development. Values ranged from less than 5 X 10^{-13} moles ^{3}H-QNB bound/mg protein at 3 days in ovo (Hamburger-Hamilton stages 19-21) to 5 X 10^{-12} moles ^{3}H-QNB bound/mg protein one day post-hatching. The relationship between innervation and changes in levels of specific ^{3}H-QNB binding will be discussed.

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1148 EFFECT OF END ORGAN REMOVAL ON ADRENERGIC DEVELOPMENT. Mark Dibner and Ira Black. Dept. Neurol., Cornell U. Med. Ctr., New York, N.Y. 10021

The role of target organs in the maturation of adrenergic neurons was studied in the neonatal rat. The superior cervical ganglion (SCG) and its end organs, the salivary glands and iris, were employed as a model system. Unilateral sialectomy and iridectomy in 3 day old animals prevented the normal development of ganglion tyrosine hydroxylase and DOPA decarboxylase activities. These enzymes are highly localized to adrenergic neurons in the SCG, and were used to monitor maturation of these cells. Enzyme activity remained depressed for at least 2 months, the longest time tested. In contrast, total ganglion protein, a measure of ganglion growth as a whole, initially developed normally. Six weeks after surgery, however, protein content was significantly lower in ganglia deprived of the normal field of innervation. Failure of normal enzyme maturation was apparently dependent on removal of ipsilateral end organs only, since bilateral sialectomy exerted no greater effect than unilateral sialectomy.

In adults, unilateral sialectomy and iridectomy did not significantly alter ganglion T-OH activity or protein in rats followed up to one month after surgery.

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1149 STIMULATION OF BRAIN DEVELOPMENT IN CHICK EMBRYO BY ELEVATED TEMPERATURE. Stephen Zamenhof. Mental Retardation Research Inst. and Brain Research Inst., Sch. Med., UCLA, Los Angeles 90024.

Experimental chick embryos were incubated at 37.5°C till day 7 and after day 10, and at 40.5°C on days 7-10; their optic lobes and cerebral hemispheres at day 10 and at hatching were compared with controls incubated at 37.5°C only. Cell numbers at day 10 were directly counted by a new method involving formalin fixation and cell disaggregation by gentle sonication. At hatching, body weights, organ weights and organ DNA (cell numbers) were the same in experimentals and in controls, for both optic lobes and cerebral hemispheres, though the protein contents were significantly higher in experimentals. However, at 10 days (end of neuron proliferation) the weights and the cell numbers in experimentals were significantly higher. Two possible explanations have been offered: 1. Elevated neuron population in experimental animals at day 10 is followed by their elevated death rate, or 2. The increment in neuron number is permanent but at hatching it is overshadowed by the population of other cells.

1150 CHARACTERIZATION OF DEVELOPMENTAL CHANGES IN SUPERIOR LARYNGEAL NERVE FIBERS OF THE POSTNATAL KITTEN. Carole Dunmire* and Arthur J. Miller. Dept. Physiol., Univ. Illinois, Chicago, 60680, Dept. Physiol. and Section Orofacial Anomalies, Univ. California, San Francisco, 94143.

The evocation of reflex responses from laryngeal sensory input bears consideration as to its potential roles in unexplained pathological conditions such as the Sudden Infant Death Syndrome. For this reason, an electron microscopic analysis in conjunction with peripheral nerve recordings was undertaken to characterize some of the developmental changes which occur in the superior laryngeal nerve (SLN) during the first 63 postnatal days of the kitten. The data obtained indicate that this nerve, which innervates the upper respiratory tract, is developed at birth with the largest fibers 5μ in diameter and fibers 4-5u comprising only 3% of the total myelinated distribution. The adult distribution of myelinated fibers with a curve heavily skewed to the right (mean 4.2μ, 1.6-13μ) is not evident until 43-61 days (mean 2.6μ, 0.5-8.0μ). Within the first month, the distribution of myelinated fibers is more normal with the largest fibers not exceeding 7.5μ. Upon electrical stimulation of the SLN, both superior laryngeal-recurrent and superior laryngeal-hypoglossal nerve reflexes were found in kittens as young as 9 days. The inducement of apnea by electrical stimulation was observed in the youngest kittens studied (at 5 days of age), although swallowing was not readily evoked until 28-30 days.

1151 DEVELOPMENT OF SPONTANEOUS SPINAL CORD BIOELECTRIC ACTIVITY IN SPINAL CHICK EMBRYOS. R. R. Provine and L. Rogers*. Univ. of Maryland Baltimore County, Baltimore, Md, 21228 and Dept. Anat., Washington Univ. Sch. Med., St. Louis, Mo. 63110.

Embryonic behavior of the chick is the product of spontaneous polyneuronal burst discharges within the ventral spinal cord (Brain Res., 1972, 45, 127). The present study describes the ontogeny of the spinal cord burst discharges in spinal embryos deprived of brain input during development. Spinalization was accomplished by removing several neural tube segments of 2-day embryos at either the cervical or mid-thoracic level. The amount and periodicity of cord burst discharges of spinal embryos were comparable to those of intact controls from 6 days until the last third of the 21-day incubation period when the activity of the spinals began to show signs of deterioration. Spinal embryos had occasional spontaneous "reverberatory" discharges which were slow to dampen. Such discharges were never observed in intact control embryos. These data indicate that brain input is not necessary for the initial development of spinal cord burst discharges but it may play an important role in subsequent maturation or maintenance of such activity.

1152 THE PATTERN OF SUPRASPINAL AND DORSAL ROOT CONNECTIONS IN THE LUMBO-SACRAL SPINAL CORD OF THE NEWBORN RAT. Mark Gilbert* and Dennis J. Stelzner (SPON: D. C. Goodman). Dept. Anat., Upstate Med. Ctr., Syr., N. Y., 13210.

Light and electron microscopy were used to study the pattern of degeneration of supraspinal and dorsal root connections in the lumbosacral spinal cord of the neonatal rat. To study dorsal root connections, unilateral spinal root lesions were made at the fourth - fifth lumbar vertebrae in 4 day old, weanling, and adult rats. To study supraspinal connections, mid-thoracic spinal cord hemisections were made in albino rats at the same ages as above. After short postoperative survival times (6 hrs. to 3 days), sections of lumbosacral spinal cord were stained by a non-suppressed modification of the Fink-Heimer reduced silver stain and the pattern of degeneration mapped. The lumbosacral spinal cords of a number of experimental animals were prepared for electron microscopy and were examined for the location of degenerating axons and synaptic knobs.

Our results indicate that the distribution of degeneration in the newborn rat is similar to weanling and adult rats with two exceptions. One is the absence of the corticospinal pathway in the dorsal funiculus and dorsal grey. Second is the absence of a monosynaptic projection of dorsal root afferents into the lateral motor region. These findings suggest that, at least in terms of the distribution of supraspinal and dorsal root connections, the lumbosacral spinal cord is relatively mature at birth. The behavioral immaturity of the neonatal rat may be due more to a lack of local connections in the grey matter than to the absence of supraspinal or dorsal root connections.

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1153 NEURO-ONTOGENY OF POSTURAL REFLEXES IN NORMAL KITTENS. Jeri A. Sechzer, Ronald F. Mervis and George L. Cooper*. Dept. Psychiatr., Cornell U. Med. Coll. and E.W. Bourne Behavioral Research Laboratory, New York Hospital, White Plains, New York 10605, and Dept. Anat., Mt. Sinai Sch. Med. of CUNY., New York 10029.

In order to evaluate neurological development, postural reflexes were tested in normal kittens from birth through seven (7) weeks of age until stable adult patterns of performance were reached. These reflex tests included: body and air righting, visual placing, chin placing, tactile placing, hopping, and locomotion. A protocol for scoring responses was designed for each individual reflex to differentiate progression. The first reflexes to mature were visual placing, chin placing, and body righting which attained a maximal score during the third week. This was followed by maturation of the forelimb hopping reflex and independent locomotion during the fourth week. Forelimb tactile placing responses, hindlimb hopping reflexes, and air righting developed by the fifth week. Hindlimb tactile placing responses were the last to mature. They became fully expressed in the sixth week. The pattern of ontogeny of these reflexes proceeds in a cephalocaudal direction. This is in agreement with earlier observations of Langworthy (1929); Windle (1930); and Tilney and Kubie (1931) who correlated maturation of reflex responses in kittens with myelination in the central nervous system.

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1154 GROWTH OF THE CORTICOSPINAL TRACT AND THE DEVELOPMENT OF PLACING REACTIONS IN POSTNATAL RAT. JANET M. DONATELLE NEUROSCIENCES PROGRAM, DEPARTMENT OF ANATOMY, UNIVERSITY OF WISCONSIN, MADISON, 53706

The purpose of this study was to determine the temporal relationship between the growth of the corticospinal tract (CST) into the spinal cord and the development of placing reactions. Unilateral injections of radioactive proline and unilateral ablations of motor-sensory cortex were done in neonatal and young rats. Subsequently autoradiographic and Fink-Heimer degeneration techniques were used to trace CST fibers. CST axons in the dorsal funiculus were found to extend as far as the cervical enlargement at day 1, mid-thoracic segments at day 3 and as far as upper lumbar cord at day 5. At 5 days of age, CST axons extend only into the most ventromedial portion of the dorsal horn in segments rostral to mid-thoracic cord. At 9 days, CST fibers are found in the dorsal funiculus of coccygeal segments and invade further into the spinal gray extending as far caudally as the lumbar enlargement. By 14 days, CST fibers reach the spinal gray of sacral cord and show more extensive distribution in the gray matter of lumbar cord. The distribution of CST fibers in the spinal gray at 21 days was similar to that seen at 14 days. Tactile placing reactions were not seen during the first postnatal week. Forelimb placing appeared during week 2 and hindlimb placing during week 3. Thus the presence of CST fibers in the gray matter of the spinal cord can form a basis for the appearance of the placing reactions. Supported by PHS-APTA Training Grant #1A02-AH00401-01.

1155 PERIPHERAL EFFECTS ON EARLY DEVELOPMENT OF CHICK SPINAL GANGLIA.
Virginia McMillan Carr and Sidney B. Simpson, Jr.* Dept. Biol. Sci., Northwestern University, Evanston, Ill., 60201.

Previous studies (Hamburger and Levi-Montalcini, 1949) based on cell counts have demonstrated that peripheral structures can influence the differentiation of dorsal root ganglia. Various interpretations have been placed on this data. It has been suggested that: 1) the developing limb does, or does not, influence the production of neurons in limb ganglia; and that 2) the primary influence of the developing limb on the developing limb is mediated exclusively via the control of cell death in the ganglion.

We have reinvestigated the role of the developing limb on spinal ganglion differentiation in the developing chick embryo using ^{3}H-TdR incorporation and autoradiography to assess proliferation. Cell degeneration was based on counts made from the same slides stained by the Feulgin reaction.

In contrast to the earlier findings, our analysis indicates that in both the mediodorsal(MD) and lateroventral(LV) regions of brachial and nonbrachial ganglia labelling indicies(LI's) do not peak at 5-6 days but are continuously high in the early stages. They fall to lower values at 6½ days in the brachial and 7½ days in the nonbrachial ganglia. However, by 9½ days the LV LI's have risen again significantly. All MD LI's decrease to very low levels where they level off at 7½ days. Following wing bud extirpation, significantly lower experimental LI's are seen only in brachial LV LI's. These occurred from 7½ days on rather than at 4½ to 5½ days.

In our hands the degeneration indicies (DI's) of control nonbrachial LV regions show a 5½ day peak thus confirming the results of Hamburger and Levi-Montalcini. Contrary to previous findings, however, is the presence of a 5½ day brachial LV DI peak although it is a small peak. Furthermore, neither of these LV DI's has decreased to zero by 9½ days. We also find degenerating cells, indistinguishable from those in the LV regions, present in the MD regions of both brachial and nonbrachial ganglia. In addition, rather than decreasing, the MD DI's begin to rise at about 7½ days reaching values of about 3% by 9½ days in both brachial and nonbrachial ganglia. Wing bud extirpation results in significantly higher DI's at all times for brachial LV's, at all but 6½ days for brachial MD's, and at 7½ and 8½ days for nonbrachial MD's. Only nonbrachial LV regions show no change.

Following limb bud addition no obvious differences between control and experimental ganglia are seen for any LI's or DI's. Furthermore, while in only a few cases are any differences in size of innervating ganglia observed, innervation of the grafts has occurred in all cases.

1156 NORMAL AND INDUCED NEURON DEATH IN GANGLIA DURING EMBRYOGENESIS. G. Pilar and L. Landmesser. Biol. Sci. Group, Univ. Conn., Storrs, Conn. 06268 and Dept. Biol., Yale Univ., New Haven, Conn.

An ultrastructural study of normally occurring neuron death and that brought about by prior removal of the peripheral target organ was made on embryonic chick ciliary ganglia in order to better understand the mechanism and significance of cell death. Before the period of cell death, the neurons in normal ganglia all developed a well organized rough endoplasmic reticulum (RER) which coincided with increased synthesis of Cholineacetyltransferase (Pilar, Chiappinelli, Uchimura & Giacobini, Physiologist 17:3, 1974) and peripheral synapse formation. None of the peripherally deprived neurons underwent this change, suggesting that some interaction with the periphery, possibly synapse formation per se, triggers these neurons into a secretory state. Nuclear changes first signalled cell death in peripherally deprived neurons, followed by freeing of ribosomes from polysomes with presumable cessation of protein synthesis. In contrast, normal cell death appeared to be brought about by dilation of the RER with eventual cytoplasmic disruption. It is possible that failure to form or maintain peripheral synapses by such neurons could result in the accumulation of transmission-related proteins and consequent dilation of the cisternae. A comparison of axon and cell number during normal development indicated that all cells had sent axons to the peripheral target organ before cell death. Further, from conduction velocity measurements, it could be deduced that all ganglion cells sent axons to the proper peripheral target and were synapsed upon by the proper class of preganglionic fiber. It can be concluded that cell death does not remove improper connections but results from failure to form a sufficient number of synapses. (Supported by NIH grants NS 10338 and NS 10666 and the Univ. of Connecticut Research Foundation).

1157 SELECTIVE INNERVATION OF TARGET TISSUES IN SPINAL CORD AND MEDULLA EXPLANTS BY ISOLATED DORSAL ROOT GANGLIA. Stanley M. Crain and Edith R. Peterson*. Depts. of Neuroscience and Physiology, Albert Einstein College of Medicine, Bronx, N.Y. 10461

In recent studies of 14-day fetal rodent spinal cord explants with attached dorsal root ganglia (DRGs), we observed marked augmentation of characteristic sensory-evoked synaptic network discharges in dorsal cord regions following DRG-hypertrophy induced by nerve growth factor (NGF) (Br. Res. 79:145,'74). Some of the NGF-enhanced DRG neurites grew through the cord tissue and, in mimicry of dorsal column fibers in situ, established similar functional synaptic connections with specific types of "target" neurons in nearby medulla explants (Science, 188,275'75). Earlier attempts to innervate explants of spinal cord cross-sections with isolated DRGs showed that "peripheral-type" neurites, invested by Schwann cells, did not invade and appeared to avoid separate CNS explants (Peterson et al., Z. Zellforsch. 66,130,'65; Bunge and Wood, Br. Res. 57, 271,'73).

We have now demonstrated that under favorable geometrical conditions, and in the presence of high NGF levels, neurites from isolated DRGs can grow across a collagen-film substrate and invade separate spinal cord and medulla explants (see Peterson and Crain, this issue). During the week after pairing these explants, DRG neurites readily grow into and innervate isolated slabs of dorsal cord tissue, in contrast to relatively poor innervation of similarly apposed ventral cord slabs. Extracellular recordings were made with Ag-AgCl electrodes via saline-filled micropipettes (3-5 μ tips) and electric stimuli were applied via similar pipettes with 10 μ tips. Focal stimuli to initially isolated DRGs evoked prominent negative slow-wave potentials in dorsal cord explants, as in dorsal cord with attached DRGs, arising abruptly after latencies of a few msec, with amplitudes up to 2 mV, and often lasting more thab 500 msec, resembling "primary afferent depolarization (PAD)" responses in situ. These PAD-like potentials were not elicited by stimuli to DRGs paired with ventral cord explants, although longer-latency, positive slow-wave or spike barrages were occasionally evoked. All of the DRG-evoked ventral cord responses were rapidly blocked in 10^{-3}M γ-aminobutyric acid (GABA), whereas the DRG-evoked 'PADs' in dorsal cord were unaffected or enhanced at this GABA concentration. The DRG-evoked 'PADs' were, however,depressed in 10^{-5}M bicuculline and blocked in 10^{-2}M Mg^{++}, as in cord with attached DRGs.

In two cases where longitudinal slabs of whole spinal cord were used, neurites from isolated DRGs formed prominent fascicles directly towards dorsal cord target zones, whereas no evidence of functional DRG neurites could be detected by systematic focal stimulation in the adjacent growth zone associated with ventral cord regions. Furthermore, focal stimuli within nearby ventral cord regions (100-200 μ away) evoked only early-latency spike potentials in dorsal cord, whereas large, PAD-like potentials could be readily elicited with stimuli to distant DRGs (>1 mm away).

Under the above culture conditions, neurites from isolated DRGs can also innervate dorsal-column-nuclei target zones in separate explants of medulla cross-sections. Focal stimulation of DRG neurites located 1-2 mm from the medulla explant could evoke characteristic 'PADs' in dorso-lateral medulla target zones, whereas similar stimuli applied within nearby ventral or medial regions of the medulla explant (100-200 μ away) were often ineffective.

The remarkable degree of regional specificity of the sensory-evoked spinal cord and brainstem networks which can form under these isolated conditions in culture provides the basis for a powerful new model system for analyses of cellular mechanisms regulating formation and development of specific synaptic connections in the mammalian CNS.

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1158 THE NATURAL DEATH OF MOTONEURONS IN THE CHICK EMBRYO: A QUANTITATIVE AND QUALITATIVE ANALYSIS OF VENTRAL ROOTS BY ELECTRON MICROSCOPY. I-Wu Chu-Wang and Ronald W. Oppenheim. Dept. of Mental Health, Raleigh, N. C. 27611.

Ventral root axons were counted in the caudal half of the lumbar spinal cord (seg. 26-29) of the chick at 4, 5, 6, 7, 8, 9, 13 days of incubation, 1 and 10 days and 5 weeks post-hatching.

At 4-days, there was an average of 800 axons per segment. The number increases very rapidly reaching a peak of 4,300 axons at 6 days of incubation. In other words, by d 6 most axons of motoneurons have already reached the ventral root region. Between d 7 and d 9, the number rather drastically declines to 2,200 axons per segment, a 48% decrease in ventral root axons. After d 9, there is only a minor and rather slow additional decrease in axons reaching 1,730 in the 13-d embryo and 1,500 at 1-day posthatching. In brief, during embryonic development about 65% of the axons are depleted in the ventral roots, i.e., only one out of every 3 axons in the ventral root is retained.

The degenerating axons are detected as early as at d 4; but the major occurrence of degenerating axons is found between 6 and 9 days of incubation. The axons are progressively lost from the ventral root by a process of self-autolysis. The necrotic symptom is found at the bulging part of the axons which contains clustering vesicles, lysosomes and autophagic vacuoles inside. The final neural debris is phagocytosed by surrounding Schwann cells. We have found degeneration in mylinated axons in the post-hatching stage, although it is a rare event.

Compared with the cell counts made in the lateral motor column by Hamburger (J. Comp. Neur., 160: 535-546, 1975), we draw the following conclusions from our data:

1. All the motor neuroblasts send axons out into the ventral roots.
2. There is an approximate one to one ratio between the number of motoneurons and axons in the ventral root before, during and following the period of maximal cell death.
3. The degeneration process probably begins in the axons and then proceeds back to the perikaryon.
4. The autolysis in the ventral root of the chick embryo is a very rapid and complete process.

1159 DEVELOPMENT OF MOTOR INNERVATION IN SUPERNUMERARY HINDLIMBS OF CHICK EMBRYOS. Deborah G. Morris* (SPON: L. Landmesser) Biol. Dept., Yale Univ., New Haven, Conn. 06520.

It has been demonstrated that muscles of the hindlimbs of mammals, birds, and amphibia are innervated by motoneurons with characteristic positions in the spinal cord. In chicks, previous experiments have shown that each hindlimb muscle is innervated by specific spinal nerves and that a general innervation pattern exists with respect to the cranial - caudal position of the motoneurons. It is not known however, what mechanisms govern initial axon outgrowth and synapse formation. In order to determine what pattern of innervation would emerge when hindlimbs were innervated by foreign motoneurons, limb buds were grafted in various positions along the flanks of St. 16 - 18 (Hamburger and Hamilton) host embryos. The development of innervation was studied from St. 25 - 43 by sequentially stimulating brachial through lumbosacral spinal nerves and the innervation of selected muscles was assessed by visual observation, tension, and nerve action potential recordings. A cranial - caudal innervation pattern similar to normal occurred regardless of the innervation source (thoracic or lumbosacral). Furthermore, when lumbosacral motoneurons grew into the limb in abnormal positions, they did not seek their appropriate muscles but formed synapses in the limb in accord with their cranial - caudal position. It can be concluded that supernumerary limb muscles do not attract appropriate motoneurons, but rather that the limb is capable of directing the growth and synapse formation of even inappropriate motoneurons in a generally normal pattern. (Supported by NIH grant NS10666).

1160 THE DEVELOPMENT OF MOTONEURONS AFTER LIMB-BUD REMOVAL IN THE CHICK. Ronald W. Oppenheim and I-Wu Chu-Wang. Dept. of Mental Health, Raleigh, N. C. 27611

Using light and electron microscopical techniques various aspects of motoneuron development were studied in the lateral motor column of the lumbar spinal cord following radical limb-bud removal at 2½ days of incubation. Confirming earlier reports we found that the proliferation and migration of motoneurons proceeded normally in the absence of the leg. Differentiation, as indicated by development of axons in the ventral root and the formation of dendrites centrally, also proceeded normally up until the time the cells started to die (i.e. days 6-7). In the absence of the leg peripheral motor and sensory nerves were caught in a tangle of nerve endings (neuroma) at the base of the missing limb. HRP injection into the neuroma at 5½ days demonstrated the ability of these fibers to transport material back to the cell body. Comparison of synaptic counts from the ablated and intact sides of the spinal cord indicated that prior to their death motoneurons deprived of their periphery receive a normal complement of synapses. There also may be a transneuronal degeneration of processes presynaptic to the motoneurons once the latter start to die.

1161 MAINTAINED FUNCTIONAL HYPERINNERVATION OF GOLDFISH EXTRAOCULAR MUSCLES. Sheryl A. Scott* (SPON: J. R. Cooper). Yale Univ., New Haven, Conn. 06520.

Previous behavioral observations suggested that the superior oblique extraocular muscle (SOM) of the goldfish could be cross-innervated by a foreign nerve (cranial NIII), but that the function of foreign synapses was repressed immediately after reinnervation by the original nerve (cranial NIV). It was suggested that this repression ultimately resulted in selective reinnervation of the SOM by its own nerve. These behavioral observations were repeated, and in addition, tension produced by the SOM in response to stimulation of foreign NIII and regenerated NIV measured. It was found that even after behavioral repression the SOM contracted in response to stimulation of both NIII and NIV. Thus, regeneration of the original nerve does not repress synapses from the foreign nerve, at least for several months after reinnervation. Junction potentials from both nerves were recorded in single fibers, indicating that individual fibers were hyperinnervated.

In the above experiments NIII was surgically crossed to the SOM and its own muscle removed to enhance cross-innervation. When NIII and NIV were simply cut and allowed to regenerate each selectively reinnervated the appropriate muscle. Since this could result from each nerve having an anatomical advantage in reinnervating its own muscle, the cut nerves were sutured together to give them equal opportunity to innervate either muscle. In this case neither nerve preferentially synapsed with its appropriate muscle. Thus, it appears that reinnervation of the SOM is not necessarily selective. Inappropriate foreign synapses are made and remain functional on SOMs reinnervated by their appropriate nerves. (Supported by NS10666 and NS06768 and NSF Predoctoral Fellowship).

1162 STOCHASTIC PROCESSES IN THE DEVELOPMENT OF DENDRITIC STRUCTURES. Robert D. Lindsay. Dept. Anat., Sch. Med., UCLA, Los Angeles, 90024

An understanding of the rules for the development of the complicated structures of dendrites will require adherence to the scientific approach of observation, generalization, hypothesis, theory, prediction, and experimentation. Dendritic structure was studied in the cerebral cortex, hippocampus and thalamus of humans, monkeys, cats, rats and mice using the Golgi impregnation technique. A number of generalizations have been made from classical descriptions and quantitative analysis of computer reconstructed neurons. Analysis of the branch length data strongly suggests that the process of bifurcation is stochastic. The probability of forming a branch node appears equally probable over the dendritic surface. However, once a branch node is formed, there is a decreased probability for a second node to develop in proximity. Hence there is a stochastic inhibition to further local node development. The analysis of branch angles again suggests a stochastic process. Smaller angles are more frequent, therefore sucessive branches tend to radiate from the soma with spherical symmetry. In many areas of the CNS this tendency towards sperical symmetry of dendritic structure is modified. A strong polarizing force appears to be present during dendritic development which directs growth in a preferred direction. In the outgrowth of a dendritic process from the soma, again polarization is suggested. Stochastic inhibition to the further outgrowth of dendritic processes appears evident in the observations. Also, branch length analysis reveals a stochastic inhibition of branch node formation near the soma. Linear growth of dendritic fibers appears to take place by two processes, expansion and extnesion. Dendritic fibers also increase in diameter during the growth period. The following concepts may be used to from a basic set of hypotheses for a stochastic theory of dendritic structural development: a stochastic process of bifurcation with equal surface area probability, stochastic inhibition proximal to branch nodes and initial outgrowth, polarization of dendritic growth pattern and location of initial outgrowth, and growth by expansion and extension. The determination of explicit time dependencies of the processes that underlie these concepts may lead to an understanding of the many varied structural forms observed in the mammalian CNS.

1163 DENDRITIC ALTERATIONS OF DEVELOPING PURKINJE CELLS DURING NEONATAL MALNUTRITION IN RAT. W.S.T. Griffin & D.J. Woodward, Dept. Physiol., Univ. of Roch., Rochester, N.Y. 14642

Postnatal malnutrition in the rat is known to result in a decrease in number of granule cells due to a slowing of the rate of cell proliferation. In this study we asked whether parallel structural alterations occur in Purkinje cells, which do not undergo mitosis postnatally, but which could be influenced directly by changes in cell constituents or indirectly through an alteration in the appearance of parallel fiber afferents. Rat pups from several dams were pooled and separated into experimental groups (20 pups/dam) or control groups (6 pups/dam). Five or six animals were sacrificed on postnatal days, 8,11,14 or 17 and Purkinje cells stained by Golgi-Cox impregnation. A total of 3,462 cells from 44 animals were scored by a single observer as to whether a number of simple "abnormal" characteristics were more prevalent after malnutrition. Morphological aberrations included: dendrites extending below the soma; elongated primary dendrites; dendrites ascending in the molecular layer via unusual directions; and dendrites with nodules at branch points. Significantly greater percentages of cells in experimentals were found to exhibit abnormalities. Such changes in nonmitotic cells might account, in part, for altered protein and RNA to DNA ratios observed in related studies. Our general conclusion about dendritic aberrations in malnourished animals is that growth and a tendency to branch have been retarded, and, in addition, the appropriate zones for dendritic growth have been altered. Supported by Grant No. NIH NS09820.

1164 UNDERNUTRITION AND PURKINJE CELL DEVELOPMENT. J. J. Pysh and R. E. Perkins*. Dept. Anat., Northwestern Univ., McGaw Medical Center, Chicago, Ill. 60611.

The effect of postnatal undernutrition in the development of Purkinje cell dendrites was investigated in Golgi preparations. Postnatal undernutrition was produced in C58 mice by the method of increasing litter size during the suckling period and restricting access to solid mouse food after weaning. Underfed mice and littermate control animals were sacrificed at 35 and 42 days postnatally; afterward parasagittal vermal sections, prepared by Golgi and Nissl methods, were analysed morphometrically. Underfeeding produced on the average a reduction in body weight of 65%, in cerebellum weight and vermis cross-sections of about 20%. Purkinje cell packing density was reduced 19% and Purkinje cell body size was slightly reduced in Nissl preparations. Comparisons of Golgi sections of underfed and control mice revealed a reduction of about 20% in the Purkinje dendritic field area and no change in the packing density of dendritic spines in underfed animals. These data suggest that the growth of Purkinje cell dendrites is altered by postnatal malnutrition. Supported by NIH Grant 1 R01 NS 10657-01.

1165 QUANTITATIVE ELECTRON MICROSCOPY OF SYNAPSE DEVELOPMENT IN DISPERSED CELL CULTURES OF RAT CEREBELLUM. Richard W. Burry* and Robert S. Lasher, Dept. Anat., Univ. of Colorado Sch. Med., Denver, 80220.

Germinal cells in cultures of dissociated 2 day old rat cerebellums differentiate to form primarily granule and stellate types of neurons and non-neuronal cells (Lasher, Brain Res. 69:235, 1974). In addition, these neurons have been shown to form synapses in culture (Burry & Lasher, Brain Res. 88:502, 1975). It was therefore of interest to determine whether synapse formation between differentiating cultured neurons occurred at the same rate and with the same morphological changes as seen in situ. Cultures of 2 day old rat cerebellums, 3 to 36 days in vitro (DIV) were fixed in 1.6% gluteraldehyde and post-fixed in either 1% osmium tetraoxide (OsO_4) or 1% ethanolic phosphotungstic acid (E-PTA). Thin sections were photographed, and various structures were counted and divided by the photographed area of the neurophile (axons, dendrites and terminals) to give a density per μm^2. The density of presynaptic terminals in OsO_4 stained cultures increased in a linear manner from 0.0024 presynaptic terminals per μm^2 at 3 DIV to 0.1829 presynaptic terminals per μm^2 at 28 DIV. No further increase was seen after this time. The ratio of the density of whole synapses (presynaptic terminals, cleft and postsynaptic terminals appearing together) to that of presynaptic terminals was 0.1515$\pm$ 0.0063 ($\bar{X}\pm$ S.E.M.) for cultures 5 to 36 DIV. The changes in density of whole synapses seen in both OsO_4 and E-PTA stained cultures followed the same linear time course for all ages. However, the measurements of the length and width of the postsynaptic thickenings in both OsO_4 and E-PTA stained cultures were not equivalent. In the OsO_4 stained synapses, the postsynaptic thickening most frequently had a width of about 10-15 nm, while in E-PTA stained synapses, the postsynaptic thickening most frequently had a width of 15-20 nm. The mean length of the postsynaptic element at, for example, 21 DIV was 386 $\pm$ 27 nm for OsO_4 stained synapses and 270 $\pm$ 21 nm for E-PTA stained synapses. The following characteristics were not found to vary significantly during the time course examined: size and shape of synaptic vesicles (OsO_4), area of the presynaptic terminal (OsO_4), length of the postsynaptic thickening (either OsO_4 or E-PTA), or the cleft width (either OsO_4 or E-PTA). The mean number of presynaptic dense projections (E-PTA), the dimensions of the presynaptic dense projections (E-PTA), and the mean number of vesicles per presynaptic terminal (OsO_4) did increase from 7 to 36 DIV. With the exception of these changes, our results suggest that in culture maturing synapses show little change in their morphology once they are formed. In addition, the changes in morphology of synapses, the time of onset of synapse formation and the rate of formation are similar to those reported to occur in situ (Woodward et al., Brain Res. 34:73, 1971). Research support: NIH Training Grant GM 01981 (RWB) and NIH Grant NS09641 (RSL).

1166 PARALLEL FIBER DEVELOPMENT IN THE RAT CEREBELLAR CORTEX: EFFECTS OF EARLY HYPO- AND HYPERTHYROIDISM. Jean M. Lauder. Lab. of Neuromorphology, Dept. Biobehavioral Sciences, Univ. Connecticut, Storrs 06268.

Parallel fiber length was measured in the developing cerebellar cortex of controls and rats made hypo- or hyperthyroid from birth using a lesioning and degeneration staining method (Brand et al., Anat. Rec. 178: 315, 1974).

Parallel fibers grow rapidly until 30 days in controls and continue to increase more slowly until 90 days when they reach an average length of 4.7 mm. Initially, the fibers in the inner half of the molecular layer are longest, but eventually the outer fibers surpass the inner ones in length and by 30 days are distinctly longer.

In hypothyroidism, parallel fiber growth is permanently retarded, whereas hyperthyroidism accelerates parallel fiber development, resulting in adult parallel fibers which are 0.5–1.0 mm longer than in controls. This raises the possibility of aberrant cerebellar output in hyperthyroid animals due to excitation of laterally placed Purkinje cells which have different projections than those in the vermis.

Changes in the rate of growth and final length of cerebellar parallel fibers in hypo- and hyperthyroidism suggest that thyroid hormone may play an important role in the postnatal regulation of axonal growth, especially in the cerebellum.

1167 ONTOGENESIS OF THE CLIMBING FIBER AFFERENT SYSTEM OF THE RAT CEREBELLAR CORTEX. D. G. Puro and D. J. Woodward. Dept. Physiology, Univ. Rochester, Rochester, New York 14642.

An analysis of responses of Purkinje cells in developing rat cerebellum to climbing fiber input was conducted to determine which identifying properties of this afferent system are established early in development and which specific features mature with age. Rat pups at various ages were anesthetized with 0.5% halothane and unit recordings made with glass micropipettes. By the third postnatal day electrical stimulation of the motor cortex and limbs at low stimulation rates (<1/sec) could elicit distinct burst responses appearing at long latencies (175 msec). The presence of these responses indicates that pathways of both ascending and descending climbing fiber systems are intact early in cerebellar cortical development. A distinctive feature maturing over the first 1½ weeks was the characteristic of the all-or-none burst response. Before about day 11 variation of the stimulus intensity influenced the interspike interval, amount of inactivation, and number of spikes in evoked burst responses. Mean latencies decreased from 175 msec at day 3 to 50 msec by day 10 but did not achieve the adult value of 20 msec until the fourth week. Typically, climbing fiber responses could only follow at stimulation rates of less than 0.2/sec at day 3. By day 14 they could follow up to 10/sec, which is the same as in the adult. An analysis of spontaneous activity revealed that the mean burst rate and intervals between bursts were adult-like by day 15. In summary, the climbing fiber system establishes connectivity from diverse sources and elicits Purkinje cell responses having identifying characteristics similar to the adult early in cerebellar development. Most aspects of the maturation of transmission can be explained by a decrease in the time scale of function of the synapses involved, mainly those in the inferior olive. (Supported by N.I.H. grant 5-R01-6M00133 and N.S.F. grant GB 43301.)

1168 MITOTIC BEHAVIOR DURING POSTNATAL HISTOGENESIS OF THE CEREBELLAR CORTEX. Ian S. Zagon. Dept. of Anatomy, The M. S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033.

The proliferative characteristics of cells comprising the external germinal layer (EGL) of the cerebellum were examined in 3, 6, 10, and 15-day-old Sprague-Dawley rats; observations were confined to vermian lobules VI, VII, and VIII. The EGL was divided into a superficial area (EGL-S) composed of round cells and a deeper region (EGL-D) consisting of spindle-shaped cells that were elongated in the long axis of the folium. At least 200 anaphases and telophases were recorded under each plane of section examined. Mitoses were observed in all layers of the EGL in transverse sections (i.e., parallel to the long axis of the folium), but generally confined to the upper two-thirds in sagittal sections. This pattern of mitotic location was corroborated by experiments employing H^3-thymidine autoradiography and mitotic arresting agents (vinblastine sulfate and colchicine). In transverse sections of the EGL-S, over 60% of the mitotic spindle fibers were perpendicular to the pial surface (i.e., plane of cleavage parallel to the pia) at all ages, while over 75% of the mitoses recorded in the EGL-D exhibited a parallel orientation of their spindles. Sagittal sections of the EGL-S revealed that in cerebella of ages 3 and 6 days, 68% of the dividing cells were oriented in a perpendicular direction; only 58% were seen at day 10 and 40% by day 15. In sagittal sections of the EGL-D, parallel mitoses predominated at all ages investigated. The frequency of anaphases and telophases per mm^3 at each age shows that except for 6-day cerebella, there were more figures per mm^3 in the transverse plane than in the sagittal plane. Comparison of mitotic frequency between the EGL-S and EGL-D indicates a decreased frequency in the latter. The orientation of the mitotic spindle apparatus was also evaluated in another germinative region located in proximity to Purkinje neurons. Examination of transverse and sagittal sections of 6-day vermis (lobules VI, VII, and VIII) showed that 90% of the anaphase and telophase figures analyzed were dividing in a perpendicular fashion; the frequency of these mitoses was similar to that seen in sagittal sections of the EGL-D. These observations support and extend previous research (Zagon, J. Cell Biol. 59:374a, 1973) and may provide insight into the mechanism(s) of cellular proliferation, commitment, and topographical positioning during cerebellar morphogenesis. (Supported by ACS #DT-30)

1169 LACK OF EVIDENCE FOR GLIAL CELLS ORIGINATING FROM THE EXTERNAL GRANULAR LAYER IN THE MOUSE CEREBELLUM. Jeffrey R. Swarz and Manuel del Cerro. Center for Brain Research, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y. 14642.

The external granular layer (EGL) is the germinal cell layer in the cerebellum that is the precursor of basket cells, stellate cells, and internal granular cells. Its role in the formation of glial cells in the cerebellar molecular layer has been a subject of dispute for the last eighty years. Thus, a study of late postnatal development of the external granular layer in the mouse cerebellum was undertaken to determine if the external granular cells were multipotential, i.e. differentiated into both neurons and glia.

The cerebellum of C57BL/6J mice was studied by autoradiography following cumulative labelling with H^3-thymidine. Mice were given two injections of H^3-thymidine (10μc/gm, i.p.), 12 hours apart, on either postnatal days 13, 14, or 15. Animals were sacrificed by perfusion (4% glutaraldehyde) 24 hours after the first injection or at 25 days of age. One micron thick plastic sections of the area surrounding fissura prima were cut, placed on acid cleaned slides, dipped in Kodak NTB-2 nuclear track emulsion and exposed for 14-28 days. Sections were stained with methylene blue-azure II-basic fuchsin (Humphrey and Pittman 1974). Results show that at 24 hours post-injection the EGL cells were heavily labelled with H^3-thymidine. Capillary endothelial cells and pericytes were also labelled in both the molecular and internal granular cell layers (IGL). A few internal granular cells and oligodendroglia were labelled in the IGL and medullary layer. Animals sacrificed at 25 days of age exhibited labelled stellate cells and endothelial cells in the molecular layer together with many IGL cells and some oligodendroglia in the medullary layer. Glial cells, labelled or unlabelled, were not found in the molecular layer at either time period. A quantitative electron microscopic study to determine the cell types present in the molecular layer of the young adult mouse cerebellum and their respective ratios is in progress.

These results do not support previous reports (Shaper 1894, Fujita et al. 1966, Fujita 1967, Meller and Glees 1969) and others who claim that the external granular layer is a multipotential germinal cell layer. Instead our data are in agreement with the hypothesis proposed by Cajal (1911) and the EM observations of del Cerro and Snider (1972). They suggest that the EGL appears to be a unique germinal cell layer in the developing mammalian nervous system which only differentiates into neurons, while glial cells in the cerebellum represent dislocated neuroepithelial cells that arise from the subventricular layer of the fourth ventricle. (Supported by NIMH training grant MH-08034 and NIH grant H-34.6214)

1170 FUNCTIONAL SURVIVAL OF SYNAPSES ON AKARYOTIC CRUSTACEAN NEURONS. Franklin B. Krasne and Sun Hee Lee. Dept. Psychol., UCLA, Los Angeles, 90024.

It is commonly observed that neurons degenerate after removal of their somas. This is presumed to be due to loss of capacity for protein renewal and perhaps also to derangement of metabolic control and/or loss of undefined trophic influences that depend on the nucleus. Recently, however, it has been shown that some crustacean neurons remain capable of spike propagation and transmitter release and replenishment for weeks or months after removal of their parykaria (Hoy et al., 1967, Science, 156, 251.). Here, we complete this picture of survival of function in "akaryotic" neurons by reporting that postsynaptic reactions to chemical synaptic input also can persist for long periods after removal of the cell body of the postsynaptic neuron.

Interneuron A of the crayfish abdominal cord receives chemically transmitting terminals of ipsilateral tactile afferents of the tail fan (Kennedy, 1971, Physiologist, 14, 5.). Interneuron A's soma lies contralateral to its axon and dendritic field at the caudal margin of the last abdominal ganglion in a sector of tissue bounded by entering roots 3 and 6. This region was removed unilaterally or bilaterally or an incision was made where the neurites cross the midline. The interneuron, unambiguously identified by receptive field, location, and size, survived and continued to respond sensitively to tactile input in better than fifty percent of the cases examined for more than eight weeks. Cobalt filling of the active fiber in several eight week old preparations ruled out the possibility that the severed neurite had reconnected with a foreign soma. These observations and their predecessors raise the question of what functions are disrupted in akaryotic neurons. Experiments in progress will determine whether regenerating sensory axons can find and make synaptic contact with an akaryotic Interneuron A; results should be available at the meeting. (Supported by USPHS Grant 2 R01 NS 08108)

1171 DO REGENERATING MOTOR AXONS RETURN PRECISELY TO THEIR ORIGINAL POSTSYNAPTIC SITES? M.S. Letinsky*, K. Fischbeck*, and U.J. McMahan*. Dept. Neurobiology, Harvard Medical School, Boston, Ma. 02115 (SPON: A.D. Grinnell).

The frog neuromuscular junction is characterized by an arborization of unmyelinated axon terminals, each of which runs longitudinally in its own shallow gutter in the muscle fiber surface. Following nerve crush, it is known that regenerating axons tend to form junctions with the original gutters. We have determined the precision of reinnervation of the original synaptic sites in the cutaneous pectoris muscle by light and electron microscopy, using cholinesterase (ChE) stain to mark the gutters and a mixture of zinc iodide and osmium to stain the terminals. In normal muscles, fewer than 1% of the terminal branches extend beyond the ends of the gutters and less than 5% of the gutter length is not covered by terminals. After prolonged (3 months) denervation, the length of ChE-stained gutters is unchanged (±10%). Thus, ChE stain is a reliable marker for original postsynaptic sites. On reinnervation, regenerating axons cover 95% of gutter length within 4 weeks, showing that axons return precisely to their original sites. At this time, up to 50% of the terminals extend beyond the ends of the gutters. However, terminal and muscle are closely apposed only at gutters; overgrown tips either end in connective tissue or grow to other muscle fibers, where they again form synapses at gutters. Thus, original postsynaptic sites need not cause cessation of axon growth. Axonal geometry is different from normal --e.g., two or more terminal branches often lie in the same gutter in reinnervated muscles. These results demonstrate a strong and specific affinity between old postsynaptic sites and regenerating motor axons. (Supported by NIH grants NS 02253 and NS 70606)

1172 A MORPHOLOGIC BASIS FOR TRANSCELLULAR TRANSFER OF MACROMOLECULES.
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Coated vesicles which form as invaginations of neuronal surface membrane have often been described, but a certain proportion of coated vesicles pinch off a small amount of the adjacent cell. These cytoplasm-containing coated vesicles (CCV) occur at a low frequency in material freshly removed from the animal or incubated in normal physiological saline, but their frequency is increased by increases of the external Ca concentration or by substitution for Ca of other multivalent cations (Co^{++}, La^{+++}) known to compete with Ca^{++}. These effects of altered external ionic composition on the frequency of CCV have been observed in Aplysia ganglia where neurons ingest portions of glial cytoplasm and between Schwann cells and the squid giant axon where transfer of newly synthesized protein has been demonstrated (Lasek, et al., 1974, PNAS 71, 1188). This transfer also occurs between neural elements at or near synapses in cultured embryonic mouse spinal cord and in the adult frog sympathetic ganglion. CCV have been seen going from pre-synaptic to post-synaptic element and vice-versa. The literature also shows examples of this process in other neuronal populations, e.g. between elements of the glomerulus of the mouse cerebellar cortex (Landis and Reese, 1974, J. Comp. Neurol. 155, 93). This phenomenon provides an apparent morphologic basis for transcellular transfer of macromolecules between glia and neurons and between neurons at synapses. The modulation of its frequency (and therefore the amount of material transferred) by the external Ca concentration suggests that it could be regulated in synchrony with the physiological state of either the whole animal or of particular neuronal circuits, but whether this represents a transfer of information remains an open question.

1173 INDUCTION OF TASTE BUDS IN THE DUCTS OF THE GLANDS OF VON EBNER.
A. A. Zalewski. NIH, Bethesda, Md. 20014.

Taste buds appear in the vallate papilla when tongue grafts are combined with sensory ganglia grafts (i.e., nodose ganglia) in the anterior chamber of rats' eyes. In those studies, the grafts were inserted through a midline corneal incision and were pushed laterally until they rested between the iris and cornea. All taste buds disappeared within two weeks and some reappeared subsequently. After survival times of 50 days, many of the tongue grafts (but not the ganglion grafts) developed cysts, their epithelium atrophied, and fewer taste buds were found in them than at earlier postoperative times. A study was therefore undertaken to determine whether exteriorizing the tongue graft (i.e., facing its epithelial surface outward) would better preserve the morphology of long-term grafts. In isogenic adult Lewis rats tongue grafts were inserted within the confines of a corneal biopsy opening and examined after 90 days. The tongue grafts were better preserved irrespective of whether they were transplanted with or without a nodose ganglion. In addition, taste buds were found in the epithelium of the ducts of von Ebner's lingual salivary gland. These glands lie just below the vallate papilla and because their ducts empty into the base of the papilla, portions of the ducts are unavoidably contained in tongue grafts. Finally, it appears that intrinsic nerve fibers of the eye, just like nerve fibers from ganglia, induced the formation of ductal buds. Taste bud induction is nerve-dependent and ductal buds appeared in 8 of 12 tongue grafts transplanted without added ganglia. These results indicate that exteriorization of tongue grafts better preserves their structure, that epithelium in the ducts of von Ebner's gland can be transformed into taste bud cells, and that intrinsic nerve fibers of the eye can induce taste bud formation.

1174 THE EFFECT OF CHRONIC IMMOBILIZATION ON DEFINED TYPES OF MOTOR UNITS IN CAT MEDIAL GASTROCNEMIUS. R. E. Burke, K. Kanda* and R. F. Mayer. Lab. of Neural Control, NINCDS, NIH, Bethesda, Md 20014 and Dept. of Neurology, U. Maryland Sch. Med., Baltimore, Md. 21201.

In 4 adult female cats (2.3 - 4 kg), the knee and ankle joints were immobilized in one hindlimb with steel pins driven through the joints (provided by Dr. V. R. Edgerton, UCLA). Approximately 6 months later, all animals showed impaired mobility of the pinned leg during walking. The pattern of gross EMG activity was normal bilaterally during walking but rectified EMG amplitudes were decreased on the pinned side. At the time of acute experiments under pentobarbital anesthesia, the wet weight of pinned MG muscles was 56 - 61% that of the unoperated MG. Whole MG twitch contraction times were shorter and twitch tensions somewhat less on the pinned side.

The MG motor unit population in the pinned MG was surveyed in each cat using intramotoneuronal recording and stimulation to ensure functional isolation of single motor units. Motor unit types were classified by criteria described elsewhere (1) and the results were compared with data from an extensive sample of MG units obtained in normal animals of approx. the same body weight. The Table below summarizes mean values for various physiological properties of 78 units of identified type in pinned MG and of 221 units in the normal "control" population.

UNIT TYPE	FF		FR		S	
	Pin	Con	Pin	Con	Pin	Con
Number units studied	42	101	8	51	22	57
Percent in pop.	53.8%	45.7%	10.3%	23.1%	28.2%	25.8%
Tw. contr. time, msec	31.3*	34.9	41.2	40.9	60.5*	73.3
Tw. tension, gm	29.5*	43.2	2.8*	12.7	0.75*	2.0
Tetanus tens., gm	52.7*	74.6	5.3*	28.7	1.4*	7.6
Tw/Tet ratio	0.55	0.55	0.53	0.43	0.59*	0.27
Cond. veloc. m/sec	96.4	98.8	98.5	99.6	82.1	83.4
MG Ia EPSP, mV	3.5	4.1	5.2	7.5	6.9*	9.1

* - difference from control mean significant at 0.05 level

The unit sample in pinned MG muscles included 4 F(int) units with fatigue resistance intermediate between FF and FR (1), or 5.1% of the sample as against 5.4% in normals. Only 2 units were found in pinned MG which could not be classified according to the usual criteria.

MG muscles from both legs were removed and quick frozen after each experiment for later histochemical analysis. Muscle fiber types were identified by histochemical profiles (ATPase activity at alkaline and acid pH, and NADH diaphorase activity: see 1.) and the characteristic profiles appeared unchanged in pinned MG. The proportion of fibers identifiable as type FF in comparable, well-preserved regions of pinned and contralateral MG were 55 - 63% versus 51 - 56%, respectively. Ranges for presumed FR fibers were, respectively, 12 - 18% versus 17 - 24%: and ranges for presumed S fibers were 21 - 26% versus 23 - 27% on the unoperated side. Mean fiber areas of individual presumed FF fibers in pinned MG ranged from 42% to 65% of fiber area in comparable regions of unoperated MG: mean areas of presumed FR fibers measured similarly were only 12 to 37% of unoperated; and presumed S fiber areas ranged from 19% to 58% of the mean area of presumed S fibers in unoperated MG.

The results suggest that atrophy following prolonged limitation of limb movement affects the fatigue-resistant FR and S motor units more severely than the fatigue-sensitive type FF, and both physiological and morphological effects appear to be most profound in the type FR unit group.

1. Burke, R.E., Levine, D.N., Tsairis, P. & Zajac, F.E. J. Physiol. 234, 723-748, 1973.

1175 ALTERATIONS IN FAST AND SLOW MUSCLES OF THE RAT AFTER SPINAL INJECTION OF 6-AMINONICOTINAMIDE. Sharad S. Deshpande*, Edson X. Albuquerque, Jordan E. Warnick and Frederick C. Kauffman. Dept. of Pharmacology and Experimental Therapeutics, Univ. of Maryland, School of Medicine, Baltimore, MD 21201.

The 6-amino analog of niacinamide (6-aminonicotinamide; 6-AN) causes significant morphological changes in anterior horn cells of rat spinal cord (Science 127: 644, 1958). In nervous tissue, 6-AN is converted to the 6-AN analog of NADP, which is a potent inhibitor of 6-phosphogluconate dehydrogenase. A complete and irreversible spastic paralysis of both hind limbs of adult female rats develops 24-36 hours after one injection (50 µg in 10 µl) of 6-AN into the lumbar subarachnoid space (L_2-L_4). Paralysis could be prevented by intraperitoneal injection of niacinamide administered immediately and repeated during the latent period. Extensor digitorum longus (extensor) and soleus muscles were removed at intervals after injection of 6-AN for electrophysiological and contractile studies. Four days after injection of 6-AN, the extensor muscle was depolarized with a maximal depolarization of 15-20 mV at 2 weeks. Recovery began by 40-60 days, and by day 70, membrane potentials of about -65 to -70 mV were recorded, i.e., 90% of control. The soleus was not depolarized until day 14 but then the membrane potential gradually decreased to 75% of control by day 70. An increase in miniature endplate potential amplitude and a decrease in frequency began on day 7 in both muscles. Indirectly elicited action potentials (IAPs) could not be generated in the extensor muscle after day 4 unless the cells were hyperpolarized to -75 mV. As the membrane potential recovered (day 70) IAPs could easily be evoked but their rates of rise were diminished. In the soleus muscle, IAPs could be evoked up to day 14 but 30 days after injection, when the muscle was greatly atrophic, these potentials could no longer be evoked. After 14 days, the threshold of directly elicited action potentials in the soleus muscle increased and their amplitude and rates of rise decreased. Tetrodotoxin-resistant action potentials and extrajunctional acetylcholine sensitivity appeared in extensor muscles between days 4-7 and in the soleus muscle after 14 days. Indirectly elicited twitches and tetani could be evoked in extensor muscles but always at tensions lower than control. These findings are consistent with the suggestion that the more differentiated extensor muscles are more sensitive to loss of neurotrophic influence. The technique of injecting chemical substances into the subarachnoid space of the spinal cord offers an additional experimental approach to delineating the nature of neurotrophic factors. (Supported in part by USPHS Grants NS-12063 and NS-12113.)

1176 EFFECT OF NERVE EXTRACTS FROM HIBERNATING HAMSTERS ON MUSCLE DEVELOPMENT IN CULTURE. T. H. Oh and S. S. Goldman*. NIH, Bethesda, Md. 20014.

During hibernation, skeletal muscle of animals undergoes some denervation-like changes, such as loss of weight, spread of extrajunctional acetylcholine sensitivity and decrease in the frequency of miniature endplate potentials at neuromuscular junctions (Lyman and O'Brien, Sym. Soc. Exp. Biol. 23: 489, 1969; Moravec et al., Pflügers Arch. 345: 93, 1973). These muscle changes are thought to occur because axoplasmic flow is interrupted which results in the loss or decrease of a neurotrophic factor. The present study was carried out to test this hypothesis. Soluble nerve extracts were prepared from warm-adapted and hibernating hamster sciatic nerve and spinal cord tissue by centrifuging homogenates (20%, w/v) in Hanks' balanced salt solution (HBSS) for 2 hr at 105,000 g and added to the culture medium to give a concentration in 0.5 mg protein per ml of culture medium. Dissociated thigh muscle cells taken from 12-day-old chick embryos were maintained in collagen-coated plastic dishes. The culture medium consisted of 63% Dulbecco's modified Eagle's medium, 10% horse serum, 5% chick embryo extract (brain and spinal cord excluded), 20% HBSS and 2% glucose (20% solution). Addition of the nerve extracts of warm-adapted hamster stimulated DNA and protein synthesis and morphological development of muscle cells. Nerve extracts from hibernating hamsters did not produce such effects. However, the extracts from both animals increased acetylcholinesterase activity of muscle cells. These results indicate that a neurotrophic factor may be lacking or reduced during hibernation which causes denervation-like changes in skeletal muscle.

1177 IS BIOELECTRIC ACTIVITY A TROPHIC MESSAGE IN SKELETAL MUSCLE? Neal Baumbach and Raphael Gruener. Department of Physiology, College of Medicine, University of Arizona, Tucson, Arizona 85724.

Submechanical-threshold electrical stimulation of denervated extensor digitorum longus (EDL) reduces acetylcholine (ACh) supersensitivity (Nature 248: 68: 1974). We report here effects of submechanical threshold stimulation on the resting potential (RP) of denervated EDL, and the effects of endplate blockade by α-bungarotoxin (α-BGT) on tetrodotoxin (TTX) sensitivity. Surgically denervated EDLs were electrically stimulated via implanted electrodes attached to the muscle tendons. The stimulus amplitude was adjusted under visual observation (80X) to 15% below contraction threshold. The contralateral EDL was wired but not stimulated. Stimulation started immediately after denervation and continued for 4 days. RPs were measured in-vivo in 17 stimulated and 17 control muscles. RPs from 340 stimulated and 340 unstimulated fibers were -62.1 ± 0.5 mV and -58.3 ± 0.5 mV, respectively. This is a significant hyperpolarization ($p < 0.01$). In another group of animals both EDLs of each animal were bathed in-situ for 30 min/day for 3 days with α-BGT (0.25 mg/ml) dissolved in balanced salt solution (BSS) to block neuromuscular transmission. Control EDLs were bathed in BSS. Five days after the initiation of α-BGT treatment, the muscles were excised and mounted for conventional double microelectrode recording. Action potentials (AP) in control muscles were abolished in less than 5 min by 1×10^{-6} M TTX while APs in muscles which had been blocked by α-BGT were not abolished at all. We conclude that artificially induced bioelectric activity restored the resting potential towards normal and furthermore that neuromuscular blockade by specific masking of ACh receptors causes the appearance of TTX-insensitive APs. Supported by NIH grant (NS-10417) and MDA grant to RG and Training grant (HL-05884).

1178 EFFECTS OF INACTIVITY ON MUSCLE MEMBRANE PROPERTIES IN RATS. A. Cangiano, L. Lutzemberger* and D.S. Zorub*. Dept.Physiol. Sch. Med., Pisa, Italy.

It is known that muscle disuse following blockade of impulses along peripheral nerves of rats, induces muscle supersensitivity to acetylcholine similar to denervation. It is however debatable whether factors other than nerve impulses partecipate in the neural control of the skeletal muscle. We have approached this problem by extending the comparison between nerve conduction-block and nerve section to other denervation-induced changes such as membrane depolarization, fibrillation and action potential resistance to tetrodotoxin (TTX). Complete and stable conduction block was obtained by placing around one sciatic nerve a silicone cuff exerting mild compression. The contralateral sciatic nerve was sectioned. At various times after these procedures in a series of rats, membrane potential and fibrillatory activity were recorded intracellularly in vivo from EDL muscle fibers bilaterally. With each recorded fiber the nerve was stimulated proximal and distal to the block and absence or presence, respectively, of transmitted action potentials observed. Resistance of muscle action potentials to TTX was assayed in vitro. It was found that all of the above mentioned denervation-like changes develop in fibers which have lost nerve impulses but retained their innervation. However these changes are less pronounced on the cuffed than on the denervated side, the difference being statistically significant. These findings suggest that other neural influences ("trophic") may share, with nerve impulses, control of muscle membrane properties.

1179 EFFECTS OF NERVE LIGATION ON AXONAL SIZE AND MUSCLE HISTOCHEMISTRY. Raymond B. Wuerker. Department of Pathology (Neuropathology), UCLA Hospital, Los Angeles, 90024

Methods for assessing the long term effects of nerve ligation on axonal size and muscle histochemistry were developed by applying sutures to the sciatic nerves of 39, 10-15 day old albino rats. In 13 animals, the nerves and triceps surae muscles from both the experimental and contralateral control sides were examined approximately 2 months after ligation. Grossly, the muscles were hypoplastic and histochemical reactions showed high levels of succinic dehydrogenase (SDH), myosin ATPase (mATPase), periodic acid-Schiff (PAS) and phosphorylase B (Phos). Nerves above the ligation were smaller than those of the controls, and there was a marked decrease in caliber below the lesion. In 8 animals, these nerves showed a uniform longitudinal distribution of SDH and acetylcholinesterase both proximal and distal to the ligature rather than the piling up of reaction product that characterizes acute ligation.

Ligatures on 18 animals were removed and their muscles and nerves examined 2-4 months later. Nerves distal to the ligature were only slightly smaller than their proximal, parent axons. Both proximal and distal fibers remained smaller than the controls. The muscles contained increased numbers of large fibers with decreased SDH activity. Although mATPase, PAS and Phos activity remained high, groups of lightly staining fibers were evident with the mATPase reaction.

Long term nerve ligation appears to produce changes in nerve size rather than axoplasmic flow. Furthermore, the changes produced in muscle histochemistry by nerve ligation are more closely related to changes in axonal size than to disturbances of flow. (Supported in part by USPHS Grant HD 05615)

1180 FUNCTIONAL AND STRUCTURAL CHANGES IN MAMMALIAN SYMPATHETIC NEURONS FOLLOWING INTERRUPTION OF THEIR AXONS. Dale Purves, Department of Physiology and Biophysics, Washington University Medical School, St. Louis, Missouri 63110.

In principle, a neuron's properties could be trophically controlled by the fibers which innervate it (as the properties or vertebrate skeletal muscle fibers are controlled by motor neurons), or by an effect stemming from an interaction with the target which the neuron innervates. The purpose of the present study was to examine the latter possibility by determining the extent to which the properties of superior cervical ganglion (SCG) neurons in adult guinea pigs are changed by interruption of their axons, and whether these changes are reversed by axon regeneration.

Within 72 hours of crushing the postganglionic nerves (thus allowing prompt regeneration) the amplitude of excitatory postsynaptic potentials (e.p.s.p.s) recorded in SCG neurons in response to preganglionic stimulation declined. E.p.s.p.s were maximally reduced (by more than 70% on average) 4-7 days following interruption, and failed to bring many cells to threshold. However, e.p.s.p.s recorded in nearby neurons whose axons remained intact were unaffected. Mean e.p.s.p. amplitudes began to increase again after about 1-2 weeks. One month after the initial injury, many neurons had e.p.s.p.s of normal amplitude, and by two months, affected neurons were generally indistinguishable from control cells. Stimulation of the cervical sympathetic trunk in lightly anesthetized animals showed that functional peripheral connections were reestablished during the period of synaptic recovery within the ganglion.

The mean number of synapses identified per unit area in electron microscopic sections from ganglia whose postganglionic branches had been crushed decreased by 65-70% in parallel with synaptic depression measured by intracellular recording. Thus loss of synaptic contacts appears to be the major cause of impaired ganglionic transmission. In addition, the mean amplitude of spontaneously occurring synaptic potentials was reduced in neurons whose axons had been crushed 4-7 days previously. This finding indicates an abnormality of the residual synapses on affected neurons which also contributes to synaptic depression.

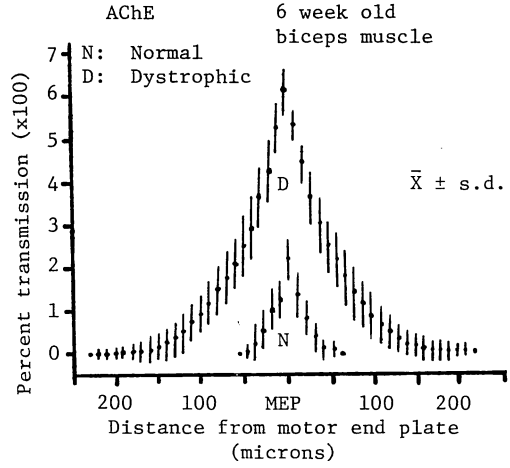
During the period of maximal synaptic depression (4-7 days after nerve crush), numerous abnormal profiles containing accumulations of vesicular and tubular organelles, dense core vesicles, and mitochondria were observed in electron microscopic sections. Injection of horseradish peroxidase into affected neurons demonstrated dendritic swellings which probably correspond to these profiles. The complement of organelles seen in these profiles is similar to that found in the interior of mammalian growth cones and suggests rapid membrane turnover. Dendritic action potentials were also observed during this period, but were not seen in normal ganglion cells.

In spite of full recovery of synaptic transmission in virtually all cells impaled two months after nerve crush, counts of the number of neurons in experimental and control ganglia showed that many neurons (about half) had degenerated during this time, although cell counts at the end of one week were similar on both sides. If, however, axons were chronically ligated to prevent them from growing back to their target organ, surviving neurons whose axons were enclosed by the ligature did not recover a normal synaptic response to preganglionic stimulation even after two months. A large majority of the neurons whose axons were ligated died within a month.

These results indicate that the integrity of the axon of a ganglion cell is necessary for the maintenance of its preganglionic synaptic contacts, and ultimately for neuronal survival. The basis for neuronal recovery from the effects of axon interruption appears to be some aspect of regeneration to peripheral target.

1181 RELATIONSHIP OF SARCOPLASMIC ACETYLCHOLINESTERASE TO MOTOR END PLATES OF NORMAL AND DYSTROPHIC CHICKENS. Gary T. Patterson* and Barry W. Wilson. Dept. Avian Sciences, Univ. of Calif., Davis, CA 95616.

There have been many studies of the distribution of acetylcholine (ACh) sensitivity and ACh receptor along muscle fibers, and little study of the distribution of acetylcholinesterase (AChE). An especially suitable system for such problems is the fast twitch fibers of dystrophic chickens. AChE activity is localized at or near the motor end plate in normal chick fast twitch muscle fibers. However, in dystrophic chick biceps muscle fibers AChE activity is also found in the sarcoplasm away from the motor end plate. AChE specific histochemical staining of serial cross sections of frozen muscle samples was performed to study the distribution of sacroplasmic AChE activity and its relationship to the motor end plate in individual muscle fibers of one, two, and six week old normal and dystrophic chickens. A photographic cytophotometry technique was developed to determine AChE activity. The results showed that at both one and two weeks of age there was no difference between normal and dystrophic muscle fibers in the localization of level of AChE activity. However, by six weeks of age, AChE activity in dystrophic fibers had almost tripled and had spread to either side of the motor end plates for approximately 250 microns (five times the distance found in normal fibers).



AChE higher, spreads further from motor end plates (MEP) of dystrophic muscle. Compilation of 40 fibers each line. Frozen serial sections. Stained according to Karnovsky.

Of interest is the fact that the distribution of AChE activity around the end plate of dystrophic fibers was similar to the distribution of ACh sensitivity of denervated muscles even though ACh sensitivity has not been found to be altered in dystrophy nor are dystrophic muscles denervated. These findings suggest that dystrophic chicken muscle develops similarly to normal muscle with respect for at least two weeks post-hatch, and then the AChE activity spreads along the muscle fiber from the motor end plate. The data are consistent with the view that there is a myogenic defect in maturation of AChE regulation associated with the motor end plate of dystrophic chickens. (Supported by NIH Grants 10957 and 16716 and the MDA.)

1182 PHARMACOLOGICAL AND DIETARY MANIPULATIONS OF FETAL BODY AND BRAIN AMINO ACIDS. Mark F. Nelson*, Robert A. Howd* and Loy D. Lytle. Dept. Nutrition and Food Science, MIT, Cambridge, Massachusetts 02139.

Previous work in our laboratory has shown that the concentrations of the amino acid precursor tryptophan (TRYP) and the neurotransmitter serotonin (5HT) in the brains of fetal rats can be increased by the subcutaneous (sc) injection of TRYP to gravid female rats. In the present experiments, 19 day post-conception pregnant rats were injected with 100 mg/kg of TRYP sc and killed one hr later, or were fasted for 12 hr and then given two hr access to an 18% protein diet, or to a protein-free, high carbohydrate diet and then killed. Injection of TRYP to the pregnant mothers increased greatly the concentration of the amino acid in the brains and bodies of the fetuses, increased fetal brain 5HT levels, and decreased the fetal brain and body concentrations of the amino acid tyrosine (TYR). Fetal brain and body concentrations of TRYP increased approximately 70% in animals whose mothers consumed the high carbohydrate meal when compared with animals consuming the 18% protein diet.

Since TYR and TRYP are precursor amino acids for putative brain neurotransmitters in mammals, it appears that drugs or diets that affect amino acid patterns in pregnant animals may alter fetal brain neurotransmitters. The significance of these changes for the developing organism are currently under investigation. (These studies were supported in part by a grant from the National Foundation and by an NIH postdoctoral fellowship 1 F22 NS01158-01).

1183 BIOELECTRIC PROPERTIES OF NEWBORN AND ADULT HYPOTHALAMIC DENDRITES. Clara Torda. Mt.Sinal School Med.,N.Y.,N.Y.,10029.

At birth the hypothalamic dendrites are morphologically immature. The surfaces of these dendrites are covered by ephemerical shaggy spinelike excrescences, pleomorphic in appearance and variable in length. Between the fourth postnatal week and the end of the second month (rat, cat) these spines become slender, shorter, and more regularly distributed. During the first postnatal month synapses have not been recognized. The extended surfaces of the protospines of dendrites seem to act as postsynaptic membranes. The differences in cell shapes and dendritic course in the various hypothalamic areas have also been studied. The resting potentials of morphologically immature and mature dendritic membranes were comparable (-56 and -59 mV respectively). Conduction velocities were also comparable (0.27 and 0.29 mm per 1 msec. respectively). The morphologically immature dendrites were hypersensitive to both stimulation with rectangular pulses and to exposure to norepinephrine. Stimuli of near-threshold intensity generated graded excitatory postsynaptic potentials(in both the morphologically mature and immature dendrites). With higher stimulus intensity spiking occurred. The site of origin of generated spikes has also been studied by observations a)of the effect of hyperpolarizing current on spike generation; and b)on tissue cultures. Due to hypersensitivity to stimuli,stimuli of intensity within the physiological range generated both spikes originating at the axon hillock and at the dendritic membrane in the morphologically immature dendrite. In adult preparations stimuli of intensity within physiological range generated mainly spikes of axon hillock origin. The differences in sensitivity to stimuli were statistically significant. The hypersensitivity to stimuli of the morphologically immature hypothalamic neurons enables these neurons to fulfill a physiological function:signaling to the environment the arousal of a vital need of the newborn (hunger, thirst, temperature and blood pressure changes).

1184 ANDROGEN AND ESTROGEN BINDING MACROMOLECULES IN DEVELOPING MOUSE BRAIN. Thomas O. Fox. Depts. of Neuropathology, Harvard Medical School and Neuroscience, Children's Hosp. Med. Cntr., Boston, Mass. 02115

High affinity binding of both estradiol and testosterone is detected in extracts of hypothalamus and preoptic area from prepubertal mice. Lower amounts of binding of both steroids are also detected in these tissues from neonatal mice. Analyzed on sucrose gradients these "receptors" sediment at 5-7.5 S ("6S") without salt and at 4-4.5 S ("4S") in 0.15 M NaCl. Buffers containing 0.15 M NaCl and 10% glycerol were used because they optimized detectable testosterone "receptor" in prepubertal mice and estradiol "receptor" in neonatal mice.

The physiological specificity of the testosterone "receptor" is demonstrated by its low levels (Fig. 1) in extracts of hypothalamus and preoptic area from the androgen insensitive, sex-linked mutant mouse, testicular feminization (Tfm). Extracts of the affected Tfm/Y animals do, however, bind normal or greater than normal amounts of estradiol. The normal androgen "receptor" binds testosterone and dihydrotestosterone with near equal affinities and also may bind estradiol.

Studies of the estradiol "receptor" in neonatal mice were initially complicated by the presence in neonatal animals of a "neonatal binding protein" for estradiol. This protein sediments at 4S in 0.15 M NaCl, but in contrast to the prepubertal "receptor" remains 4S even in the absence of salt. Prepubertal "receptor" binds to DNA on DNA-cellulose columns and has high affinity for the non-steroidal estrogen, diethylstilbesterol (DES). In contrast, most bound estradiol in neonatal extracts does not bind to DNA and does not have high affinity for DES. A small fraction of bound estradiol in neonatal extracts that does bind to DNA is eliminated by DES. Therefore, a small amount of estradiol "receptor" is present in neonatal tissues and can be separated from "neonatal binding protein" by DNA-cellulose chromatography.

By separating "neonatal binding protein" from "receptors" on DNA-cellulose columns (Fig. 2), we have determined the apparent affinities of the two proteins for estradiol. Estradiol "receptor" from prepubertal tissues saturates at about 5 nM estradiol; "neonatal binding protein" is not saturated even at 140 nM. In neonatal extracts, the "receptor" saturates at about 40 nM estradiol, about 8-fold higher than in prepubertal extracts. Thus "neonatal binding protein" competes appreciably with estradiol "receptor" in neonatal extracts, reducing its apparent affinity. "Neonatal binding protein" may protect the neonatal brain from high maternal estrogen levels. Sufficient exogenous estradiol causes sterilization of neonatal rodents. It is possible that sterilization by maternal estrogens might always occur if estradiol sensitive tissues were not protected.

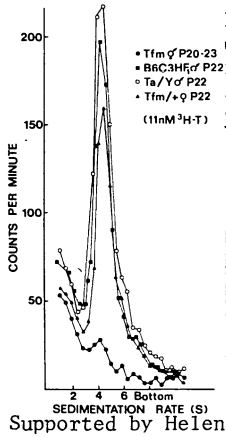


Fig. 1. Sucrose gradients of bound ^{3}H-testosterone in extracts of hypothalamus and preoptic area from prepubertal Tfm/Y mutants and controls.

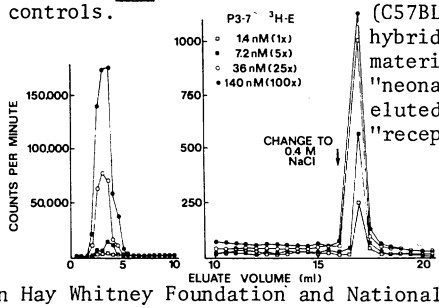


Fig. 2. DNA-cellulose chromatography of bound ^{3}H-estradiol in extracts of hypothalamus and preoptic area from "neonatal" (C57BL/6J x C3H/HeJ)F_1 hybrid mice. Breakthrough material on the left is "neonatal binding protein"; eluted material on right is "receptor".

Supported by Helen Hay Whitney Foundation and National Foundation.

1185 ELECTROPHYSIOLOGICAL STUDIES DURING SYNAPTOGENESIS IN CULTURES OF FETAL MOUSE OLFACTORY BULB. William A. Corrigall*, Stanley M. Crain and Murray B. Bornstein. Depts. of Neuroscience, Physiology and Neurology, Albert Einstein College of Medicine, Bronx, N.Y. 10461

Explants of olfactory bulb from 18- and 19-day fetal mice have been cultured in Maximow slide chambers for periods up to 4 weeks. During the first 3-4 days *in vitro* compound spike potentials representing conductile activity are the only bioelectric responses which can be evoked. By the end of the first week in culture, however, characteristic synaptically generated slow wave potentials may be evoked, and in older cultures these slow wave discharges also occur spontaneously.

Whereas young cultures (up to 7 days *in vitro*) show no evidence of synaptic slow waves in balanced salt solution (BSS), these complex network discharges are readily elicited after addition of antagonists of γ-aminobutyric acid (GABA), e.g. bicuculline or picrotoxin (10^{-5}M), or after substituting propionates for the chlorides in BSS. Furthermore, in older cultures, slow waves occurring in BSS are depressed by the addition of GABA (10^{-4}M). The data suggest development in these isolated olfactory bulb explants of GABA-mediated inhibitory synaptic circuits, as well as complex excitatory synaptic networks, resembling organized bioelectric activities observed during maturation of cerebral neocortex and hippocampus explants in culture (Crain and Bornstein, Br. Res. 68:351,'74). In these relatively simpler bulbar cultures, however, it may be possible to identify the granule-to-mitral synapse as a specific component of the inhibitory network.

In addition to slow wave activities, positive or positive-negative unit spikes, with amplitudes reaching several millivolts, have been recorded extracellularly (via saline-filled micropipettes, *ca.* 3 μm tips) from presumptive mitral cells in the olfactory bulb explant. These large unit spikes may occur spontaneously by 1-2 days *in vitro*, well before the first slow wave network discharges can be detected, and may also be evoked, at 3-10 msec latency, following stimulation of the explant or the neuritic outgrowth. These spikes frequently exhibit an inflection on the rising phase, resembling A-B fragmentation seen in giant extracellular spikes recorded from mitral cells of the olfactory bulb *in situ* (Phillips, C. et al, J. Physiol. 168:65,'63). The large positive (or positive-negative) unit spikes in the bulb explants are in marked contrast to the smaller, predominantly negative spikes which are generally recorded from other types of CNS tissues under similar experimental conditions in culture. Since these spikes have been seen even in the youngest cultures, olfactory bulb explants provide a valuable model system in which the development of synaptic network activity may be analyzed in relation to an identified type of cerebral neuron. Furthermore, introduction of olfactory epithelium to these deafferented olfactory bulb explants should permit direct studies *in vitro* of the effects of sensory innervation on this specialized cerebral tissue. (Supported by a Fellowship from the Medical Research Council of Canada, NINDS grants NS-06545 and NS-06735, and NSF grant BMS75-03728.)

1186 DEVELOPMENT OF SYNAPSES IN THE NUCLEUS INTERPEDUNCULARIS OF THE ALBINO RAT. Nicholas J. Lenn, Dept. Neurol., Pediat., Carnegie Embryol. Lab., Sch. Med., U. Calif., Davis, 95616.

The habenulointerpeduncular afferent axons form a prominent horizontal plexus within the interpeduncular nucleus (IPN). These axons form synapses en passant of 2 types. At S synapses the axon contains spherical vesicles, and the contact is asymmetrical. At crest synapses 2 axons form similar contacts paired and coextensive on opposite sides of attenuated dendritic processes, either side branches (crests) or in synaptic complexes.

On postnatal day 0 (PO) IPN contains horizontal axon bundles and occasional synapses en passant. Because these synapses contain spherical vesicles and minor contact asymmetry they are interpreted as early forms of S synapses. Apparent dendritic growth cones are also present. At P4 there is some increase in the postsynaptic densities. At P6 an increase in dendrites and S synapses results in a more complex neuropil. At P8 the earliest crest synapse is found, and the horizontal axons are more mature, with longitudinal neurotubules appearing. By P14 crest synapses are more frequent, and early synaptic complexes are forming. At P21 myelinated axons are present in IPN, many more S and crest synapses are seen, and probable variability of synapse maturity is apparent.

Destruction of the habenular nuclei on PO causes marked disruption of this sequence of development with delayed ingrowth of axons, marked decrease in S and crest synapses, early (P3) presence of flat vesicle containing synapses, and no myelinated axons by P21. With unilateral habenular destruction on PO disruption is partial and intact crest synapses are found by P10. Thus loss of a predominant afferent input unilaterally causes major but differential response in different synaptic types which is obscured with bilateral destruction.

1187 HISTOGENESIS OF THE VISUAL CORTEX IN RHESUS MONKEY: DIFFERENCES IN TIME OF ORIGIN AND IN EVENTUAL DISTRIBUTION OF NEURONS IN AREAS 17 AND 18. Pasko Rakic, Dept. of Neuropathology, Harvard Med. Sch. and Dept. of Neuroscience, Children's Hospital Medical Center, Boston, Mass. 02115.

The time of origin and eventual disposition of neurons in the visual cortex were studied by autoradiography in two- to five-month-old rhesus monkeys that had been exposed to a single pulse of H^3-thymidine at selected embryonic (E) and early postnatal (P) days. Neurons which entered their last cell division at the time of H^3-thymidine injections, and, as a result, became permanently heavily labeled were plotted from autoradiograms in selected regions of area 17 and 18. The position of neurons in the cortical laminae in both areas correlates systematically with time of cell origin; neurons destined for deeper cortical positions are generated earlier, and more superficial ones progressively later, the last neuron being generated more than two months before birth. (Gestation in rhesus monkey lasts 165 days.) Although the production of neurons destined for both cortical areas generally overlap in time, both the beginning and end of neurogenesis occurs at slightly earlier ages for neurons of area 18 than for those of area 17. Thus, in area 17, most neurons destined for layer VI are born between E43 and E62, for layer V between E62 and E70, for layer IV between E70 and E85, and for layers III and II between E80 and E102. In area 18, neurons of layer VI are generated between E40 and E54, layer V between E54 and E62, layer IV between E62 and E80 and layers III and II between E80 and E90. From this, it follows that neurons that are generated simultaneously in adjacent regions of the proliferative zones end up in different cortical layers and therefore eventually assume different morphological and functional characteristics in the two cytoarchitectonic areas. (Supported by NIH Grant NS11233.)

1188 TIME OF ORIGIN OF NEURONS IN THE HIPPOCAMPAL REGION OF THE RHESUS MONKEY. R.S. Nowakowski* and P. Rakic. (SPON: M. Schachner). Depts. of Neuropathology, Harvard Medical School and Neuroscience, Children's Hospital Medical Center, Boston, Massachusetts 02115.

The time of origin of neurons in the hippocampal region was determined in a series of 26 rhesus monkeys, each of which had been exposed to a pulse of ^{3}H-thymidine at a different time during ontogeny and then sacrified between two and five months after birth. No labeled cells were found in specimens exposed to ^{3}H-thymidine at embryonic day 30 (E30), E33, or E36, but in specimens injected at E38 a few labeled neurons were present in all of the hippocampal subdivisions. Although neurogenesis in the rhesus monkey begins simultaneously throughout the entire hippocampal region, it terminates at variable times in each of its subdivisions. Thus, in the entorhinal area and in the presubiculum the last neurons are generated between E70 and E80, whereas in the subiculum neurogenesis ends about two weeks earlier, between E54 and E62. Within the hippocampus proper the last pyramidal cells are generated between E70 and E80 in area CA 1, between E54 and E62 in area CA 2, between E62 and E70 in area CA 3, and between E70 and E80 in area CA 4. Granule cells of the fascia dentata are generated during a five month period extending from E38 through the first postnatal month. There is a clear inside-to-outside spatiotemporal gradient of neurogenesis in the entorhinal area, presubiculum, and in the stratum pyramidale of the subiculum and hippocampus. However, the spatiotemporal pattern of granule cell origin is outside-to-inside. Furthermore, those granule cells generated between E38 and E80 are distributed in a distinct suprapyramidal-to-infrapyramidal spatiotemporal gradient, while those generated at later ages are distributed evenly throughout the fascia dentata. (Supported by NIH Research Grant #NS 11233.)

1189 DEVELOPMENT OF HIPPOCAMPAL AFFERENTS. Rebekah L.C. Smith, Gary Lynch and Carl Cotman. Dept. Psychobiology, Univ. Calif. Irvine, Ca 92664

While many investigators have analyzed the cytoarchitecture and afferent lamination of the adult hippocampal formation, studies dealing with the development of this structure are relatively scarce, and no one to date has dealt experimentally with the manner of ingrowth of hippocampal afferents and their establishment in the characteristic laminae. We have examined this question in the rat hippocampus using Fink-Heimer staining methods. Animals received either unilateral hippocampal or entorhinal cortex lesions every other day of age between 2 and 26 days after birth. Operated animals were sacrificed after 1 or 3 days, and the brains stained by a modification of the Fink-Heimer method omitting the initial suppression steps (as per C.M. Leonard, J. Comp. Neurol. 156, 435, 1974).

Preliminary data analysis suggests that the entorhinal cortical afferent arrives in the molecular layer of both dentate gyrus and hippocampus proper (CA1) before that from the contralateral hippocampus. The entorhinal fibers appear to invade the dentate gyrus even by birth; at the earliest ages tested degeneration argyrophilia appears throughout the molecular layer. The first evidence of commissural axon or terminal staining in the dentate gyrus appears between days 8 and 10. At 10 days the terminal field covers about 30% of the 170 μ wide molecular layer. Thus, a zone of about 50 μ contains commissural terminals at the earliest age in which this afferent appears. Considering that this afferent covers approximately 75 μ in the adult rat dentate gyrus, we must conclude either: (1) that the dendrites grow along their lengths to accomodate the added 25 μ during development; or (2) that the established entorhinal terminals are displaced outward along the growing dendrites as these grow at their tips. (Supported in part by NIH predoctoral fellowship #MH 57363-01 to R.S. and NIH grant # NS 11589-01 and NSF grant #BMS 7702237-2 to G.L.)

1190 THE ORGANIZATION OF THE AFFERENTS TO THE DENTATE GYRUS OF THE HIPPOCAMPAL FORMATION IN THE REELER MUTANT MOUSE. Brent B. Stanfield*, V.S. Caviness, Jr.*, and W.M. Cowan. Dept. Anat. & Neurobiol., Sch. Med., Washington Univ., St. Louis, Mo., 63110, and E.K. Shriver Inst., Harvard Med. Sch., Waltham, Mass. 02154.

As in certain other cortical structures in the brain of the reeler mutant mouse, the cells in the hippocampal formation are arranged abnormally. The defect in the positioning of the cells in the hippocampus itself appears to be somewhat less severe than that seen in the neocortex and cerebellum, but the disposition of the granule cells of the dentate gyrus is markedly abnormal. Thus, in the reeler the pyramidal cells of the regio superior of the hippocampus (field CA_1) are distributed in two more-or-less distinct laminae, while those in the regio inferior (fields CA_2, CA_3 and CA_4) tend to be arranged in a more dispersed yet fairly continuous layer. In the dentate gyrus the molecular layer and the "V"-shaped granule cell zone are well defined. However, collections of granule cells are also spread throughout the hilar region so that this normally granule cell-free zone is virtually obliterated. In normal mice, as in other mammals, the afferents to the molecular layer of the dentate gyrus are distinctly laminated. The outer three-fourths of the layer receives an input from the entorhinal cortex by way of the perforant path. This projection has distinct outer and inner laminar subdivisions, representing the projections from the lateral and medial entorhinal areas, respectively. The inner one-fourth of the molecular layer receives associational and commissural fibers from the pyramidal cells in fields CA_{3c} and CA_4 of the hippocampus of the ipsilateral and contralateral sides, respectively.

We have examined the distribution of the various afferents to the dentate gyrus in the reeler mutant using both the Fink-Heimer method (after lesions of the entorhinal cortex and the contralateral hippocampus, and the autoradiographic method (following stereotaxic injections of ^{3}H-proline). In the reeler mouse the termination of the perforant path is contained within the molecular layer and, as in normal animals, the projections of lateral and medial entorhinal areas are distributed to distinct outer and inner laminae. However, the lamination pattern in the reeler animals is abnormal in that the perforant path fibers extend all the way from the hippocampal fissure or pial surface to the outer margin of the granule cell layer; they thus occupy the inner zone of the molecular layer which in normal mice is free of entorhinal input. The commissural and associational inputs are similarly displaced in that their termination fields extend from the outer margin of the granule cell layer (where they abut upon the zone of termination of the medial entorhinal afferents) throughout the diffuse granule cell zone. There is thus virtually no commissural or associational input to the molecular layer of the gyrus. But again, as in normal mice, the termination fields of the commissural and associational inputs are coincident.

These observations suggest that at least some of the factors responsible for the distribution of the afferents to the dentate gyrus in normal animals operate in the reeler mutant despite the fairly gross abnormality in the positioning of the target cells. Thus in the reeler, as in the normal mouse, the commissural and associational inputs share the same termination field and this is proximal (with respect to the granule cell somata) to the zone occupied by the entorhinal inputs. Furthermore, the entorhinal and commissural/associational zones are as sharply defined as in normal animals, with the afferents to each zone being strictly confined to their own domain. (Supported in part by NIH grants NS-10943 and HD-04147, and the Joseph P. Kennedy, Jr., Memorial Foundation.)

1191 SEX HORMONE INDUCTION OF PREOPTIC/HYPOTHALAMIC DEVELOPMENT IN THE NEWBORN MOUSE IN VITRO: C. Dominique Toran-Allerand, International Institute for the Study of Human Reproduction and Dept. Neurol., Columbia Univ., Coll. of P & S, New York, New York 10032

The influence of the post-natal steroid environment on sexual differentiation of the brain and on gonadotropic regulatory mechanisms has been extensively studied in the rodent. The neural sites and morphological correlates of these effects are, however, largely unknown. This study was designed to investigate, in cultures of the newborn mouse hypothalamic/preoptic area, the neurogenesis of sexual differentiation of the brain. Coronal explants of the genetic male and female newborn mouse were maintained in vitro as organotypic cultures for periods up to 60 days. Explants were halved to produce homologous pairs. Testosterone was added at explantation to ½ of each of some pairs in the standard nutrient medium for a final concentration ranging from 1-5µg/ml. The homologous half received an equal volume of 0.5% BSA diluent as control. Antibodies to estradiol and testosterone were added to the nutrient medium of ½ of each of other pairs in order to diminish intrinsic sex steroid content. Addition of testosterone results in an intense neuritic proliferation radiating out from the margins of some portions of the preoptic/anterior hypothalamus and mammillary regions accompanied by enhanced neuronal maturation and myelinogenesis. The presence of antibodies to estradiol and testosterone produces the exact opposite developmental effect only in regions previously shown to be stimulated. These findings suggest that the maturation of certain hypothalamic regions is selectively advanced by the addition of androgen at birth and that both male and female patterns of neural differentiation may require neonatal imprinting by steroid. Since the development of the nervous system is dependent on well ordered sequences of phenomena, interrelated both spatially and temporally, timing is critical and deviations may alter the entire subsequent pattern of neural differentiation.

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1192 EFFECTS OF NEONATAL STRESS UPON CELLULAR AND SYNAPTIC MEASURES IN OCCIPITAL CORTEX OF MALE AND FEMALE RATS. Timothy J. DeVoogd and William T. Greenough. Depts. Psychology and Neural & Behavioral Biology Program, University of Illinois, Urbana-Champaign, 61820.

Considerable research indicates that there are long term behavioral effects of neonatal stress in rodents. However, possible anatomical concomitants of such stress have received little attention. One study (Schapiro & Vukovich, Science 167:292, 1970) indicated that multi-modal sensory stimulation increased both the density of spines and the density of cells impregnated by the Golgi method. In the present study, similar multi-modal stimulation (handling, loud sound, temperature extremes, electric shock, and other tactile and vestibular stimulation) was given to rats for the first 8 postnatal days. Behavioral tests indicated greater open field exploration in neonatally-stressed female, but not male, 60 day old rats. Animals were sacrificed at 2 months of age. In light microscope analysis of paraphenylenediamine stained sections, the occipital cortex of stressed rats tended to be thinner in both males and females. In most layers, neonatal stress increased cell density in females, whereas males had either a decrease or no difference from controls. Nonstressed females generally had greater occipital cortical cell density than nonstressed males. Initial results of analysis of electron micrographs from occipital cortex have also suggested a sex by neonatal stress interaction in the size of synaptic contacts. The results extend previous work on the anatomical effects of neonatal stress and demonstrate sex differences in both behavioral and morphological responses to the stress.

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1193 NEOCORTICAL ORGANIZATION IN HUMAN CEREBRAL MALFORMATIONS: A GOLGI STUDY. R.S. Williams*, R.J. Ferrante*, and V.S. Caviness, Jr. Harvard Medical School, Boston, Ma. 02115.

Two cases of lissencephaly-pachygyria were studied by Golgi methods. This human malformation is believed to represent an arrest of neocortical migration. A wide field of heterotopic neurons underlies the incompletely formed cortical plate. The post-migratory neurons of the true cortex are segregated by class into laminae comparable to III-VI in the normal. Polymorphic neurons typical of layer VI lie deep, and medium and large pyramids typical of normal layers III-V are more superficial. Pyramids are in general radially aligned with ascending polarity. By exception, the most superficial pyramids tend to have descending polarity. Neurons of all classes, including spiny stellates and pyramids representative of laminae II-V, are found intermixed in the heterotopic zone. Though not segregated by class into laminae, heterotopic pyramids are in general radially aligned with ascending polarity. Both cortical and heterotopic neurons have well differentiated dendritic arbors and are rich in spines. These observations imply that cell class is an inherent property of the post-mitotic neuron and a property independent of cell position, that neurons segregate by class into laminae only if migration to the cortex is completed, and that local factors play an important role in the shaping and alignment of dendrites.

1194 FACTORS INVOLVED IN THE STIMULATION OF PARASYMPATHETIC NERVE OUTGROWTH. Michael D. Coughlin and Michel P. Rathbone, MRC Group in Developmental Neurobiology, Dept. of Neurosciences, McMaster Univ. Med. Ctr., Hamilton, Ontario, Canada L8S 4J9.

Homogenates of salivary glands and formalin-fixed glands were assayed for ability to elicit parasympathetic axon outgrowth. The outgrowth of axons from the parasympathetic ganglion of the mouse submandibular gland has previously been shown to depend of the presence of a target epithelium (M. Coughlin, Devel. Biol. 43: 140, 1975). Whole salivary glands from 15d embryos were frozen, homogenized and added to culture medium at dilutions ranging between 1:100 and 1:100,000 (w/v). In no case was an increase in axon outgrowth observed.

Since extracellular materials are thought to be active in numerous morphogenetic events, whole submandibular glands were cultured in the presence of enzymes that have been shown to disrupt the basal lamina. Glands cultured in medium containing collagenase at concentrations of 1 to 10 ug/ml or hyaluronidase at 5 ug/ml showed epithelial growth despite the disruption of the normal branching pattern. Axon outgrowth from the ganglion was extensive but diffuse, and lacked the pattern seen in control glands.

Ganglion-free glands were cultured 3 to 5 days, fixed in formalin and washed thoroughly. When ganglia were cultured with such fixed tissue, axon outgrowth occurred into the tissue. Outgrowth also occurred when fixed glands had been treated with various enzymes. Disruption of the pattern of axon outgrowth by collagenase and hyaluronidase and the stimulation of outgrowth by fixed glands indicate that such stimulation does not depend directly on the metabolic activity of the epithelium but is due rather to some epithelial product. (Work supported by the Medical Research Council of Canada and the Dysautonomia Foundation.)

1195 REGENERATION OF NORADRENERGIC AXONS IN RAT SCIATIC NERVE. Irvine G. McQuarrie, Bernice Grafstein, and Michael D. Gershon. Departments of Physiol. and Anat., Cornell Univ. Medical Col., New York, N.Y., 10021.

The outgrowth rates of regenerating noradrenergic and sensory axons were examined following a crush lesion in the sciatic nerve of rats. Noradrenergic axons were identified by two methods: histofluorescence of norepinephrine (NE) in whole mounts of teased nerve, and the specific uptake of ^{3}H-NE. Pinch testing, the classic method of locating the leading sensory axons in regenerating nerves, was also found to locate the leading 1% of fluorescent noradrenergic axons. Following a crush lesion, the outgrowth rate of these axons (as determined by the pinch test) was 4.0 mm/day. The use of a priming lesion (a cut of the major sciatic nerve branch 2 weeks prior to crushing the proximal sciatic nerve) resulted in a 28% increase in this rate ($p < 0.05$).

The specific uptake of ^{3}H-NE in regenerating nerves was found to decrease linearly distal to the crush lesion. The point on the nerve at which uptake was reduced by 50% was found to advance at a rate of 1.5 mm/day during normal regeneration, and at a rate of 0.5 mm/day during regeneration influenced by a priming lesion ($p < 0.02$). These results suggest that while a priming lesion stimulates the outgrowth of leading noradrenergic axons, it may depress the outgrowth of most noradrenergic axons.

We were unable to demonstrate any effect of dibutyryl cyclic adenosine monophosphate on the outgrowth rate of the axons under study.

This research was supported by USPHS grants NS-09015, NS-01043, and NS-07436.

1196 EFFECT OF VINBLASTINE SULFATE ON CHICK EMBRYO TIBIAL NERVE MATURATION. Betty G. Uzman, Gloria M. Villegas* and J. M. Curnutt*. Vet. Admin. Hosp., Shreveport, La., 71130, LSU Med. Sch.-Shreveport, and Inst. Venez. de Invest. Cient., Caracas, VZ.

Vinblastine sulfate (Velban, VBS) injected into the chorio-allantoic sac of chick embryos (C.E.), 0.5μg VBS/gC.E. weight, at 11 days incubation (dinc) deleted ∿ 50% bundle axons in 48 hrs. with percents of myelinating fibers in the remaining nerve fiber population relatively increased. Electron microscopic observations of early effects of VBS on bundle axons will be described, as will the long term functional and morphological sequelae in C.E.'s sacrificed at 18 dinc, at hatching, and at intervals after hatching.

Possible sites of action (on satellite cells, neurones, axons) as well as mechanisms of injury (disassembly of microtubules, alteration of cell surface moieties, et al) will be discussed.

1197 CHANGE IN STRUCTURE FROM "SLOW" TO "FAST" OF MOTH MUSCLE FIBERS DURING DEVELOPMENT. Mary B. Rheuben. Department of Biology, Yale University, New Haven, Conn. 06520.

The thoracic dorsal longitudinal muscle of Manduca sexta is present in the larva, functioning in locomotion and postural control, and then partially degenerates (with loss of contractile structures) during metamorphosis. It re-develops in the pupa to become one of the major flight muscles. During both degeneration and re-development, the motor nerve remains in contact with the muscle. Casaday (1974, Ph.D. thesis) showed that the same 5 identifiable motor neurons innervate both the larval and the adult muscle. The larval myofibers do not disappear completely during degeneration; during pupal development their remnants fuse with "myoblasts", a cell type whose origin is unknown (Stocker, Exper. 30: 896, 1974), and which have been found floating in the hemolymph. The fused cells then develop into the adult fibers.

The ultrastructure of adult and larval muscles were examined with standard electron microscopic techniques. The neuromuscular junctions of the adult and larva are qualitatively similar. The synaptic release sites occur within a synaptic complex consisting of interdigitating processes from the muscle cell and the glial cells accompanying the nerve. Within each complex are numerous release sites. Innervation is multiterminal.

The structures of the larval and adult forms of the muscle fibers differ significantly. The larval fibers resemble the typical "slow" arthropod muscle: 10-12 thin filaments arranged around each thick filament, few small round mitochondria, poorly developed sarcoplasmic reticulum, irregularly aligned Z bands. The adult muscle is "fast": 6-8 thin filaments/thick, large mitochondria surrounding the myofibrils, well developed sarcoplasmic reticulum, and evenly aligned Z bands. This moth muscle is an example where innervation by a particular nerve is clearly not the sole factor in determining whether the morphological properties of a muscle are slow or fast.

The cell type corresponding to the "myoblast" as described by Stocker and by Crossley (J. Embryol. exp. Morph. 27: 43, 1972) in pupal fly tissues, was also found here in the larval muscle. The myoblasts are spindle-shaped, mononucleate cells, characterized by large numbers of ribosomes and by microtubules which are aligned parallel to the long axis of the cell. In Manduca they were seen intermingled and possibly fusing with the larval muscle fibers. They have only been rarely observed in the adult muscle. Since they are present prior to the degenerative and metamorphic changes, they may be analagous to the vertebrate satellite cells which are hypothesized to provide myoblasts for continuing muscle growth or regeneration after injury.

This work was done during the tenure of a Research Fellowship of Muscular Dystrophy Associations of America, and was partly supported by NIH Research Grant NS08966 to M.J. Cohen.

1198 REDUCTION OF NORMALLY OCCURRING MOTOR NEURON DEPLETION FOLLOWING SUPERNUMERARY LIMB TRANSPLANTATION IN CHICK EMBRYO. Margaret Hollyday and Viktor Hamburger. Dept. Biology, Washington University, St. Louis, Mo. 63130.

In normal development, the lumbar lateral motor column of the chick embryo is reduced from approximately 17,000 neurons to approximately 10,000 neurons between stages 29 and 35+ (6 - 9 days), (Hamburger, '75). Neuronal death is generally attributed to the inability of some neurons to compete successfully at the periphery. To test this hypothesis, supernumerary leg buds were transplanted immediately rostral to the normal leg bud at stage 17+ (2½ days). Cell counts of identified motor neurons were made at stages 37 - 38 (12 days) on both sides. At 12 days, the counts on the operated side were 12% to 27% higher than those on the contralateral side. Preliminary observations indicate that the distribution of the additionally surviving neurons is not restricted to the rostral segments of the lateral motor column, as might have been expected. Motility of the supernumerary limbs was partly homologous and partly non-homologous with movements of the normal ipsilateral limb.

Since the birthdates of lumbar lateral motor neurons are from stages 17 to 23 (unpublished autoradiographic data), proliferation could possibly be influenced by the transplant. This possibility is currently under investigation.

1199 BEHAVIORAL AND MONOAMINERGIC EFFECTS OF PRENATAL CARBON MONOXIDE EXPOSURE IN NEONATAL RATS. L.D. Fechter and Z. Annau. Dept. Environ. Med., Johns Hopkins Univ., Baltimore, 21205

This series of experiments was designed to determine the effect of mild CO-produced hypoxia in the prenatal period on neonatal behavior, development, and central catecholamine activity.

Pregnant rats were chronically exposed to 225 ppm CO throughout gestation and were removed from the exposure chamber once they had given birth. The neonates were counted, weighed, and examined for malformations and behavioral observations were carried out at various ages between 1 and 14 days. When necessary, litter sizes were reduced to 8 pups on the first post-natal day.

Carbon monoxide exposure produced decreases in body weight apparent on the 10th and 14th post-natal day while litter size (number of pups) and fetal mortality were not significantly different from air exposed controls. Teratogenic effects of CO were not observed.

From age 1 day, neonates were challenged by an injection of 100 mg/kg 1-DOPA so that measurements of locomotor activity could be made. Offspring of the CO mothers demonstrated significantly lowered activity levels. Following testing, the animals were sacrificed and the brains taken for flourimetric assays of DOPA, dopamine (DA), and nonadrenaline (NA). A correlation between the behavioral and central neurochemical data was observed.

1200 ELECTRICAL COUPLING IN DEVELOPING STRIATED MUSCLE. Nancy S. Peters* and Henry A. Lester. Div. of Biology, C.I.T., Pasadena, Ca. 91125.

We are investigating the role of electrical coupling in the interhydoideus of Xenopus laevis during tadpole development and metamorphosis. In the tadpole the interhyoideus consists of a right and left muscle which extend from the ceratohyale to the ventral midline. Intracellular recording and current pulsing were performed on both in vitro and anaesthetized, whole animal preparations. In the whole tadpole, muscle fibers exhibit large variations in the amplitudes and durations of synaptic potentials and nerve fibers often are observed in contact with several contiguous muscle fibers. Synchronous synaptic potentials have been observed from two or more adjacent fibers within one muscle and can also be observed in two opposing fibers from the right and left muscles even though each muscle appears separately innervated. Electrical coupling, which can be measured by the decrement of the electrotonic depolarization produced by a pulse of current longer than the membrane time constant, exists within groups of two or more fibers within a muscle. Although the groups of coupled fibers are interspersed within groups of non-coupled fibers, they are more readily detected in specific anterior-posterior strata of the muscle during development. Electrical coupling also occurs between the right and left muscles across the midline. Members of an opposing pair of coupled fibers are sometimes coupled with one or more adjacent fibers in the same muscle. The amount of inter- and intramuscular electrical coupling decreases as the tadpole approaches metamorphosis and is not seen in the newly formed toad. (Supported by grants from USPHS NS11756 and the Sloan Foundation. N. Peters is a Spencer Foundation Fellow.)

1201 MONOSODIUM GLUTAMATE-INDUCED OBESITY AND ACTIVITY CHANGES: EVIDENCE OF AN AGE DEPENDENT EFFECT. William J. Pizzi and June E. Barnhart*. Neuropsychology Lab, Northeastern Ill. Univ., Chicago, Illinois, 60625.

A number of reports have attributed neurotoxic properties to monosodium glutamate (MSG), a common flavor enhancer. Concomitant with this CNS damage there have been reports of developmental, physiological, and behavioral abnormalities. The abnormalities reported include weight gain, stunted skeletal growth, sterility in females, damage to the retina, abnormal activity levels, and learning deficits. The present studies attempt to resolve a number of conflicting reports regarding MSG-induced obesity and activity changes, and to explore the possibility that these effects are age dependent. To this end mice 1, 5, 15 and 25 days of age (N=183) were administered MSG subcutaneously for 10 days according to a dose schedule described by Potts et al. (Amer. J. Ophthalmol., 50: 900, 1960).

Significant weight gains were found in both male and female mice in the 1-day and 5-day groups, while in the 25-day groups significant weight gains developed only after an extended period of time and then only in the males. No data were collected for the 15-day groups due to an extreme mortality rate which persisted following a reduction in dose level to that tolerated by younger animals.

Three measures of activity were taken between 150 and 200 days of age including wheel running, open field, and the Boissier poke test. Significant decreases in activity were observed for MSG treated mice. The 1-day groups showed the decrease in activity on all three tests. Male and female mice in the 5-day groups showed significant decreases in activity on the poke test but not in wheel running or the open field. The MSG treated males in the 25-day group showed a significant decrease on the poke test, while the 25-day females failed to show decreases in any of the activity tests.

These findings clearly support previous research demonstrating increased weight gain and decreased activity levels following high doses of MSG administered during the neonatal period. The data further support the hypothesis that younger organisms are more susceptible to these effects. The 1-day groups preceded the 5-day groups in showing significant weight gains, and the effect became mixed in the 25-day groups. Likewise, the 1-day groups showed much greater decreases in activity and exploratory behavior on all three tests, while the 5-day groups showed significant decreases only on the poke test, and the 25-day groups again showed mixed results.

1202 CHANGES IN THE IONIC BASIS OF THE ACTION POTENTIAL IN ROHON-BEARD NEURONS DURING DEVELOPMENT. Nicholas C. Spitzer and Paola I. Baccaglini*. Dept. Biol. UCSD, La Jolla, CA 92037 Support: NS 11311 & A.P.Sloan Foundation.

Rohon-Beard (R-B) cells are found in the dorsal spinal cord of tadpoles of *Xenopus laevis*. They have been birthdated at Nieuwkoop & Faber stage (NF) 13 (15 hours after fertilization of the egg): their last round of DNA synthesis is completed prior to neurulation (J. Spitzer). Their large size (20μ) and characteristic position permit intracellular recordings early enough to study changes in electrical excitability during development. Embryos at different stages were dissected to expose the spinal cord. The preparations were perfused with a simple Ringer's solution (NaCl, 125mM; KCl, 3mM; $CaCl_2$, 10mM). Cells were impaled under direct visualization with a Nomarski interference contrast microscope. To examine the role of different ions in carrying inward current during the action potential, specific ions were isoosmotically substituted by others, or blocking agents were added to normal Ringer.

At NF 19-25 (20-30 hours) R-B neurons have regenerative responses to injected currents. These are unaffected by replacement of Na^+ with Tris or choline, and are blocked by Co^{++} or La^{+++}, which block inward Ca^{++} currents in other excitable cells. The size of the overshoot depends on the [Ca^{++}] in the perfusion solution. At NF 25 the action potential abruptly develops a new component with a rapid rate of rise. This is blocked by removal of Na^+ or addition of tetrodotoxin. A shoulder on the falling phase persists under these conditions, but is eliminated by Co^{++} or La^{+++}. This shoulder is lost gradually, and may have disappeared by NF 42/43. At NF 46-49 (5-12 days) the impulse is unaffected by Co^{++}, La^{+++}, or the external Ca^{++} levels, but is abolished by the removal of Na^+. The duration of the action potential has changed from 40-50 ms (NF 22) to 0.5 ms (NF 49). These observations indicate that the inward current is initially carried by Ca^{++}, later by Na^+ and Ca^{++}, and ultimately by Na^+ alone.

1203 SELECTIVE GROWTH OF NEURITES FROM ISOLATED FETAL MOUSE DORSAL ROOT GANGLIA TOWARD SPECIFIC CNS TARGET TISSUES. Edith R. Peterson* and Stanley M. Crain. (SPON: E.R. Masurovsky). Depts. of Neuroscience and Physiology, Albert Einstein College of Medicine, Bronx, N.Y. 10461

It has been noted in previous tissue culture studies (Peterson et al, '65; Bunge and Wood, '73) that fetal rat dorsal root ganglia (DRG) detached from spinal cord and explanted in close proximity to deafferented spinal cord explants did not invade the CNS tissue. Attempts have now been made to clarify the mechanisms underlying these complex cellular "barriers" by more systematic alterations of the experimental conditions under which DRG and cord neurons, and their associated supporting cells, may interact in culture. Nerve growth factor (NGF) added once (1,000 units/ml) at explantation to 13-14 day fetal rodent DRGs leads to vastly increased survival of neurons and an exuberant neuritic outgrowth. When a series of individual fetal mouse DRGs are positioned at various sites around a cross-section of deafferented fetal mouse spinal cord, the NGF-stimulated neuritic growth pattern in relation to the cord explant is essentially similar to that observed without NGF. Although the DRG neuritic outgrowth is greatly enhanced, it still appears to bend away from the CNS tissue. (Meningeal covering of CNS tissues which would present a formidable block to DRG invasion were totally stripped prior to explantation.)

In order to provide experimental conditions more favorable to NGF-stimulated DRG invasion of CNS tissues, regions of the CNS rich in sensory target neurons, e.g. strips of dorsal cord instead of whole cross-sections, were placed in close proximity (ca. 0.5 mm) to clusters of 3-6 DRGs. Under these conditions neurites from at least some DRGs of a cluster readily invaded the target CNS explants and formed characteristic functional connections (see Crain and Peterson, this issue). The variability in the degree of DRG innervation of CNS target explants in our present experiments may be due in part to the fortuitous orientation of the central vs. peripheral branches of the individual DRGs. In contrast to the successful DRG innervation of CNS target tissues, when strips of ventral cord were presented to similar DRG clusters, most of the DRG neuritic outgrowth appeared to be deflected from the CNS explant. Neuritic invasion of CNS target tissues (including cuneate and gracilis nuclei of the medulla, as well as dorsal cord) appeared to be further enhanced by positioning non-target CNS tissues, e.g. ventral cord, so as to restrict the DRG outgrowth which might otherwise occur in directions away from the target explant.

Besides the greatly increased density of outgrowing DRG neurites in high NGF, the rate of neurite elongation is also markedly enhanced. Migration of Schwann cells and meningeal tissue (the latter explanted with the DRGs) lags far behind the neuritic outgrowth. Under these conditions, the DRG neurites are essentially naked for at least the first 3 days and they are therefore unencumbered by cellular elements which might interfere with successful DRG invasion of CNS explants. When the slowly migrating Schwann cells finally arrive at the zone of glial outgrowth from the CNS tissue they stop abruptly.

Under the equivalent geometrical conditions provided by these explant arrays, the apparently selective growth of DRG neurites toward specific regions of CNS tissue rich in sensory target neurons provides further support for the theory of "chemoaffinity in the orderly growth of nerve fiber patterns and connections" (Sperry, PNAS 50:703,'63) which may now be accessible to more direct analysis in CNS tissue cultures.
(Supported by NINDS grants NS-08770 and NS-06545, and NSF grant BMS75-03728.)

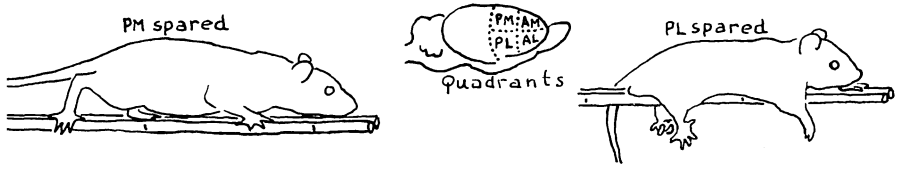
1204 NON-SOMATOTOPIC ORGANIZATION OF THE DEVELOPING MOTOR-SENSORY CORTEX (MSC). Constance J. D'Amato and Samuel P. Hicks. Dept. Pathology, Univ. Michigan, Ann Arbor, 48104.

Previous experiments showed that the motor-sensory cortex of the rat was organized into regions subserving different components of locomotor movement relatively independent of the classic somatotopic maps (Hicks, D'Amato, Amer. J. Anat. 1975). Dividing the MSC arbitrarily into anteromedial (AM), anterolateral (AL), posteromedial (PM), and posterolateral (PL) quadrants, and ablating single quadrants unilaterally or bilaterally in newborn, juvenile, or mature rats showed that positioning the limbs and feet on irregular terrain, such as narrow paths made of long round or rectangular wood rods, was markedly dependent on PM and PL. Bilateral removal of AM or AL had no effect on locomotion as we tested it. Bilateral removal of PM produced continual slipping of all limbs and feet off the narrow paths and inability to retrieve them effectively, apparently because of insensibility to their abnormal positions. Ablation of PL bilaterally produced slovenly application of the feet to the paths and impaired grasping to hold on; attempts to reposition the limbs on the paths were characterized by groping, poorly aimed movements. Major projections of PM and PL differed considerably, PM coursing especially to striatum, diencephalon, midbrain, pons and spinal cord, while PL went largely to diencephalon, pons, and medullary reticular formation. Removal of PL and PM bilaterally at birth led to a summing of the deficits described, much as expected, but an unexpected result of the combined ablations was an extremely hyperactive tactile placing response of the limbs, as that response is ordinarily tested.

To explore further the regional representation of movement in the cortex, three quadrants were ablated bilaterally in newborn, juvenile, or mature rats, leaving the PL or PM quadrants alone bilaterally. Animals with PM's remaining (N = 9) negotiated the narrow paths with skill approaching normal, but when limbs slipped off they were sometimes retrieved with difficulty. This latter contrasted with the behavior of normal litter-mates who recovered from slips and missteps with alacrity, accuracy, and grace. Animals with PL remaining (N = 9) were severely handicapped on the narrow paths, making clumsy progress on a round dowel (28 mm diam.) and being nearly incapacitated on two approximated dowels (8 mm diam.). Hind limbs, especially, dangled, sometimes grasping each other, possibly reflecting the strong somatotopic representation of hind limbs in PM. Forelimbs also dangled at times but were somewhat more effective.

Rats having bilateral three-quadrant ablations sparing PL walked and ran virtually normally on flat surfaces, as had rats with the whole MSC ablated bilaterally. Those operated on at birth, however, developed this locomotor ability more slowly than normals as had rats with the whole MSC removed at birth. Rats having three-quadrant ablations at birth with PL remaining, like rats with both MSC's wholly removed at birth were unable to jump from one platform to another, while rats in whom these operations were performed in the adult period (2 months old), retained their ability to jump. Operations sparing the PM's at any age did not interfere with jumping, but when the operations sparing the PL's were done at 4 weeks, the animals lost their jumping ability in contrast to adults.

Below, tracings of single slow-motion movie frames illustrate effects of MSC ablations at 4 weeks sparing PM or PL quadrants bilaterally, and the quadrants are diagrammed. (Supported by USPHS Grant NS 10531)



1205 EFFECT OF NERVE GROWTH FACTOR ON ETHYL NITROSO UREA CARCINOGENESIS. J. R. Perez-Polo and S. A. Vinores*. Zoology Department, University of Texas at Austin, Texas, 78712.

Following prenatal treatment with ethyl nitroso urea (ENU), adult rats develop malignant tumors of the nervous system. Of these, over 90% are of glial origin and the remainder are neuronomas. The actual neural tissues involved vary dramatically as a function of the stage of development at which ENU administration takes place. Pretreatment of rat embryos with the nerve growth factor protein (NGF) prior to ENU treatment brings about a response which is indistinguishable from the organ unspecific response observed when adult rats are treated with ENU alone. Prenatal treatment of rats with antibodies directed against the NGF followed by the ENU treatment alters the organotropic ENU effect and accelerates the appearance of tumors. Tumors now appear in those regions of the nervous system known to have high levels of NGF and to be primarily affected by immunosympathectomy.

When embryonic primary cultures of different parts of the central and peripheral nervous system of rats are treated with ENU, NGF, anti-NGF, ENU and NGF, and ENU and anti-NGF similar results ensue in that ENU plays a cell transforming role as measured by increases in cell proliferation and high levels of NGF coincide with neurite proliferation. The results are discussed in terms of a new probable theory of NGF action. Supported by NINDS research grant NS-11211.

1206 Immunochemical Measurement of Myelin Basic Protein in Developing Rat Brain: An Index of Myelinogenesis, Steven R. Cohen* and Michael Guarnieri, Dept. of Neurology, Johns Hopkins University, Baltimore, Maryland 21205.

We have developed a radioimmunoassay for myelin basic proteins that is quantitative, specific and sensitive (Cohen, McKhann and Guarnieri, J. Neurochemistry, in press). In the optic nerve, the appearance of basic protein correlates with the morphological appearance of the myelin membrane and the activity of cerebroside sulfotransferase. Therefore, immunochemical measurement of the basic protein appears to be a precise and sensitive technique to measure myelination. We quantitated basic protein in eleven areas of developing rat nervous system. The period of rapid basic protein synthesis occurred first in the trigeminal nerve at day 5 postnatally followed by the optic nerve at day 9, the spinal cord at day 10 and the cerebrum, cerebellum, olfactory bulb, midbrain and brainstem at day 12. The area which myelinated most rapidly was the optic nerve. The amount of myelin in brain was calculated from the immunochemical measurement of the basic protein and compared to the weight of myelin that can be isolated by a standard technique. During early development (10-20 days) when the membrane is rapidly changing, a smaller proportion of the total myelin is recoverable by isolation methods than in older, more mature animals. Supported by USPHS Grants No. NS 10920,NS10465 and a grant from the John A. Hartford Foundation.

1207 BEHAVIORAL DEVELOPMENT IN MONKEY INFANTS FOLLOWING FORELIMB DEAFFERENTATION OF EXTERIORIZED FETUSES AT THE END OF THE SECOND TRIMESTER OF PREGNANCY. Edward Taub, Gilbert Barro*, Robert Heitmann*, H. Cannon Grier*, John W. Boretos*, and John L. Cicmanec*. Inst. for Behavioral Research, Silver Spring, Md. 20910; NIH, Bethesda, Md. 20014; Litton Bionetics, Inc., Kensington, Md. 20795.

In previous work, severe quadriparesis was observed in rhesus monkey infants which, as fetuses, had been exteriorized at the end of the second trimester of pregnancy, placed in a temperature-controlled saline bath, subjected to forelimb deafferentation, and then replaced in utero for completion of fetal development. At postmortem examination, the pedicles of the vertebrae were found to have flared outwards and spread to 3 times their normal width. This malformation permitted the overlying muscle to compress and severely damage the spinal cord in the cervical region. In current work a prosthetic bridge was emplaced at time of fetal operation (unilateral forelimb deafferentation, C_1 or C_2-T_4) to substitute for the portions of the vertebrae removed by laminectomy. The prosthesis consisted of a left and right leaf, one of which slid over the other, thus permitting expansion of the device to accommodate growth of the fetus.

After birth the original prosthesis, which was rigid along its long axis, was replaced by a second device consisting of separate segments. The design permitted dorsiflexion and ventriflexion of approximately 30°, as well as some flexibility from side to side. X-ray examination and direct observation of the operative site at time of postnatal surgery indicated that the original prosthesis did not shift in position and was successful in preventing the type of spinal cord damage previously noted.

In the 2 surviving infants, motor function at 3 months of age (the time of this writing) was normal in the hindlimbs and in the intact forelimb. The deafferented forelimb exhibited a clear motor deficit but was used for a wide variety of purposes, including postural support during standing and sitting, ambulation (with placement on the wrist dorsum), and reaching toward objects. In one infant, purposive use of the deafferented limb developed spontaneously; in the other it emerged after restraint of the intact forelimb.

The results demonstrate that neurosurgery in the fetal primate is feasible and can be used to study the development of the nervous system and behavior. The extensive use that the infants could make of a forelimb deafferented two-thirds of the way through gestation indicates that spinal reflexes and local somatosensory feedback are not necessary for the subsequent development of many of the movements performed by the forelimb musculature in monkeys. If there is a critical period during which somatic sensation is required for the elaboration of the observed motor patterns, it has already passed by the end of the second trimester of pregnancy. (Supported by NIH Grants HD 08579 and FR 5501RR05636.)

1208 MANIPULATION OF THE ACETYLCHOLINE SYSTEM IN CNS OF DEVELOPING RAT: NEUROCHEMICAL AND BEHAVIORAL CONSIDERATIONS. Clyde B. Mathura*, Gilbert W. Meier, and Williamina A. Himwich. Univ. Nebr. Med. Ctr., Nebr. Psych. Inst., Res. Div., Omaha, Nebraska 68106.

Although several investigators have shown correlations between the early environment and central acetylcholine (ACh) components (as dependent variables), and although the temporal course of development of cholinergic inhibitory mechanisms has been clearly delineated, no efforts have been directed at experimentally investigating the early availability of differing ACh levels (as the independent variable) on later learning performance. These shortcomings are magnified because of the tremendous amount of data signifying the early postnatal period in the rat to be the most prolific in central nervous system (CNS) development and therefore most susceptible to hypothesized manipulative effects. A study was conducted, therefore, to investigate the effects of an early increase of ACh levels in the CNS of infant rats on later performance on both active and passive avoidance tasks. The experimentally induced increase in ACh levels was studied from the perspective of age-related developmental patterns of the cholinergic system in the rat brain.

Litters were injected with 0.75 mg/kg physostigmine salicylate and 0.025 mg/Kg methscopolamine HBr (I), 0.10 mg/Kg physostigmine salicylate and 0.025 mg/Kg methscopolamine HBr (II), and distilled water in like amounts to groups I and II, (III). Littermates were injected daily on Days 2-15 of age, or 15-28 days of age. On Days 16 and 29, 24 hours after the final daily injection of their respective drug regimen, some brains were analyzed for ACh, AChE (acetylcholinesterase), and ChAc (choline acetyltransferase). Finally, animals were tested on Days 60-65 on a GO:NO-GO discrimination avoidance task (active versus passive avoidance)within the same shuttlebox. All procedures (injections, sacrifice for chemical assays, behavioral testing) occurred, when scheduled, within the same time period of the day.

The neurochemical findings showed an increase in ACh levels dependent on age period injected. This increase after a 24 hour interval is contrasted with previous evidence of a peak increase in ACh 30 minutes after acute injections. AChE significantly decreased in both a dose related fashion and in both age-at-injection groups. Surprisingly, ChAc, the synthesizing enzyme mimicked AChE activity levels and not ACh.

Behaviorally, superior performance on both active and passive avoidance tasks was demonstrated by the highest dose group (I) over II and III.

Thus, success was achieved in manipulating the "ACh system" in CNS of infant rats. The ability of the high dose group to perform better than controls on tasks designed to measure opposing behavioral tendencies further serves to eliminate the alternative explanation of peripheral activation variables. The ACh system was discussed in its relationship to other putative transmitter systems.

1209 SIX-HYDROXY-DOPAMINE (6OHDA) ALTERS DEVELOPING CORTEX AND LOCOMOTION IN RATS. S. P. Hicks and C. J. D'Amato. Dept. Pathology, Univ. Michigan, Ann Arbor, 48104.

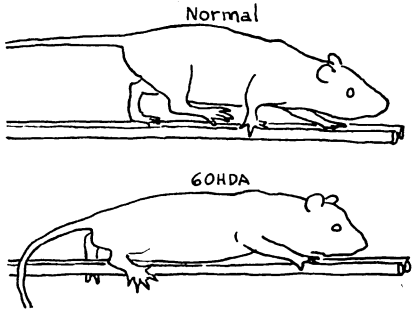
6OHDA (100 to 300 mg/Kg) given subcutaneously to newborn rats altered the differentiation of neurons in the isocortex and the alignment of their apical dendrites. Normally many of the neurons in layers VI to II of the cortex were grouped in vertical columns, and their apical dendrites ran together in loose bundles in the columns, precisely perpendicular to the brain surface. The columnar arrangement was fairly even and characteristic in a given region, a number of neurons from each layer contributing to the bundle as seen in dye, silver and Golgi stained histologic sections. After 6OHDA, the number of neurons contributing dendrites to a bundle increased variably, especially cells in the outer layers. The vertically columnar appearance of the cortex became both exaggerated and irregular. The small neurons of layer IV were also less compactly and distinctly arranged than normal.

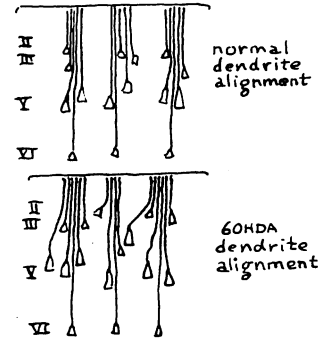
In mid-third week when a rapid transition from infant to mature locomotion normally occurred, affected animals developed locomotor defects. These were characterized by slovenly positioning of the feet as the animals walked and ran on narrow pathways (long round or rectangular wood rods). The result was frequent misstepping and slipping the feet and limbs off the paths. The disorder was reminiscent of certain locomotor abnormalities that followed ablation of parts of the motor-sensory cortex (Hicks, D'Amato, Amer. J. Anat. 1975).

In previous experiments, asphyxia or low levels of radiation in newborn rats produced abnormalities of differentiation of neurons and alignment of their dendrites in the cortex (Hicks et al 1962; D'Amato, Hicks 1965). Certain similarities between these effects and those of 6OHDA were noted. The alterations produced by asphyxia and radiation were attributed not only to a direct action on the involved cells, but indirectly to failure of a normal trophic effect of afferent thalamocortical axones growing into the cortex in the postnatal period. The asphyxia and radiation delayed or diminished the ingrowth of these afferents.

Recently Maeda et al (1974) showed that destruction of the region of the locus coeruleus (lc) in newborn rats was followed by markedly impaired development of the cortex, implying a trophic effect of lc axones on cortical growth. Thus the action of 6OHDA on cortical development might be through its specific damage to the developing adrenergic terminals of the lc axones that ramify through the cortex. However, 6OHDA has other modes of action, not specifically on adrenergic neurons (Sachs and Jonsson, 1975). A direct "non-specific" effect on the outer neurons of the cortex in infancy, which are least mature and vulnerable in that period, has not yet been ruled out.

Below, tracings of single slow-motion movie frames illustrate 6OHDA (150 mg/Kg) locomotor deficit, and the 6OHDA cortical dendrite alignment is diagrammed. (Supported by USPHS Grant NS 10531)





1210 ANDROGEN-DEPENDENT SEXUAL DIMORPHISM IN DENDRITIC BRANCHING PATTERN (CLUSTERING) OF PREOPTIC AREA NEURONS IN THE HAMSTER. William T. Greenough and Carol Sue Carter. Depts. Psychology and Ecology, Ethology, and Evolution, and School of Basic Med. Sciences, University of Illinois, Urbana-Champaign, 61820.

Dendritic branching was examined in neurons of the dorsal preoptic area (dPOA) of 6 groups of hamsters: normal males and females, neonatally castrated males (within 36 hr of birth), testosterone propionate-injected females (20 µg/animal, sc., within 36 hr of birth), oil-injected females, and neonatally sham-castrated males. All animals were gonadectomized (or sham-operated) in adulthood and they all received the same sequence of steroid hormones. Brains were rapid Golgi stained (Ramon-Moliner method) and neurons whose cell bodies were located in the dPOA were drawn at 1000 X with the aid of a camera lucida. No gross (i.e., structure to structure distance) dimorphism existed in this region. About 100 neurons were drawn from each group of animals (at least 6 animals per group). A modified analysis (after Sholl) was performed, in which the intersections between dendrites and an overlay of concentric rings at 20 micron intervals in pie-shaped octants around the cell body was counted. The results indicated that: a) males, in general, had slightly greater total dendritic mass per neuron (sum of intersections) than females; b) dendrites from males tended to be concentrated (clustered) in a region dorsolateral to the peak clustering region in females and the female dendritic distribution was more diffuse along this oblique axis than that of the male, regardless of cell body position; and c) castrates, in general, paralleled the females while androgen-treated females resembled males. These data are not inconsistent with the notion that preoptic area neurons connect differentially with various afferent sources depending upon the presence of postnatal testosterone.

Supported by NIH grants HD06862 and HD07496.

1211 EFFECTS OF SUBCUTANEOUS ADMINISTRATION OF 6-HYDROXYDOPAMINE (6-OHDA) IN NEONATAL RATS ON DENDRITIC MORPHOLOGY IN THE HIPPOCAMPUS. David G. Amaral*, John A. Foss, Carol Kellogg, and Donald J. Woodward, Dept. of Psychology and Dept. of Physiology, Univ. of Rochester, Rochester, N.Y. 14627.

Various lines of evidence have suggested that dendritic maturation is influenced by innervation. Related to our interest, the loss of the noradrenergic afferents to the somatosensory cortex has been shown to lead to aberrations in the "pyramid-like" neurons in layer VI (Maeda et al., Brain Res., 70, 515, 1974). We have tested the generality of this finding by injecting 6-OHDA (100 mg/kg) subcutaneously in rats on postnatal days 5, 6, and 7. The hippocampus of the 28 day old animals was impregnated by both rapid-Golgi and Golgi-Cox methods. Treatment with 6-OHDA led to increases in endogenous noradrenaline of 76% in the brainstem and 22% in the diencephalon with a concomitant decrease of 62% in the cortex-hippocampus. The modified pyramids of the CA4 region present the most striking modification seen thus far. In the treated animals, the apical dendrites of these cells are thicker than controls and extend for long distances out of the hilus into the molecular layer of CA3. Spine distribution is patchy. An analysis of other hippocampal regions is in progress. These data extend the possibility that the noradrenergic fibers from the locus coeruleus affect dendritic maturation in various cortical regions.

1212 CHOLINESTERASES IN THE DEVELOPING AVIAN HEART. D. Bruce Gray. Section of Neurobiology and Behavior, Cornell Univ., Ithaca, New York 14853.

A quantitative description of cholinesterase activity is a valuable step in the study of the ontogeny of cholinergic mechanisms in differentiating tissues. In this study, atrial and ventricular tissues of the embryonic chick heart (Gallus domesticus) were assayed for acetylcholinesterase [AChE, EC 3.1.1.7] and non-specific cholinesterase [BChE, EC 3.1.1.8] throughout development.

In whole atrial homogenates, specific activity of AChE increased from 45 nanomoles acetylthiocholine (ATCh) hydrolyzed/min/mg protein at 3 days in ovo (Hamburger-Hamilton stages 19-20) to 150 nanomoles ATCh hydrolyzed/min/mg protein at hatching. BChE exhibited a similar increase in specific activity from 30 nanomoles butyrlthiocholine (BTCh) hydrolyzed/min/mg protein at 3 days in ovo to 250 nanomoles BTCh hydrolyzed/min/mg protein at hatching. Ventricular homogenates exhibited a significantly lower rate of increase in specific activity of both AChE and BChE over the same developmental period.

Although the specific activity of both AChE and BChE increased monotonically in atrial and ventricular tissues, the ratio of specific activities of AChE to BChE reversed between 3 days and 7 days (Hamburger-Hamilton stages 30-31) in ovo, during which time the chick heart receives vagal innervation. Correlations between changes in specific activities and enzyme function and myocardial differentiation will be discussed.

Supported by AHA grant to K. Arms.

1213 STUDIES ON THE POSTNATAL DEVELOPMENT OF THE RAT HIPPOCAMPUS USING ^{3}H-THYMIDINE AUTORADIOGRAPHY AND LOW-LEVEL (150-200r) X-IRRADIATION.

Shirley A. Bayer* and Joseph Altman. Dept. Bio. Sci., Purdue Univ., W. Lafayette, Ind. 47907

The degree of postnatal acquisition of neurons and glia in the rat hippocampus was determined with progressively delayed cumulative labelling of precursor cell DNA. Groups of rats were injected with four successive daily doses of ^{3}H-thymidine during non-overlapping periods ranging from birth to day 19. They were killed at 60 days of age, and the percentage of labelled cells was determined. The pyramidal cells of Ammon's horn and the polymorph cells of the dentate gyrus are not labelled postnatally, confirming earlier conclusions of their prenatal origin. In the dentate gyrus, 85% of the granule cells are formed postnatally, with 45% forming during the first week. The majority of the small cells of the dentate molecular layer and of the Ammonic strata oriens, radiatum, and lacunosum-moleculare are formed during the second week.

The long term consequences of graduated interference with the acquisition of hippocampal neurons and glia during early infancy were examined with quantitative histology and ^{3}H-thymidine autoradiography. The head region containing the hippocampus was irradiated from day two on with either two (2X), four (4X), six (6X) or eight (8X) doses of 150-200r X-rays. The animals were killed at 30, 60, 90 and 120 days of age. The morphology of the hippocampus was normal in all irradiated groups. While the number of pyramidal cells of Ammon's horn was unaffected, the number of granule cells of the dentate gyrus was progressively and permanently reduced from control levels by the different dosage schedules (2X, 59% reduction; 4X, 77%; 6X, 83%; 8X, 84%). A further experiment using ^{3}H-thymidine autoradiography confirmed that the surviving granule cells in the 8X group (16%) are the prenatally formed component of the population. Incidental observations in control animals indicated a 20% increase in granule cells between 30 and 120 days of age in agreement with earlier observations of granule cell labelling after ^{3}H-thymidine injections in adult rats.

In contrast to the permanent reduction in the number of granule cells, there was partial recovery in the dentate molecular layer and the Ammonic stratum oriens; in the fimbria, recovery in cell number was complete by 60 days. In a supplementary autoradiographic experiment, cell proliferation in the granular layer and in the fimbria was determined at 60 days of age after a single postnatal injection of ^{3}H-thymidine on either day 15 or day 20 in the control, 2X, 4X and 6X groups. The number of labelled cells in the irradiated groups was always well below control levels in the granular layer, but it was either above or at the same level as controls in the fimbria. Tentative interpretations were offered for the differential long-term effects of variable X-ray schedules on the neuronal and glial populations of the hippocampus.

1214 DEVELOPMENT AND METABOLISM OF THREE SUBFRACTIONS OF MYELIN IN RATS. Mary J. Druse and John H. Hofteig*.Dept. of Biochem. and Biophys.,Loyola Univ. Med. Ctr., Maywood, Ill. 60153.

Purified myelin (Norton, JNC 21:749,1973) isolated from rat brain was fractionated into light, medium and heavy myelin (Matthieu, BBA 329:305,1973). The development of the subfractions, as reflected by protein accretion, was determined. The metabolism of lipids and proteins in the subfractions was also examined at intervals from 1 h to 85 d after intracerebral injection of 150 µCi (^{3}H)leucine and 20 µCi (^{14}C)glucose into 12-day-old rats.

Between 12 and 97 days of age, the content of the 3 myelin subfractions increased tremendously,with the largest increase occurring in the light and medium subfractions. The proportion of heavy in the total myelin fraction declined during the age period studied.

Both the ^{3}H and the ^{14}C radioactivity in the light and medium subfractions increased until approximately 52 d after injection. Interestingly, the specific radioactivity (SA) (dpm/µg protein) of ^{3}H and ^{14}C in the medium and heavy subfractions increased markedly between 1 and 4 d after injection and declined thereafter. The SA of the light subfraction declined beyond 12 h after injection.

The results are consistent with the idea that there is a membranous precursor to the light subfraction of myelin (Druse, BR 76:423,1974; Agrawal, BJ 140:99,1974).

This research was supported in part from grants from NIH, SO1 RRO5368, and from the Soceity of Sigma Xi.

1215 PHYSIOLOGICAL CORRELATES OF SYNAPSE FORMATION IN THE IMMATURE MAUTHNER CELL SYSTEM. R. C. Eaton and R. D. Farley*. Dept. Neurosci., UCSD, La Jolla, 92037 & Dept. Biol., UCR, Riverside, 92502.

Previous morphological, behavioral and electrophysiological studies demonstrated in newly hatched zebrafish that the tail-flip startle reflex is mediated by the giant Mauthner cells (M-cells) of the hind brain. In the present study, single unit extracellular recording was done from embryos before hatching (35 to 96 hr after fertilization). In response to a vibrational stimulus, an M-cell spike could be distinguished in some cases by 40 hr, and in most cases by 48 hr. Thus, there are functional synapses on the M-cell from the beginning of, or before, the formation of dendrites. The M-cell spike became progressively larger in amplitude and shorter in duration from 40 - 96 hr, thus suggesting maturational changes in the cell's membrane. In embryos it was possible to elicit the M-cell spike at stimulus rates of 0.2/sec, but after 110 hr, the animals responded only to the first of a series of such stimuli. This failure to respond was not due to a developmental change in threshold, which didn't change, or to fatigue, since the failure could be overcome by increasing stimulus intensity or by microinjections of strychnine. Failure to respond was thus due to an active inhibitory process which became functional during the 4th day. The inhibition could be at the M-cell or the sensory receptors, but it is probably due to newly formed or newly functional inhibitory synapses.

1216 AUDIOGENIC SEIZURES: RELATION TO AGE AND CENTRAL MONOAMINERGIC MECHANISMS. C.Kellogg, Dept. of Psychology, Univ. of Rochester, Rochester, N.Y. 14627.

Audiogenic (AG) seizure activity in mice has been utilized as a model with which to analyze the development of the monoamine systems in the brain in relation to a neurologic disorder which is manifest at specific ages of development. Three forms of the model have been utilized: 1) Genetically determined audiogenic seizures in a sensitive strain. Maximal elaboration of the seizures in response to an intense sound stimulus occurs at 21 days postnatal age. 2) Primed sound-induced seizures in a genetically resistant strain. These animals are exposed to the intense sound at 16 days of age and tested for seizures at 21 days. 3) H13/04-evoked sound-induced seizures. Previous work with this drug (see Naunyn-Schmiedeberg's Arch. Pharmacol. 285, 257-272; 1974) demonstrated the effectiveness of this agent in eliciting sound-induced seizures in resistant strains of mice at 21-28 days of age.

Initially, a developmental study on the ontogeny of the monoamine system was conducted in sensitive and resistant animals. The in-vivo rate of tyrosine and tryptophan hydroxylase was analyzed in forebrain and hindbrain regions (produced by a pre-collicular cut) by measuring the accumulation of 3,4-dihydroxyphenylalanine(DOPA) and 5-hydroxytryptophan (5-HTP) following the inhibition of L-amino acid decarboxylase. The amines noradrenaline (NA), dopamine (DA), and 5-hydroxytryptamine (5-HT) were analyzed in control animals. A significant interaction was noted between strains and time after injection of the inhibitor in the accumulation of DOPA at 14 days only, with the faster rate of accumulation at this age occurring in the sensitive strain (forebrain and hindbrain). The development of NA levels in control animals was significantly slower in the sensitive strain. The achievement of DA levels followed a similar pattern of development in both strains. The accumulation of 5-HTP was found to be significantly faster in the resistant strain at 21 days of age indicating a faster rate of tryptophan hydroxylase in this strain. Significant non-linear regression was present at 14 and 21 days in the sensitive strain suggesting different influences on tryptophan hydroxylase in this strain. The achievement of 5-HT levels was similar between strains with the maximum increase occurring after 28 days.

Pharmacological manipulation of the process of priming in the resistant strains was employed to elucidate mechanisms involved in elicitation of seizures. Drugs were administered prior to a 30 sec exposure to the stimulus at 16 days. The animals were tested at 21 days by exposure to the same stimulus. These results indicate that the process of priming can be effectively prevented by activation of NA neurotransmission. Activation of 5-HT neurotransmission did not prevent the process of priming even though such manipulation will prevent the elaboration of the primed seizure at 21 days. Also activation of DA transmission did not prevent priming.

The in-vivo rates of tyrosine and tryptophan hydroxylase were analyzed in brains of resistant mice (28 days of age) given the AG seizure-inducing agent H13/04. The brains were dissected into 3 regions: telencephalon, diencephalon, and brain stem. H13/04 was administered 30 or 90 min prior to administration of a centrally effective decarboxylase inhibitor, since behaviorally, seizure elaboration is maximal 30 min after H13/04 (150 mg/kg) but absent 90 min after the drug. H13/04 given 30 min before the inhibitor markedly reduced the rate of accumulation of 5-HTP in all brain regions and enhanced the accumulation of DOPA in the diencephalon and brain stem. Given 90 min before the inhibitor, H13/04 did not change the rates of accumulation compared to controls.

All studies stress the importance of NA-neurotransmission in preventing priming and elaboration of AG seizures. DA transmission appears to have little effect in preventing the seizure but may have a role in recovery following a seizure. 5-HT transmission is effective in preventing seizures at 21 and 28 days but is ineffective in preventing priming at 16 days. This observation may be related to the slower achievement developmentally of 5-HT noted in both strains.(Research supported by Grant No. NS-10777.)

1217 MULTIPOTENTIALITY OF SCHWANN CELLS FOR MYELIN FORMATION. L. Charron*, J. Epps*, A. Aguayo*, and G.M. Bray* (SPON: D.G. Lawrence). Dept. Neurology and Neurosurgery, McGill University, Montreal General Hospital, Montreal, Quebec, Canada.

Cross anastomosed and autografted unmyelinated and myelinated nerves were examined by electron microscopy and radioautography to determine if Schwann cells are multipotential with regard to their capacity to produce myelin or assume the configuration seen in unmyelinated fibers.

In adult white mice: (A) the myelinated phrenic nerve and the unmyelinated cervical sympathetic trunk (CST) were cross anastomosed in the neck; (B) in other mice, the CST was grafted to the myelinated sural nerve in the leg.

(A) From 2 to 6 months after anastomosis previously unmyelinated distal stumps contained many myelinated fibers, while phrenic nerves joined proximally to CST's became largely unmyelinated. Radioautography of distal stumps indicated that proliferation of Schwann cells occured mainly in the first days after anastomosis but was present also in isolated stumps. (B) A month after being grafted to the sural nerve, the unmyelinated CST became myelinated but there was no radioautographic indication of Schwann cell migration from sural nerves to CST.

Thus, after joining myelinated and unmyelinated nerves the regenerated distal nerves resembled proximal stumps. The early Schwann cell proliferation in distal stumps is a local response independant of axonal influence; at later stages, axons from the proximal stump cause indigenous Schwann cells in distal stumps from previously unmyelinated nerves to produce myelin while those in myelinated nerves become the Schwann cells of unmyelinated fibers.

1218 DEVELOPMENTAL AND LONGITUDINAL CHANGES IN BRAINSTEM AUDITORY FUNCTION IN MAN. A. Salamy and C.M. McKean, Brain-Behavior Research Center, Univ. of California, Eldridge, California 95431

It is now possible to monitor brainstem activity through the intact skull (animal or man) via a single scalp electrode. Using the technique of Jewett (EEG clin. Neurophysiol. 28:609,1970) we recorded brainstem (far field) potentials evoked by brief acoustic stimuli from human subjects ranging in age from a few hrs after birth to adulthood. Marked differences in latencies of the various peaks of the brainstem evoked response (BER) revealed that the peripheral and central components of auditory transmission mature at different rates. Peripheral transmission essentially reached adult values by around the 3rd mo whereas central transmission required 1-2 yr for full maturation. In a more detailed analysis it was shown that the 6 wk old differs significantly from the newborn but not the 3-6 mo old in terms of both peripheral and central transmission, although a progressive shortening of all BER latencies can be seen with increasing age.

The waveform of the BER also changes as a function of age. This is best reflected in the total distribution of energy under the response curve and the appearance of a negative wave seen from birth to 6 wks of age. However, in contrast to central transmission, the time course for topological maturity is much shorter (around 3 mo).

The neurological instability of the neonatal period is most apparent in the inter and intra subject variability of the waveform and amplitude despite consistent latencies. Interestingly, the BER does not exhibit habituation to prolonged stimulation irrespective of age. Longitudinal data obtained between birth and 3 mo faithfully parallels the developmental changes.

1219 CHARACTERISTICS OF SYNAPSES IN CEREBRAL NEOCORTEX OF NEWBORN RAT - A QUANTITATIVE ULTRASTRUCTURAL STUDY. Donald A. Kristt and Mark E. Molliver
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Recent studies from this laboratory have shown that - in immature dog and human neocortex - there are bands of high and of low synaptic density, which are parallel to the pial surface. These studies of the development of neuronal connectivity have now been extended to the rodent: we have determined the locations of synapses with respect to the cytoarchitectonic layers of immature rat neopallium at post-natal day 1 (P1) and day 6 (P6). The following 5 layers can be recognized in the rat pallium at day P1: (from pia to ventricle) marginal zone, cortical plate, subplate layer, white matter and ventricular zone. Three of these layers constitute the immature cortex: a) the marginal zone, containing very few cell bodies; b) the cortical plate, made up of densely packed cell bodies which are elongated and have a strikingly consistent radial orientation; c) the subplate layer (corresponding to the outer part of the intermediate zone), characterized by a lower density of neuronal somata which have varied shapes and orientations. By day P6, the cortical plate becomes differentiated into three layers. The distribution of synapses (as a function of depth beneath the pia) in mid-lateral (somatosensory) cortex of the rat was analyzed at P1 and P6, using a quantitative electron microscopic method. To show the spatial relations between synapse density and perikaryal layers, the synaptic distributions are presented as histograms alongside photomicrographs of cortex sections. All synapses included in these data are characterized by specialized appositions with membrane-associated densities and synaptic vesicles (ca. 40 mμ).

In the newborn rat, most synapses have asymmetrical membrane specializations and are presumably axo-dendritic; axo-somatic synapses are rarely encountered. Most synapses in newborn cortex have very few synaptic vesicles and do not contain mitochondria. Preliminary measurements at day P1 reveal that synapses in the marginal zone have a smaller cleft width and a shorter appositional length than do synapses beneath the cortical plate.

At age P1 there are two strata of high synaptic density: in the marginal zone and in the subplate layer. At age P6 there are three synaptic strata: in the marginal zone, in the deep third of the cortical plate, and in the subplate layer. Thus, in immature rat cortex, synapses are not uniformly distributed, but are arranged in strata which have a clear-cut, spatial relationship to perikaryal layers. In early stages of development, synapses are concentrated at the superficial and deep borders of the cortex (i.e., above and below the cortical plate), as previously reported in other species. In subsequent developmental stages, multiple synaptic peaks are formed, starting in the deep layers of cortex and ascending towards the pia. This sequential, ascending pattern of synapse formation closely matches the sequence of neuronal birthdays, for cortical neurons in progressively more superficial layers (as determined by thymidine autoradiography). The alternating peaks and valleys of synaptic density are indicative of a discontinuous and selective process in synapse formation: either a set of axons growing into the cortex may seek a specific layer of termination, or, conversely, synapse formation may be determined by the sequential maturation of dendrites in successively formed cortical layers.

These descriptive data were obtained to provide a basis for further experimental studies of circuitry development in immature rat cortex. (For preliminary results see the abstract by Molliver and Kristt in this volume). In particular, we are attempting to classify cortical synapses into different types using morphologic criteria, and to identify the cell bodies of origin of synapses in different strata.

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1220 AXONAL TRANSPORT OF UNINCORPORATED ^{3}H-PROLINE AND ITS RELEASE FROM LABELED PROTEIN BOTH CONTRIBUTE TO TRANSNEURONALLY LABELED PROTEIN.

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The efficient incorporation of intraocularly(IO) injected ^{3}H-proline into retinal ganglion cell protein and its relatively poor transport into the brain via the blood make it uniquely suitable for radioautographic identification of presynaptic fields following axonal transport. Such studies may also show radioactive protein beyond the known ganglion cell presynaptic terminal region in the brain, leading to speculation on the possible transneuronal migration of intact macromolecules. We report here two more probable explanations.

To examine the possible breakdown of transported labeled protein and local incorporation of released ^{3}H-proline in neighboring brain cells, the precursor was injected IO into goldfish, a species with completely crossed optic tracts. Radioactivity in ipsilateral(IOT) and contralateral optic tectum(COT) were measured at 3,6,12,24 and 48 h. A Triton X-100 purified nuclear fraction was prepared from each tectal homogenate. Since nuclei are not transported axonally, an increase in the specific activity of nuclear protein COT relative to that IOT could only be due to reutilization of precursor. A COT-IOT difference in nuclear protein specific activity was observed at 6 h and continued to increase from 12 to 48 h, during which time the homogenate specific activity had reached a plateau. With ^{3}H-leucine as precursor, no such difference was seen. Confirmation that the labeling of nuclear protein in the COT was due to reincorporation of precursor ^{3}H-proline was obtained by examining the effect of a protein synthesis inhibitor. Cycloheximide (10 μg) was injected intracranially at 6 h intervals beginning 12 h after IO administration of ^{3}H-proline. The specific activity of the nuclear protein in the COT at 40 h was decreased 37% relative to saline treated controls. These results indicate that a significant fraction of the rapidly transported protein has a relatively rapid turnover and that the labeled proline released is efficiently reutilized for protein synthesis.

Another possible source of free ^{3}H-proline in the tectum is via its axonal transport. While this possibility has often been discounted, we have performed experiments with the labeled proline analog, ^{3}H-hydroxy-L-proline, which is not incorporated into protein. Results suggest that transport of small amounts of free amino acid might additionally account for some of the radioactive protein that is not associated with primary afferent terminals.

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1221 CHARACTERIZATION OF TRANSPORTED RNA IN NORMAL AND REGENERATING OPTIC NERVES OF GOLDFISH. N.A. Ingoglia and R. Tuliszewski*. Dept. Physiol., New Jersey Medical School, Newark, N.J. 07103.

When goldfish optic nerves regenerate large amounts of RNA, apparently synthesized in the retina, accompany fibers returning to the tectum. The present experiments were performed in order to determine what molecular species of RNA are associated with these growing fibers. Both optic nerves of 45 fish were crushed and 6, 12, or 18 days later both eyes were injected with 4 μc ^{3}H-uridine. Five days later intracranial injections of 0.5 μc ^{14}C-uridine were made (to monitor local tectal RNA synthesis) and fish were sacrificed 1 day later. Fifteen control fish were injected as above without crushing the nerve. Tecta were rapidly removed, homogenized, and RNA was extracted with hot phenol, and precipitated with ethanol. Purified RNA was electrophoresed on 2.0% polyacrylamide gels and gels were scanned at 260 nm, frozen, cut into 2 mm segments, and counted in a liquid scintillation counter. Greater than 80% of the radioactivity was confined to ethanol soluble and RNA fractions. At 18 and 24 days of regeneration the amount of ^{3}H-RNA was increased by 5 and 12 xs controls respectively, while at 12 days (before reinnervation) ^{3}H-RNA levels were less than controls. ^{14}C-RNA was 2 xs control regardless of whether re-entry had or had not occurred. In fish in which the optic nerves were regenerating for 24 days ^{3}H-radioactivity was found to be approximately 5 times controls in both the 28S and 18S RNA fractions but increased to greater than 13 times control in the 4-7S region. These results suggest that large amounts of small molecular weight RNA may be contained in growing optic axons. Supported by NIH (NS 11259).

1222 RNA DISTRIBUTION IN GOLDFISH OPTIC TECTUM AFTER INTRAOCULAR INJECTION OF ^{3}H-URIDINE. AN EM AUTORADIOGRAPHIC STUDY DURING OPTIC NERVE REGENERATION. P. Gambetti*, N. Ingoglia, P. Weis* and L. Autilio-Gambetti*. (Spon. M. Murray). Div. Neuropath., U. of Pa., Phil., Pa. and Depts. Physiol. and Anat., New Jersey Med. School, Newark, N.J.

After intraocular injection of ^{3}H-uridine, ^{3}H-nucleotides and ^{3}H-RNA are found along the entire retino-tectal pathway of the goldfish. The way by which this pathway is labeled is poorly understood, but labeling via blood or by passive diffusion of the precursors through extracellular spaces appears unlikely. In the goldfish, regenerating optic nerves establish functional reconnections 18-24 days after crushing the nerve. At this time, the amount of labeled RNA found in the optic tectum after intraocular injection of ^{3}H-uridine is more than 10 times greater than normal (Ingoglia et al., J. of Neurobiol. in press). In the present experiments the distribution of this labeled RNA was studied by quantitative EM autoradiography. Goldfish were sacrificed 24 days after bilateral optic nerve crush. Six days prior to sacrificing, ^{3}H-uridine was injected into the right eye. Samples from both optic tecta were studied. In the tectum contralateral to the side of injection, the highest density of grains related to ^{3}H-RNA were found over growth cones and adjacent glial cells, whereas other structures were much less labeled. Virtually no radioactivity above background was found in the tectum homolateral to the side of injection. (Supported by NIH grants NS 08933 and NS 11259.)

1223 ^{3}H-N-ACETYLGALACTOSAMINE IS INCORPORATED INTO GLYCOPROTEINS WITHIN THE AXON OF R2, A GIANT NEURON OF APLYSIA CALIFORNICA. R.T. Ambron and S.N. Treistman, Div. of Neurobiol., Coll. of Phys. & Surg. of Columbia Univ., New York, N.Y. 10032, and Friedrich Miescher Inst., Basel, Switzerland.

It has previously been shown that glycoproteins synthesized in the cell body of R2 are exported into the axon where they are rapidly transported toward nerve terminals (Ambron et al., J. Cell Biol. 61, 665 (1974). To see if sugars can be incorporated into protein within axons, we injected ^{3}H-N-acetylgalactosamine (^{3}H-GalNac) directly into the axon of R2 as it courses through the right connective. By 15 h after injection 13% of the radioactivity in the connective was incorporated into soluble and particulate glycoproteins and into glycolipid. Autoradiography indicated that at least 40% of the radioactivity was in the axon of R2. The remainder was in glia and connective tissue. Within the axon, silver grains were found associated primarily with membranous structures, including vesicles and smooth membranes. Five labeled macromolecules were resolved in the lipid-depleted particulate fraction by disc gel electrophoresis in SDS. These could be digested by pronase. Only one of the components had a mobility similar to that of a glycoprotein synthesized in the cell body. Incorporation of ^{3}H-GalNac into axonal glycoprotein was unaffected by anisomycin, a potent inhibitor of protein synthesis in Aplysia, whereas incorporation into surrounding glia and connective tissue glycoproteins was greatly inhibited. To examine the distribution of the glycoprotein along the axon, we cut the connective into mm sections at various times after injection. Each section was analyzed for its content of labeled glycoprotein by either autoradiography or acid precipitation. The glycoproteins were found to be transported away from the injection site in both retrograde and orthograde directions. Thus, although protein synthesis occurs primarily in the cell body of neurons, mechanisms apparently exist in the axon whereby proteins originating from the cell body can be modified.

1224 THE EFFECT OF PARAOXON ON CHOLINESTERASE TRANSPORT IN RAT SCIATIC NERVE. Norman A. Ranish and Wolf-D. Dettbarn. Dept. Pharmacol., Vanderbilt Univ. Sch. Med., Nashville, TN 37232.

Paraoxon, a cholinesterase inhibitor, has recently been shown to produce myopathies in rats which could be prevented by prior denervation (Fenichel et al., Neurol. 22: 1026, 1972). This suggested the possibility of an effect on the axoplasmic transport of a substance regulating the structural integrity of the muscles involved. As a first step in examining the relationships involved in the myopathic effects of this drug, the transport of cholinesterase was examined in rat sciatic nerve. Cholinesterase transport was evaluated by measuring the net accumulation of enzyme activity in segments proximal to ligations of the nerve. Control animals or rats receiving a single subcutaneous dose of .22 mg/kg of paraoxon two hours prior to ligation were sacrificed at 4,8,12,16 and 20 hours after ligation. In addition animals treated for 3 days with paraoxon were sacrificed between 4 and 20 hours after ligation, which was also performed 2 hours after the final dose of the drug. Four, 5 mm segments proximal to the ligations were assayed for cholinesterase activity spectrophotometrically, and enzyme activity calculated on a per mm basis.

In all three conditions (i.e. control, acute and chronic drug treatment) cholinesterase accumulation occurred in a linear fashion during the time intervals studied. In addition, the net accumulation of enzyme activity was decreased by the same percentage as the percent inhibition of sciatic nerve cholinesterase. For the single injection a net inhibition of approximately 60% of enzyme activity was observed as well as a 60% decrease in the accumulation of enzyme proximal to the ligature. In the case of chronically treated animals, the inhibition was approximately 80% and the level of accumulation was similarly reduced. The data suggest that the different levels of enzyme accumulation produced by paraoxon under these conditions are a reflection of the level of inhibition and do not indicate a change in either the rate or amount of enzyme transported. The results are in agreement with those obtained by James and Austin (Brain Res. 18: 192, 1970) in which subcutaneous injections of DFP were without significant effect on either the slow or rapid transport of precursor-labelled proteins.

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1225 AXOPLASMIC TRANSPORT OF A MYOSIN-LIKE PROTEIN IN RABBIT RETINAL GANGLION CELLS. Mark Willard* (SPON: T. A. Woolsey). Dept. Anat. & Neurobiol. and Biochemistry, Washington University Sch. Med., St. Louis, Mo. 63110.

A protein with some properties similar to those of muscle myosin has been identified in the rabbit visual system and has been shown to be one of the proteins which are transported down the axons of retinal ganglion cells. Extracts of optic nerve, optic tract, lateral geniculate and superior colliculus were mixed with F-actin purified from muscle. The mixture was centrifuged and the pellet was analyzed by electrophoresis for proteins that co-sedimented with the F-actin. This procedure revealed a protein that resembles muscle myosin in the following respects: (1) it sediments rapidly in the presence of F-actin, (2) its actin-dependent sedimentation is prevented by the presence of ATP (10^{-4}M), (3) its electrophoretic mobility is similar to but slightly lower than that of the heavy chain of muscle myosin.

In order to determine whether this protein is transported in retinal ganglion cells, ^{35}S-methionine was injected into the eyes of rabbits and after appropriate time intervals the radioactivity associated with the myosin-like protein was determined in the optic nerve and optic tract. The kinetics of labeling indicate that the myosin-like protein is synthesized in retinal ganglion cell bodies and subsequently transported down the axons in the slowest phase of axoplasmic transport at a velocity of 2-4 mm per day.

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1226 AXOPLASMIC TRANSPORT AND TRANSMITTER RELEASE. T. N. Tiedt* and S. G. Younkin. Department of Pharmacology, College of Medicine, University of Cincinnati, Cincinnati, Ohio 45267.

It has been shown (Albuquerque et. al., Exp. Neurol. 37, 607, 1972) that selective interruption of fast axoplasmic transport leads to post-synaptic changes similar to those seen with nerve section. These changes are thought to be related to some alteration in the delivery of "trophic" factors from the nerve cell body to the muscle. Such "trophic" factors may also influence the pre-synaptic function of transmitter release. Our initial experiments examined this possibility by comparing facilitation, depression, and post-tetanic potentiation (PTP) in control and denervated frog sciatic nerve - sartorius muscle preparations. Endplate potentials (EPP) were recorded using glass microelectrodes filled with 3M KCl. EPP amplitudes were measured using a PDP-8e computer. The muscle was bathed in a solution containing 112.0 mM NaCl, 2.0 mM KCl, 1.8 mM $CaCl_2$, 4.0 mM $NaHCO_3$, 0.05 mM tubocurarine chloride, and 1.0 gm glucose/L. Under these conditions, a 10 impulse train at 50/sec generates both facilitation and depression, and this train was used to assess these phenomena. A 1001 impulse train at 50/sec was used to generate PTP. In order to examine PTP and the decay of depression, impulses were delivered at suitable intervals following these trains. We have observed no statistically significant changes in facilitation, depression, or PTP in the first 99 hours after nerve section. After 99 hours it becomes difficult to locate endplates, and it appears that neuromuscular transmission ceases abruptly at about this time. To assess the effects of prolonged interruption of axoplasmic transport we have implanted nerve cuffs containing either 1% colchicine or vinblastine sulfate. The effects of these cuffs on transmitter release will be discussed.

1227 INHIBITION OF FAST AXONAL TRANSPORT OF [^{3}H]PROTEINS BY COBALT IONS. R. Hammerschlag*, A. R. Dravid and A. Y. Chiu*(SPON: J. E. Vaughn). Div. of Neurosciences, City of Hope National Med. Ctr., Duarte, CA 91010.

In previous studies of fast axonal transport in vitro we proposed that calcium ions are involved in the initiation of axonal transport within cell somata and also in the coupling of proteins to the transport system in axons (Science 188:273, 1975). This was based in part on the finding that in calcium-free medium (CFM) the amount of protein undergoing axonal transport was reduced by 40-60%. Since cobalt ions antagonize several calcium-dependent processes in neural tissue, fast axonal transport of [^{3}H]protein and of $^{45}Ca^{++}$ was studied in CFM supplemented with Co^{++}. Axonal transport was examined in vitro along the peripheral branches of primary afferent neurons of the bullfrog Rana catesbeiana. The 8th and 9th dorsal root ganglia of twin hemicord preparations were exposed to frog Ringer solution containing [^{3}H]leucine (.05 mCi/ml; 1.7 μM) and $^{45}Ca^{++}$ (0.8 mCi/ml; 1.8 mM). Following a 1 hr pulse, preparations were transferred to normal medium or to CFM supplemented with 1 mM EGTA and 1.8 mM $CoCl_2$ for periods of 6-20 hr at 18°. Axonal transport of calcium was assessed by determining levels of $^{45}Ca^{++}$ leeched into TCA from 3 mm segments of nerve trunks. TCA-insoluble [^{3}H] in each segment was taken as a measure of protein transport. Incubation in Co^{++}-supplemented CFM was found to reduce the amount of [^{3}H]protein undergoing fast axonal transport by 80-90% with no concomitant reduction in the amount of $^{45}Ca^{++}$ being transported. The Co^{++}-containing medium had no significant effect either on protein synthesis within the ganglia, or on levels of ATP within the nerve trunks. The results suggest that initiation of axonal transport involves the coupling of proteins to the transport system via Ca^{++} links. Co^{++} is seen as preventing protein from linking to Ca^{++} at a step that is independent of the attachment of Ca^{++} to the transport system. [Supported by USPHS Grants NS-09885 and NS-09226].

1228 ORGANELLE MOVEMENT IN THE NEURITES OF CULTURED DORSAL ROOT GANGLION CELLS IS DEPENDENT ON MAGNESIUM AND BLOCKED BY CALCIUM. C. N. Christian* (SPON.: P. G. Nelson). NICHD, NIH, Bethesda, Md. 20014.

Organelle movement (OM) within the neurites of cultured chick dorsal root ganglion cells was visualized by Nomarski interference-contrast microscopy, filmed and analyzed with a computer. The velocities of visualized organelles were within the range of fast axoplasmic transport. During periods of one half hour or more, normal OM was observed in media lacking Ca^{++}, Mg^{++}, K^+ or Na^+. Thus OM does not require the movement of ions across the outer neurite membrane. The ionophore A23187 was used to equilibrate internal with external concentrations of divalent cations. In the absence of divalent cations, A23187 stopped OM. The addition of Mg^{++} (threshold below 10^{-4}M) or Mn^{++} immediately reactivated OM. In the presence of the ionophore and 1 mM Mg^{++}, Ca^{++} in low concentrations (10^{-8}-10^{-6}) had no effect on OM and attenuated it at higher concentrations, irreversibly blocking it at 10^{-3}M. Sr^{++} does not substitute for Mg^{++} but does not block the Mg^{++} reactivation. The divalent cation specificity, repetitive motile force generation in the absence of ion fluxes, and OM in neurites containing microtubules but lacking neurofilaments (Breuer, et. al., JCB, June 1975), is consistent with the hypothesis that fast axoplasmic transport involves dynein-like arms attached to microtubules. (This work was supported by NIH grant 5 F02 HD55299-02).

1229 $^{45}Ca^{++}$ transport in sensory and sympathetic nerves of the frog. Jeffery L. Barker and Joseph H. Neale, Behavioral Biology Branch, NICHD, Bethesda, Md. 20014

Axonal transport of Ca^{++} was examined using the isolated peripheral nervous system of the frog. The dorsal root ganglion (DRG) with intact dorsal root and sciatic nerve and accompanying ventral root were placed in a plexiglass chamber and the cell bodies isolated from axons by means of silicone grease seals. The 9th and 10th sympathetic ganglia were treated similarly. Ligatures were placed at the ends of the nerves. Cell bodies of sensory nerves in the DRG and of post-ganglionic nerves were incubated in solutions containing mCi $^{45}Ca^{++}$ for 1 hr. During the incubation the other elements of the preparation were continuously flushed at a high rate with label-free oxygenated Ringer. In several experiments the hemisected spinal cord was isolated and incubated in $^{45}Ca^{++}$ to examine retrograde transport of $^{45}Ca^{++}$ along the dorsal root. Following the 1 hr incubation the cell bodies were also continuously flushed. The preparations were next chased for 12-20 hours under these conditions and the $^{45}Ca^{++}$ content of each in equal-sized segments along the nerves was determined. In some experiments ^{3}H-leucine was added to the incubation medium and the labelled trichloroacetic acetic-precipitable material in each segment was also determined. $^{45}Ca^{++}$ and ^{3}H-leucine-labelled protein were transported along the dorsal root and sciatic nerve in the sensory system and along post-ganglionic sympathetic axons, as judged by the accumulation of labelled material at the ligatures. No accumulation of $^{45}Ca^{++}$ was found at the ventral root following DRG incubation. No evidence of retrograde transport of $^{45}Ca^{++}$ along the dorsal root was found. Experiments are currently in progress to test the notion that $^{45}Ca^{++}$ transport by these systems is specific and possibly related to the contributing of $^{45}Ca^{++}$ to spike generating mechanisms.

1230 FAST AXOPLASMIC TRANSPORT OF CALCIUM BINDING COMPONENTS IN MAMMALIAN NERVE. Zafar Iqbal and Sidney Ochs. Indiana University Med. Cent. Dept. Physiol., Indianapolis, Indiana, 46202.

Calcium is considered to play an important role in the "transport filament" model for axoplasmic transport (Ochs, S., Ann. N.Y. Acad. Sci. 228, 202, 1974). The supply of ATP as a source of ~P energy needed for transport may require the MgCa ATPase present in nerves to utilize the ATP. Recently, a report of a fast transport of ^{45}Ca in frog nerve was reported (Hammerschlag, R., David, A.R., and Chiu, A.Y., Science 188, 273, 1975). In this communication we report independently initiated studies of fast axoplasmic transport of ^{45}Ca in the mammalian nerve. Such studies were made by injecting ^{45}Ca into the L7 dorsal root ganglion of cats and the transport of labeled materials in the sciatic nerves studied by the methods used in this laboratory. Bound ^{45}Ca was found transported at a fast rate, though with some difference in the outflow pattern compared to the transport of proteins. The ^{45}Ca transported was associated with two components isolated from the high speed supernatant fraction. The soluble fraction was separated on Sephadex G-100 columns into two peaks, peak I characterized by higher molecular weight proteins, and peak II by lower molecular weight polypeptides. Peak I on further chromatography on Biogel A5m columns showed ^{45}Ca activity associated with a subgroup (peak Ic) having a molecular weight of approximately 15,000. Peak II material was further characterized on Sephadex G-10 and Biogel P-2 columns and ^{45}Ca was found associated with polypeptides in the peak IIb subgroup (Iqbal, Z. & Ochs, S., Proc. IUPS 11, 436, 1974) having molecular weights in the range of 1100-1500. The relation of these components to the transport filaments themselves or to components bound to them is under study.

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1231 FAST TRANSPORT IN VAGUS NERVE: INFLUENCE OF THE MICROTUBULE STABILIZING AGENT, DIMETHYLSULFOXIDE. J.A. Donoso, J.P. Illanes* and F.E. Samson. Ralph L. Smith Research Center, Kansas Univ. Med. Center, Kansas City, Ks. 66103.

Dimethylsulfoxide (DMSO) has recently been shown by several laboratories to have a strong stabilizing effect on microtubules (MT). Dulak and Crist (Am. Soc. Cell Biol., 1974,Abst. 77) found that DMSO prevented the depolymerizing action upon MT under certain conditions of temperature cooling, pH, ionic strength and anti-mitotic drugs in vitro. Since these anti-microtubule agents are known to block fast axoplasmic transport (FAT) by depolymerizing the MT, the question raised was what influence DMSO has on FAT in light of its stabilizing effect on the MT. The FAT of proteins was studied in vivo, in vitro and in the presence of DMSO in the vagus nerve. The nodose ganglion of cats was injected with 3H-leucine and the vagus nerve removed and analyzed 3 to 6 hrs later. A well defined distribution of labelled material was found along the nerve, with wavefront velocity of 388 + 25 mm/day. The in vitro experiments were carried out by injecting the nodose ganglion with 3H-leucine, allowing 2 hrs for incorporation of the 3H-leucine into protein and then removing vagus peripheral to the ganglion from the cat and incubating it in a modified Krebs saline solution, at 37°C, in a 95% O_2, 5% CO_2 atmosphere. The characteristics of the FAT were the same in vitro as in vivo. Nerves were exposed to DMSO 1%, 2%, 5% and 10% for 2 or 3 hrs. A total blockage of FAT resulted in the 10% DMSO medium within 30 min. In 5% DMSO the FAT was blocked in a distinct pattern that suggests a differential susceptibility of types of axons. In 2% DMSO the FAT velocity was within a normal range. The ultrastructure of the nerves under these experimental conditions was also studied. The nerve fibers in 10% DMSO presented an extensive swelling of the glial cells. The individual axons behaved very differently, some axons were swollen but others were dramatically reduced in diameter and in these, numerous well-formed microtubules occupied almost all of the intra-axonal space. In the swollen axons the individual MT appeared normal but their distribution within the axon was somewhat more dispersed than normal. In 5% DMSO the ultrastructure was changed in a manner similar to the 10% DMSO but less pronounced. In 2% the ultrastructure appeared normal except for some swelling of the glial cells. Both the FAT blockage and the ultrastructural changes were completely reversible, with 2 hrs of washing; even the 10% DMSO treated nerves resumed a normal rate of FAT within 30 min. The maximum stabilizing effect of DMSO on MT in vitro is found at a concentration of 10%. The FAT is completely blocked at this concentration. These results are compatible with the concept that the MT must be in a "dynamic" condition to be biologically functional. The DMSO in strengthening the polymerization forces would make the MT non-functional, although structurally intact.

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1232 EFFECT OF TEMPERATURE ON RAPID RETROGRADE AXONAL TRANSPORT OF OPTICALLY DETECTABLE INTRA-AXONAL ORGANELLES. D. S. Forman, A. L. Padjen and G. R. Siggins. Lab. of Neuropharmacology, NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

Rapid movements of organelles can be visualized microscopically inside living axons in vitro. Acutely isolated single myelinated axons from bullfrog sciatic nerve were studied by darkfield microscopy. Organelle movements were recorded by cinemicrography, measured with an L-W motion analyzer system, and analyzed with the aid of a PDP-12 computer. The elongated axonal mitochondria move very infrequently, although they are capable of rapid saltations on occasion. Nearly all of the optically detectable moving organelles are spherical or ellipsoidal, with diameters about 0.2-0.6 microns. Their movements are rapid, saltatory, and bidirectional. More than 90% move predominantly in the retrograde direction, i.e., toward the cell body. Although the mean speeds of individual particles vary considerably, the statistical distribution of particle velocities is similar in different axons studied at the same temperature. The average mean speed of organelles moving in the retrograde direction at 28°C is 1.08 microns per second (S.D.=0.42), equivalent to 93 mm/day. In the range of 8°-35°C the speeds are highly temperature dependent, with a Q_{10} of roughly 3.5. Movement stops completely at about 4°C. The mean particle speeds are compatible with estimates of the rate of retrograde transport as measured by other methods, and are slower than the rate of anterograde transport. Depending on the temperature, the mean particle speed is only 15-40% of the reported rate of anterograde rapid axonal transport in the frog sciatic nerve. The average organelle speeds in myelinated axons are faster than those which are seen in neurites in tissue culture. The standard deviation of the distribution of retrograde particle velocities also varies with temperature. The standard deviation remains a constant fraction (about 38%) of the population mean at all temperatures. Remarkably, this relationship (i.e., S.D./mean=.38) is also found in axons from other species, and in neurites in tissues culture, and may reflect a fundamental property of the axonal transport mechanism.

1233 SPECIFICITY OF RETROGRADE AXONAL TRANSPORT IN THE RAT VISUAL SYSTEM. A. H. Bunt, R. H. Haschke, D. F. Calkins*, and R. D. Lund. Ophthalmology, Biological Structure, Anesthesiology and Biochemistry, Univ. of Washington, Seattle, Washington, 98195.

The general phenomenon of retrograde axoplasmic transport, by which an exogenous protein is pinocytosed by axon terminals and transported back to the lysosome system of the cell body, has been demonstrated in a number of neuronal systems. Study of sympathetic neurons has revealed uptake and retrograde transport of nerve growth factor by their axons. Certain other proteins were not transported, suggesting a specificity in axonal uptake and/or transport to the cell body (Stöckel, et al, Brain Res. 76:413, 1974). A selectivity has also been found in the rat visual system for retrograde transport by optic nerve axons from superior colliculus to the ganglion cell somata in the retina. Horseradish peroxidase (HRP), but not bovine serum albumin (fluorescent or radioactive label), alkaline phosphatase, hemocyanin or lactoperoxidase, was transported retrogradely. Modification of the HRP by acetylation did not affect the transport process. However, no evidence of retrograde transport could be found after HRP was oxidized by periodate followed by borohydride reduction (HRP still enzymatically active). This suggests that the carbohydrate moiety of the enzyme is involved in the specificity of recognition of HRP. Studies are in progress to elucidate which physical and/or chemical properties play a part in the recognition process of proteins by axons of the visual system.

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1234 VAGAL AFFERENTS - AXONAL TRANSPORT OF HORSERADISH PEROXIDASE AND LABELLING OF CELLS IN THE NUCLEUS OF THE SOLITARY TRACT. Madhu Kalia. Dept. of Physiol., Hahnemann Med. Col., Phila., PA 19102.

This study was undertaken to demonstrate the transport of horseradish peroxidase (HRP) in vagal sensory nerve fibers. In 15 adult cats and 4 kittens (1-3 weeks old), the right cervical vagus was sectioned and the cut central end was submerged in a 33% solution of HRP in saline for 7-28 hours. The animals were maintained under nembutal anesthesia throughout. The nodose and jugular ganglia of both sides and the brain stem were processed and examined for the presence of the marker under light and dark field microscopy. By 12 hours, the HRP had diffused out of the nodose ganglion towards the medulla. In all animals, labelling was found in the motor nuclei of the vagus, i.e., the dorsal motor nucleus (DMN) and the nucleus ambiguus; in addition, very marked labelling of the nucleus of the solitary tract (ST) was found. This indicates that HRP moves in the anterograde direction from the nodose ganglion towards the second order neurons in the medulla. This marker was found in the ST as early as 18 hours after the nerve was exposed to the HRP. The quantity of enzyme transported did not appear to be different in newborn and adult cats. There was no evidence of continuous transport along the axons even though the nerve remained in the solution of HRP over the entire period. It is apparent from these experiments that HRP moves in both retrograde and anterograde directions in sensory fibers of the vagus nerve and labels the synaptic connections in the nucleus of the solitary tract. (Supported by RCDA HL-00103 and NIH grant-in-aid HL-00178.)

1235 PARTIAL SUPPLY OF PROTEIN TO MYELIN VIA RAPID AXONAL TRANSPORT IN VITRO. J. F. Hines and Paula Higgins*. Department of Biology, Texas Woman's University, Denton, Texas 76204.

Several reports have appeared in the literature concerning the in vitro supply of protein to CNS myelin via axonal transport. The data presented here relates to the in vitro supply of protein to peripheral nerve myelin via rapid axonal transport. The preprration used in this study included the dorsal root ganglia, sciatic nerve and gastrocnemius. It was placed in a three part chamber. Each compartment was separated by a silicone grease barrier. The dorsal root ganglia was incubated in ^{14}C leucine for five hours in compartment A. The protein was transported down the axon in compartment B to the muscle in compartment C. Isolation of the myelin after transport and electrophoresis of myelin protein revealed that radiolabel was localized in the high molecular weight protein, but little or no label was found in the major myelin proteins. To insure that the radiolabelled protein isolated from myelin was not produced by Schwann cells, protein synthesis in the Schwann cells was inhibited by superfusing chamber B with cycloheximide (100ug/ml). Sciatic nerves incubated in ^{14}C leucine without the ganglia showed an incorporation into the major myelin proteins. It is concluded therefore, that protein may be supplied to peripheral nerve myelin either by Schwann cells or by neurons via rapid axonal transport, but the proteins supplied by each appears to be different. (Supported by Institutional Research Fund of Texas Woman's University, #959)

1236 IDENTIFICATION AT THE ULTRASTRUCTURAL LEVEL OF ACTIN-LIKE FILAMENTS IN RAT CENTRAL NERVOUS TISSUE BY MEANS OF HEAVY MEROMYOSIN LABELING.

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To identify structures within neurons possibly involved in transport of material from sites of synthesis to functional locations, a study was undertaken by means of HMM labeling. Myosin, prepared from rabbit skeletal muscle, was split into HMM and LMM by tryptic digestion. Small pieces from rat brain were incubated in glycerol solutions of decreasing concentrations at 4^{o} and then transferred into standard phosphate buffer (pH 7.0) or into tris-K^{+}-Mg^{++}-Ca^{++} buffer (pH 7.9) with or without HMM. Other pieces were immersed for an identical period in the same buffers to which were added HMM and either 2.5 mM Na^{+} pyrophosphate or 5 mM ATP. In each portion of the HMM-untreated neurons, smooth-surfaced microfilaments, 40-60 A in diameter, were intertwined in a loose network. After reaction with HMM, these filaments became decorated with HMM and increased strikingly in diameter to 180-200 A. This was observed in neurons and as well in glial and endothelial cells and especially in pericytes. A dense network was formed. Arrowheads pointing in the same direction were spaced at regular intervals (300-350 A) along short segments of the surfaces of a given filament, when longitudinally sectioned. More often, however, arrowheads could not be observed decorating obliquely-sectioned filaments, the surfaces of which were thus seen coated with polarized side-arms cross-bridging the spaces between adjacent elements at more or less regular intervals. When cross-sectioned, the microfilaments appeared as dense dots from which a material of lesser electron density radiated. Following incubation in HMM solutions containing Na^{+} pyrophosphate or ATP, no arrowhead structure was observed. Occasional, tapered myosin-like filaments, 120-180 A in diameter, were lying among the HMM-reacted filaments. They were attached to them. Undecorated, whisker-like strands, about 20 A in diameter, linked together the HMM-decorated filaments. They were tentatively interpreted as protofilaments of myosin. Of particular interest was the anchorage of the HMM-reacted filaments to the plasma membrane at many points, an association which may be analogous to the relationship of actin with Z-lines in muscle. Mitochondria, ER membranes and synaptic vesicles were attached to the actin-like filaments and enmeshed in the network. The microtubules, as well as most of the neurofilaments, were disrupted by the glycerination procedure at 4^{o}. Serial sectioning allowed, in addition, a geometrical description of the synaptic densities in E-PTA-stained nervous tissue. In tangential sections, the presynaptic dense projections (PDP's), disposed in regular hexagonal arrays, had a regular hexagonal shape and the framework on which the presynaptic grid was built consisted of juxtaposed equilateral triangles. Surrounding a central PDP were six electron-lucent hexagonal holes forming in turn the sieve of the presynaptic grid. In cross sections, the PDP's appeared as trapezoids. From the top of these small truncated pyramids, spines extended into the ground substance, forming a presynaptic filamentous network regularly stretched throughout the presynaptic bag. The microfilaments were also anchored at multiple points to the inner surface of the plasma membrane which was coated with a dense fuzz. Several procedures permitted thus the identification of a neuronal cytoplasmic network, the microfilaments of which bound HMM molecules on their surfaces, strongly suggesting the actin-like nature of the proteins which make up the microfilaments. Myosin-like filaments were also observed, though few in number. Thus, it is hypothesized that these contractile filaments are involved in transport within neurons. This could explain the axonal flow and the transport of synaptic vesicles to the presynaptic membrane by a mechanism of chemomechanical transduction.

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1237 CHANGES IN THE AXONAL TRANSPORT OF STRUCTURAL PROTEINS DURING AXONAL OUTGROWTH. Paul N. Hoffman* and Raymond J. Lasek. Dept. Anat., Case Western Reserve Univ., Cleveland, Ohio, 44106.

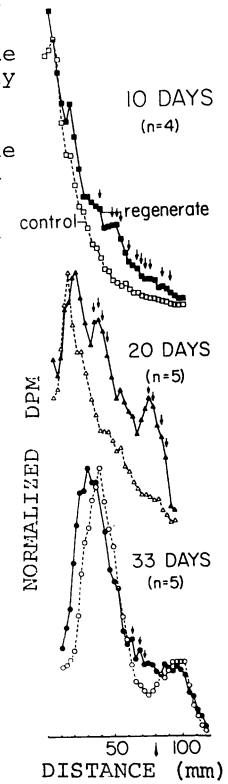
The mechanisms responsible for controlling axonal outgrowth may play an important role in determining the form of the nervous system. Axonal regeneration in the rat ventral motor neuron was used as a model of outgrowth. Having previously demonstrated the transport of structural proteins in the slow component of axonal transport, we investigated the possible role of these proteins in the outgrowth process.

The figure illustrates the transport profile of regenerating sciatic nerves which were freeze-crushed at the knee (the level of the freeze-crush is indicated by an arrow on the abscissa) 20 days prior to labeling motor neurons by the direct injection of amino acids into the ventral spinal cord. Comparison of the distributions of labeled proteins in regenerate and paired contralateral control nerves reveals significant differences. (Differences significant to the .05 level by the paired t-test are indicated by small arrows.) The data in the figure demonstrate: i) The regenerating axons contain additional labeled protein, not present in the controls, which moves slightly ahead of the slow component peak. This difference is most dramatic at 20 days. and ii) The rate of movement of the slow component peak is not altered in regenerating axons. This is best illustrated at 33 days.

The slow component is composed of five major polypeptides. Three of these polypeptides, the slow component triplet, (with molecular weights of 212,000; 160,000 and 68,000 daltons) are constitutive, and as such appear to be associated with a single structure which has been tentatively identified as the neurofilament. The two remaining polypeptides have been tentatively identified as tubulin, the microtubule protein, on the basis of their molecular weights.

It has been found that the differences in the distributions of labeled protein in control and regenerate nerves are attributable to the presence of additional polypeptides, tentatively identified as tubulin, moving slightly ahead of the slow component peak in regenerating axons. It has not yet been determined to what extent this fraction of additional tubulin reflects either alterations in synthesis or the redistribution of axonal tubulin.

This observation leads us to propose that the axonal microtubules and neurofilaments may play quite different roles in the process of outgrowth. While either a redistribution or increased synthesis of tubulin may occur, such changes do not appear to occur in the case of the neurofilament. The neurofilament may be a relatively stable element of the axonal framework in comparison to the more plastic microtubule. Alterations in the synthesis, distribution or degree of polymerization of tubulin might play important roles in the process of axonal outgrowth.



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1238 TURNOVER OF AXON-TRANSPORTED PROTEIN IN RAT MOLAR SENSORY NERVE ENDINGS. Margaret R. Byers, Stephen J. Kish*, and B. Raymond Fink. Anesthesiology and Center Res. Oral Biol., Univ. Washington, Seattle, Wash., 98195.

Injection of ^{3}H-L-proline into the trigeminal ganglion of adult rats labels rapidly and slowly transported protein in the ipsilateral maxillary nerve as determined by scintillation counting of TCA-precipitable material (Fink et al, BRAIN RES., in press). The arrival of this protein in maxillary molar sensory nerve endings (15-20mm from the ganglion) was studied in a series of rats (n=20) fixed by perfusion with buffered 4% formaldehyde at 1.5 hr to 28 days after injection of 20 µl ^{3}H-L-proline (5-10 µC/µl, NEN) into the right ganglion. The right and left maxillary teeth were excised, fixed 24 hr, decalcified, and processed for autoradiography. The left teeth served as controls against incorporation of blood-born isotope and had only weak general pulp label at all times. In right molars at 1.5 hr no nerve-born radioactivity was present but by 2 hr moderate label was present over apical and coronal pulp nerves and the coronal subodontoblast plexus, odontoblast layer, and circumpulpal dentin. From 3-24 hr heavy labelling was present over these regions. At 3 days there was in increase in label as the slower proteins began to arrive and by 7 days the molars were maximally labelled. Electron microscopic autoradiography showed silver grains at 7 days to be almost exclusively over coronal nerve endings or pulp axons, as was found at 6 hr. By 14 days radioactivity had decreased about 50%, and by 28 days very little remained in the nerve endings, although pulp nerves were still moderately radioactive. These results show that most axon-transported protein turns over every 14-28 days in molar sensory nerve endings in mature rats. A comparison will be made between these results and those found for synaptic nerve endings.

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1239 AUTORADIOGRAPHIC OBSERVATIONS ON LATE AXOPLASMIC TRANSPORT OF TRITIATED PROLINE IN DRG OF RAT. David G. Whitlock and Priscilla A. Ledbury*. Dept. Anatomy, Univ. of Colorado Med. Sch., Denver, Colorado 80220.

Concentrated tritiated proline was injected electrophoretically into a single lumbar dorsal root ganglion (L5 or L6) of anesthetized adult rats. Animals were sacrificed after post-injection survival times of 4 hours to 33 days. The injected ganglion, its proximal and distal dorsal roots, the sciatic nerve, and the contralateral control tissues were then totally removed and fixed in Bouin's solution. All tissues were blocked, embedded, serially sectioned at 3 microns and prepared for autoradiography using Kodak NTB-2 emulsion. After developing, the ARG's were stained with aqueous toluidine blue.

Microscopic examination of the ganglia from animals with short survival times showed dense accumulations of silver grains overlying most ganglion nerve cell bodies at the injection locus. In animals with progressively longer survival times, the number of silver grains overlying nerve cell bodies decreased. This apparent reduction of radioactivity in the ganglion cell bodies was interpreted as due to a depletion of labelled materials as a result of transport of the radioactivity out along the nerve processes.

Transverse sections of the dorsal root taken within 1 cm of the injected ganglion from animals surviving 3 to 33 days showed many silver grains overlying the nerve fiber sheaths but few grains were seen over the axons. Distally in the peripheral sciatic nerve, the number of labelled axons significantly increased while the number of silver grains found over the nerve sheaths markedly decreased. A similar increase in the number of labelled axons and decrease in sheath radioactivity with reference to the injection locus was seen in the proximal dorsal root.

At distances of 1-3 cm from the injected ganglion it was possible to count the number of labelled axons in the peripheral nerve and proximal dorsal root, utilizing a computerized microscope. No sections could be counted at distances less than 1 cm, because of the high amounts of sheath labelling; or at more than 3 cm, because of the onset of branching of the nerve processes. After 11 days survival time, the number of labelled axons found at 1 cm from the injected ganglion was approximately one half that found at 3 cm, suggesting a depletion of radioactive material in the proximal axon similar to that seen with increasing survival times in the ganglion nerve cell bodies.

These findings suggest that with increasing post-injection survival times, there is a progressive decrement of radioactivity both in the nerve cell body and in the proximal axons. This reduction in the proximal axons is obscured by the retention of the radiochemical residues in satellite cells and the sheath cells near the injection site and probably could not be detected by liquid scintillation counting methods. The findings also indicate that the length of the post-injection survival interval must be considered when tritiated proline, and perhaps other labelled amino acids, are employed to trace nerve pathways.

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1240 THE EFFECTS OF 2-CHLOROADENOSINE AND PROSTAGLANDIN E_1 ON THE ACTIVATION OF MOUSE NEUROBLASTOMA ADENYLATE CYCLASE PRODUCED BY Gpp(NH)p. Arthur J. Blume and Carolyn J. Foster.* Dept. Physiol. Chem., Roche Institute of Molecular Biology, Nutley, New Jersey 07110.

Studies on the adenylate cyclase (AC) in crude particulate fractions obtained from clone NS20, mouse neuroblastoma C1300, were performed at 30°, pH 7.4, 5 mM $MgCl_2$ using the two different substrates ATP and App(NH)p. All studies with ATP and some of those with App(NH)p contained an ATP regenerating system (RS). 50 µM 2-chloroadenosine (2-CA), 1.4 µM prostaglandin (PG)E_1 and 50 µM Gpp(NH)p were used to alter AC activity. With 0.1 mM ATP, AC activity was linear with time when assayed without any additions (basal activity) or with 2-CA (2-fold stimulated) or with PGE_1 (10-fold stimulated). Gpp(NH)p stimulated activity was however not linear with time, there being a 8-12 min lag before a maximum rate (4-6-fold elevated over basal) was obtained. The presence of 2-CA with Gpp(NH)p, or PGE_1 with Gpp(NH)p produced activities which exhibited reduced time lags and slightly higher maximum rates than seen with Gpp(NH)p alone. In both respects, PGE_1 was more effective than 2-CA. At 0.1 mM ATP a similar qualitative picture was observed for AC activity although the absolute activity values were smaller. Preincubation of the particulate fraction at 30°, pH 7.4, with 5 mM $MgCl_2$ (1) caused no loss in the ability of 2-CA or PGE_1 to stimulate basal activity and (2) basal, 2-CA and PGE_1 activities were all still linear with time. However, preincubation did cause the Gpp(NH)p stimulated activity to also become linear with time and now the additional presence of 2-CA or PGE_1 along with Gpp(NH)p produced only a 10% and 50% further increase in activity. The presence of a number of adenine and guanine nucleotides or the simple presence of the ATP RS during these preincubations prevented the abolishment of the Gpp(NH)p activity lags when subsequently assayed. GDP was observed to inhibit all cyclase activity dependent upon added Gpp(NH)p when Gpp(NH)p, Gpp(NH)p plus 2-CA or 2-CA or Gpp(NH)p plus PGE_1 stimulated AC activities were monitored. However GDP did not inhibit basal, 2-CA or PGE_1 stimulated activities.

A different picture was observed when 0.1 mM App(NH)p without an ATP RS was used to assay the AC activity: (1) no significant basal activity could be detected, (2) significant activity was also not detected in the presence of 2-CA or PGE_1, (3) activity was observed in the presence of Gpp-(NH)p. This Gpp(NH)p directed activity did show a small time lag (3-4 min) and was about 25% that seen with 0.1 mM ATP plus the ATP RS. The presence of 2-CA did not effect this Gpp(NH)p activity while the presence of PGE_1 did lead to an increase (about 40%) in the Gpp(NH)p dependent activity. Surprisingly, when an ATP RS was included along with 0.1 mM App (NH)p an AC activity picture was seen which resembled that seen with 0.1mM ATP and the ATP RS. Basal activity was now measureable and 2-CA and PGE_1 stimulated this activity. The Gpp(NH)p directed activity now exhibited long time lags (8-10 min) and the combination of 2-CA or PGE_1 along with Gpp(NH)p caused a reduction in the activity time lags. With 0.1 mM App (NH)p and no ATP RS the addition of GDP was seen to greatly increase the Gpp(NH)p directed activity lags. Furthermore, these GDP produced lags were overcome by the addition of PGE_1 but not 2-CA. With 0.1 mM App(NH)p and 5 min incubation times, 5 µM GDP was observed to produce a 50% inhibition of the 50 µM Gpp(NH)p directed AC activity. More GDP was required to produce a 50% inhibition at longer assay times as would be expected due to the hydrolysis of GDP during the incubations. The presence of PGE_1 and 2-CA along with Gpp(NH)p caused the concentration of GDP required for 50% inhibition, at all incubation times, to be increased 6 and 1.5-fold respectively.

We interpret our results to indicate that neuroblastoma adenylate cyclase (1) has an absolute requirement for some regulatory nucleotdie triphosphate (most probably GTP), (2) is inhibited by nucleotide diphosphates (most probably GDP) which compete with GTP binding, and (3) that PGE_1 and 2-CA stimulate the adenylate cyclase, at least partly, by decreasing the enzyme's sensitivity to GDP.

1241 SYNTHESIS AND STORAGE OF CATECHOLAMINES BY CENTRAL AND PERIPHERAL NEURONS IN VITRO. M. Schlumpf* and W. Shoemaker, NIMH, Saint Elizabeths Hospital, Washington, D. C. 20032.

We have been studying the synthesis, storage and release of catecholamines (CA) in neurons that utilize these substances as transmitters. Embryonic rat brains were used as sources for explants of locus coeruleus (LC) (a region of norepinephrine (NE)-containing neurons) and substantia nigra-midbrain (SN) (a region of dopamine (DA)-containing neurons). Superior cervical ganglia (SCG) were dissociated according to the method of Mains and Patterson (J. Cell Biol. 59: 329,1973) to provide cultures composed chiefly of NE-containing sympathetic neurons. The brain explant cultures were maintained in serum-enriched Dulbecco's Modified Eagle's Medium and routinely treated with 5-fluorodeoxyuridine. Variations in media conditions produced changes in the rate and pattern of culture growth but had little effect on CA synthesis and storage. For instance, whereas cerebellar and cerebral cortical cultures could be maintained in L-15 media, cultures of brain stem regions do not tolerate prolonged (over 1 week) culturing in L-15. Nerve Growth Factor (NGF) which is an absolute requirement for SCG cultures, seems to enhance the initial outgrowth of processes in all brain cultures. However, NGF seems to have minimal effects on CA synthesis and storage, and these effects may be due indirectly to enhanced attachment and survival of the brain explants. After 2 to 3 weeks in vitro, we measured norepinephrine and dopamine levels in both media and cells using a modification of the radio-enzyme assay that permits detection of pg quantities. More than 50% of LC cultures synthesize and store NE (ave.=600 pg/culture) whereas only 25% of SCG cultures do (ave.=250 pg/culture). SN cultures synthesize large amounts of DA but retain very little of it in the cells. The detection of 2-5 ng/ml of DA in the media of such cultures probably means that the cells are synthesizing DA at a high rate because catecholamines are degraded rapidly in culture media at incubator conditions. Although SN cultures rarely synthesize NE, LC and SCG cultures produce large quantities of DA, most of which is not retained by the cells. Attempts to increase the rate of NE synthesis from DA in these cultures by adding the known co-factors for dopamine β-hydroxylase, Cu^{++} and ascorbic acid, were not effective. The inability of many of these cells to store the CA they synthesize is further confirmed by the difficulty in obtaining consistent histochemical fluorescence. We find an average of 0.6 ng NE in LC cultures 2 weeks after explanting tissue containing 2 ng NE. Assuming that less than 50% of neurons survive the explant procedure, these results indicate that culture conditions for LC explants permit reasonable transmitter metabolism.

1242 THE SYNAPTOLOGY OF CULTURES OF CEREBELLAR CORTEX. Walter J. Hendelman. Dept. Anat., Fac. of Med., University of OTTAWA, Ottawa, Canada. K1N-9A9

The purpose of attempting cultures of cerebellar cortex only, without deep nuclear neurons, was to investigate their synaptology. These cultures were established by undercutting 2 - 3 folia of the vermis of newborn mouse cerebellum; 2 - 3 pieces were placed into the Maximow chamber and handled in the usual manner. These explants are smaller than the normally-used fragments of cerebellum; many did not attach to the collagen and others failed to thrive. Few have survived beyond 2 weeks (up to 5 weeks) and these contained a small number of Purkinje neurons, as judged in their living state; myelinated fibres were rarely seen. In contrast to thicker cultures, these explants have failed to stain with a modified Golgi-Cox technique. Electron microscopic examination of a limited number of cultures was carried out after aldehyde-osmium fixation and uranyl-lead staining. The better cultures were filled with small Purkinje dendrites and a plentitude of spines, most of which were free, but some were ensheathed by glia. Parallel fibres were seen in small fascicles and parallel fibre-spine synapses were found. Golgi-type synapses and recurrent collateral terminals were recognized by the accepted standards of vesicle population, and both contacted the smooth surface of dendrites. Stellate-type synapses were found on the smooth surface of the Purkinje soma. Occasionally, large terminals were seen filled with round vesicles and forming multiple synaptic contacts with small elements; sometimes these were recognizable as granule cell dendrites. These terminals resemble mossy fibres. Assuming that these were in fact pure cortical cultures, the present results are being interpreted as a modulation of synaptic morphology which occured under experimental conditions.

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1243 NEURON CULTURE FROM ADULT GOLDFISH. U. De Boni*, J.W. Scott and D.R. Crapper. Depts. Physiology and Medicine, Univ. of Toronto, Toronto, Ontario M5S 1A8, Canada.

The optimum time for explanation of neuronal tissue from vertebrate central nervous systems has been limited to the prenatal or immediate neonatal period, at which time cytodifferentiation begins but morphogenesis has almost ceased. This limitation is imposed by the inability of neurons of the post neuroblast stage to divide, although they may occasionally be induced to undergo mitosis (Murray and Benitez, 1967). In contrast, the CNS of teleosts has remarkable regenerative properties attributed to the presence of matrix zones of undifferentiated cells, (Maron, 1963; Segaar, 1962; Kirsche and Kirsche, 1961; Srebro, 1959; Jordan, 1958). Neurons and glia from the central nervous system of the adult teleost Carassius auratus have been grown as explant cultures of minced brain tissue and as trypsin dissociated cells. These cultures exhibit extensive neurite growth from two neuronal types, have organotypic ultrastructure and contain electrically active cells. Histological, electrophysiological and autoradiographic data suggest that a large proportion of these neurons arise by differentiation of immature cells localized in matrix zones in the brain of fishes.

1244 LONG-TERM GROWTH AND ELECTROPHYSIOLOGICAL PROPERTIES OF PERIPHERAL GANGLIA OF ADULT FROG IN VITRO. A. L. Padjen, G. R. Siggins and D. S. Forman. Lab. of Neuropharmacology, NIMH, Saint Elizabeths Hospital, Washington D.C. 20032.

We have developed a simple method for long-term cultivation of peripheral sympathetic and spinal ganglia of adult bullfrog (R. catesbeiana). Ganglia were placed on collagen-coated cover slips and kept in Petri dishes in modified McMahan-Kuffler medium (Proc. Roy. Soc. Lond. B, 77(1971) 485) in moist chambers at 21°C. Ganglia did not attach to the coverslip until up to three weeks after isolation. Cultures were first fed after attachment and re-fed at 2 week intervals. Subsequently most explants showed slow continuous outgrowth of neurites and outward migration of Schwann cells lasting several months. After 4-6 weeks in vitro, the cultures contained cells with morphology characteristic of normal ganglioneurons (elliptical or spherical cells with large eccentric nuclei and nucleoli, many cytoplasmic granules and only 1-2 processes). These cells were often found in clusters on the surface or outgrowth zone of the explants, and were highly suitable for electrophysiological studies. Intracellular recording of up to 10 hrs duration was performed using Nomarski optics, on over 60 sympathetic ganglion cells (SGC; O.D. 35-120 μ). Resting membrane potentials of uninjured cells ranged from -40 to -70 mV. All SGC generated spikes (overshoots up to 30 mV) in response to intracellular stimulation. Input resistance (R_i) calculated from largely linear current-voltage curves was 15-40 MΩ. Iontophoresis or superfusion of acetylcholine, carbachol or GABA caused depolarization in SGC with R_i reduction. These results indicate survival of mature frog ganglioneurons, with apparent retention of the morphological, electrophysiological and pharmacological properties seen in acutely isolated ganglia. Simplicity of the preparation and care of these cultures makes them an attractive model system for neurobiology.

1245 MONOLAYER CULTURES OF CEREBELLAR NEURONS FROM NORMAL AND NEUROLOGICAL MUTANT MICE. Anne Messer*. (SPON: R.L. Sidman). Depts. of Neuropathology Harvard Medical School and Neuroscience, Children's Hospital Medical Center Boston, Mass. 02115.

Cells are dissociated from cerebella of postnatal day 7 mice and plated in a modified F12 medium on glass or plastic. Preliminary identification of cell types has been made using light and electron microscopy, uptake of ^{3}H-γ-aminobutyric acid (GABA) in the presence of the differential inhibitors β-alanine and 2,4-diaminobutyric acid, and synthesis of GABA from its precursor, glutamic acid. In vivo, granule cells degenerate in the neurological mutants staggerer (sg) and weaver (wv). In vitro, cells I have identified as granule cells seem to have a greater capacity for survival under these culture conditions, although there are some morphological differences between the mutant cells and their normal counterparts in culture. Staggerer cells clump less initially, while those from weaver exhibit a more complex pattern of fiber outgrowth from otherwise normal-looking clumps. These experiments are consistent with the hypothesis that the primary pathology for these two mutants lies in cells other than the granule cells which degenerate (Purkinje cells for sg and Bergmann glia for wv). They further suggest that there are some previously unrecognized changes which take place either in the granule cells themselves or in yet a third cell type within the cerebellum of the mutant mouse, and offer a new system in which to explore these changes.

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1246 DEPRESSION BY MAGNESIUM ION OF NEURONAL EXCITABILITY IN TISSUE CULTURES OF CEREBELLUM. Kenneth C. Marshall, J. Martin Wojtowicz* and Walter J. Hendelman. Depts. of Physiol. and Anat. Faculty of Medicine, Univ. of Ottawa, Canada K1N 9A9.

The divalent cations calcium (Ca^{++}) and magnesium (Mg^{++}) synergistically depress excitability of nerve membranes, but have antagonistic effects on neurotransmitter release. Low concentrations of Mg^{++} have been used to selectively depress transmitter release. We have studied the effects of such low concentrations (e.g. 10 mM) of Mg^{++} on the excitability of neurones in tissue cultures of mouse cerebellum. The cultures were continuously perfused by a bicarbonate buffered balanced salt solution (BSS), which was substituted during tests by BSS to which $MgCl_2$ had been added. Evoked spikes of single neurones were recorded extracellularly in one part of the culture after electrical stimulation of a different region. Depression of evoked spikes was often observed, but in order to distinguish changes in membrane excitability from synaptic effects, antidromically activated spike potentials were sought. These were identified by constant latency, following at high frequencies of stimulation, and where possible, collision of evoked with spontaneously occurring spikes. In most cases, antidromic activation was reversibly blocked by 10 mM Mg^{++}; in a few, the threshold was increased by 50-100%; and occasionally no change in threshold was seen. We conclude that even low concentrations of Mg^{++} cannot be used as a test for synaptic activity unless careful controls of neuronal excitability are also performed.

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1247 LOCALIZATION OF SYNAPSES IN NERVE-MUSCLE CULTURES. William J. Betz and Molly Osborne*. Dept. of Physiology, Univ. of Colo. Sch. Med., Denver, CO 80220.

Chick embryo ciliary ganglia were cultured with dissociated chick embryo pectoral muscle. Nerve axons grew from the ganglia, contacted muscle fibers, and formed nicotinic cholinergic synapses. Synaptic sites were mapped by blocking nerve-evoked synaptic potentials with d-tubocurarine (dTC), which was applied iontophoretically to discreet sites of nerve-muscle contact. The optimum spatial resolution possible with this technique was about 10 microns. Synapses did not appear to form along the entire length of contact between nerve processes and muscle, but rather were restricted to small, discreet sites 10 microns or less in length. After a synapse was located, the sensitivity of the muscle to iontophoretically-applied acetylcholine (ACh) was mapped, and was found to be 5-20 times higher at synaptic sites than in the surrounding vicinity. Such regions of elevated ACh sensitivity ("hot spots") were found scattered along the length of both noninnervated and innervated muscle fibers. All synapses localized by dTC iontophoresis were found at "hot spots." However, not every "hot spot" on muscle fibers receiving synaptic input was innervated. (Supported by NIH Grant NS-10207).

1248 SYNAPSES ON PURKINJE SOMATIC SPINES IN ORGANOTYPIC CULTURES.
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Golgi studies of the development and growth of Purkinje neurons in organotypic cultures of mouse cerebellum have revealed that the Purkinje cell somatic spines develop in culture as in the intact animal (Aggerwal, Anat. Rec., 1974, 178: 296). Initially, electron microscopic examination demonstrated that a few Purkinje cell somatic spines remain unwed, without a presynaptic component, in culture (Aggerwal and Hendelman, Anat. Rec., 1975, 181: 298). On further electron microscopic examination of these cultures, particularly those of younger age, it was found that many of the Purkinje cell somatic spines were contacted by a large terminal. The terminal is packed with large round vesicles, aggregated near the presynaptic membrane. The inter-synaptic cleft is wide, with a prominent sub-synaptic density. This has been classified as an asymmetrical synapse. These were found to synapse with a variable number of somatic spines (from 2 - 8). Similar terminals were found contacting the spines of the large principal dendrites. Purkinje neurons in culture retain several large dendrites which emerge from the soma. These terminals are morphologically similar to those described in the literature as climbing fibers, which likewise form multiple synapse with somatic spines (during development) and dendritic spines (in the adult). In culture, these fibers could originate from neurons of the deep cerebellar nuclei. Alternatively, the morphology of some parallel fiber terminals could be altered under experimental conditions, such as tissue culture, and these might resemble climbing fibers.

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1249 ELECTROPHYSIOLOGY OF MOUSE NERVE/MUSCLE CELL CULTURES.
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Intracellular microelectrode recordings show spontaneously occurring excitatory postsynaptic potentials (EPSPs) in approximately 20% (116/538) of myotubes in combined muscle and spinal cord cell cultures (2-7 weeks after cells are dissociated from fetal mice). Synaptic incidence in individual cultures in this series ranged from 1/25 to 15/18 myotubes sampled. Regional areas of innervation occur both in the routinely used high density neuronal plates (initially 10^6 cells added to muscle) and in cultures with fewer neurons (initially 0.25×10^6 cells). Such areas are often selected for electrophysiology on the basis of contracting myotubes (frequency about 10/sec). Spontaneous and evoked EPSP amplitudes range from 0.3 to 15 mv. EPSPs and some spontaneous contractions are blocked by d-tubocurarine (10^{-6} gm/ml). The capability for innervation of more than one myotube by the same neuron is suggested by the recording of synchronously occurring EPSPs and action potentials from 7 non-electrically coupled myotube pairs; an additional myotube pair showed electrical coupling. Presynaptic neurons are rarely found within the same microscopic field of innervated myotubes which usually are in areas of nerve cells with well developed processes. On-line real time histogram analysis of spontaneously occurring EPSPs, utilizing a computer routine derived from a program by P. G. Nelson and W. Sherif, disclosed that intervals between successive EPSPs are generally less than 300 msec and are randomly distributed. Occasional discrete, and even periodic, interval histogram patterns are observed, sometimes with a correlation between amplitude and interval classes. Such histogram patterns are stable during repeated computer trials over a recording period of up to one hour.

1250 CHOLINERGIC SYNAPSES BETWEEN SPINAL CORD AND SYMPATHETIC NEURONS IN TISSUE CULTURE. Chien-Ping Ko*, Harold Burton, and Richard Bunge. Depts. Physiol. and Anat., Sch. Med., Wash. Univ., St. Louis, Mo., 63110.

Superior cervical ganglion neurons (SCGN) were taken from newborn rats, dissociated and grown in nerve tissue culture together with explants of thoracic spinal cord taken from 15 day fetal rats. Intracellular recordings were made from the SCGN after 3-12 weeks *in vitro* with 1M Kcitrate electrodes. Excitatory postsynaptic potentials (EPSP) were recorded from the SCGN subsequent to electrically stimulating the spinal cord tissue with bipolar, extracellular electrodes filled with 4M NaCl in agar. These synaptic potentials had the characteristics of a typical EPSP and could be mimicked by iontophoretic application of acetylcholine chloride (ACh) that was randomly applied to the somal region of these cells (mean sensitivity for 14 cells was 104 mV/nC ±81.2). During the application of ACh the membrane conductance increased and the receptors demonstrated desensitization. With the aid of a continuous perfusion system both the ACh potential and the EPSP from the spinal cord were shown to be blocked by nicotinic blocking agents (mecamylamine or hexamethonium 10^{-4}M). The ACh potential was insensitive to alpha bungarotoxin (10^{-5}g/cc). Both responses were insensitive to low concentrations of atropine (10^{-6}M), but high concentrations (10^{-4}M) had a postsynaptic blocking effect. Eserine (10^{-5}g/cc) blocked both potentials whereas neostigmine (10^{-5}g/cc) blocked the EPSP but only slightly reduced the ACh potential. These results suggest that the spinal cord explants form a cholinergic nicotinic synapse *de novo* in culture on the sympathetic neurons that is functionally similar to the synapse made in the intact animal. (Supported by Grants NS 09809, NS 09923, NS 11888.)

1251 CHOLINERGIC SYNAPSES BETWEEN SYMPATHETIC NEURONS IN TISSUE CULTURE. Harold Burton, Chien-Ping Ko*, and Richard Bunge. Depts. Anat. and Physiol., Sch. Med., Wash. Univ., St. Louis, Mo., 63110.

Dissociated superior cervical ganglion neurons (SCGN) from the rat grown in tissue culture together with explants of spinal cord develop an elaborate plexus of processes that extend over adjacent SCGN. Intracellular recording and stimulation studies, which were done on over 550 pairs of SCGN, showed that 10-30% of these pairs were coupled only with excitatory postsynaptic potentials and 3-6% were reciprocally joined by electrical connections. Various arrangements of synaptic coupling were observed and included reciprocal, recurrent and multiple innervations. All synaptic potentials tested were sensitive to cholinergic-nicotinic blocking agents (mecamylamine or hexamethonium 10^{-4}M) but were insensitive to an alpha adrenergic blocking drug (phenoxybenzamine 10^{-5}M). All of these intrinsic connections developed in the presence of synapses formed by explants of spinal cord onto these SCGN and the percentage of chemically interacting pairs was not changed subsequent to surgical removal of the spinal cord. Observations of similar cholinergic interactions have also been made on cultures containing only SCGN by O'Lague *et al.*, PNAS, 71: 3602, '74. Previous morphological studies (J. Comp. Neur., 157: 1, '74) have shown that the intrinsic connections in 3 week cultures have the cytochemical characteristics of noradrenergic endings, but the present observations on similar, but older (more than 4 weeks) cultures indicate that the interactions are functionally cholinergic. Physiologically similar intrinsic connections between principal neurons of sympathetic ganglia have not been observed in intact rats. These results suggest that the culture system may induce a change in transmitter production. (Supported by Grants NS 09809, NS 09923, NS 11888.)