Society for
Neuroscience

ABSTRACTS

Volume 4

8th Annual Meeting
St. Louis, Missouri
November 5–9, 1978

Published by
Society for Neuroscience
Bethesda, Maryland

SOCIETY FOR NEUROSCIENCE ABSTRACTS

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Proper citation form for this volume:
Society for Neuroscience Abstracts, Vol. 4, p. ■■■, 1978.

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Made in the United States of America
International Standard Book Number 0-916110-08-7
Library of Congress Catalog Card Number 75-7761

AUDITION

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1 MORPHOLOGY AND PHYSIOLOGY IN THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS. JOE C. ADAMS LNO, NINCDS, NIH, BETHESDA, MD 20014

In the ventral portion of the ventral nucleus of the lateral lemniscus (VNLL) there are two broad categories of cells as seen with the light microscope, electron microscope, and electrophysiological recordings. In Protargol impregnations there is a class of cells in this region that receive large terminals similar to those found in the anterior cochlear nucleus and medial nucleus of the trapezoid body. When visualized following injections of HRP into the trapezoid body these terminals resemble end bulbs of Held seen in rapid Golgi impregnations of the cochlear nucleus. Seen with the electron microscope, the two categories of cells include those whose somata are covered with terminals and those with very few terminals on their somata. Most terminals contain large, clear, round synaptic vesicles but some contain pleomorphic vesicles. Single unit response patterns in this region are mostly "chopper" and "on" types. The waveform of some "on" type cells shows a positive prepotential preceding every unit discharge. It is likely that cells having this waveform are those with the large terminals and that these terminals originate from octopus cells in the contralateral cochlear nucleus. Further, it seems likely that cells that respond with "chopper" patterns are those with few if any terminals on their somata. These findings suggest that mechanisms underlying stimulus coding in the VNLL are similar to those in the cochlear nucleus.

2 PARALLEL AUDITORY PATHWAYS FROM MIDBRAIN TO THALAMUS IN THE RAT. K. D. Ahmann* and G. C. Thompson (SPON: H. E. Heffner). Dept. of Psychology, Duke University, Durham, N. C. 27706.

Numerous parallel neural pathways exist in the central auditory system of mammals. In the hindbrain alone, the cochlear nucleus projects to the inferior colliculus 1)directly, 2)via the ipsilateral superior olivary complex ⟶ contralateral inferior colliculus pathway, 3)via the contralateral medial nucleus of the trapezoid body ⟶ superior olivary complex ⟶ inferior colliculus pathway, 4)via the contralateral ventral nucleus of the lateral lemniscus ⟶ inferior colliculus pathway, 5)via the contralateral dorsal nucleus of the lateral lemniscus ⟶ inferior colliculus pathway. It would therefore be surprising if the continuation of auditory pathways beyond the inferior colliculus was limited solely to the well-known classical route through the ventral division of the medial geniculate. Utilizing retrograde transport techniques, we have demonstrated that parallel auditory pathways do also exist from midbrain to thalamus.

Horseradish peroxidase (HRP) dissolved in dimethyl sulfoxide (DMSO) in tris buffer was electrophoretically injected into the medial geniculate of white rats. Each animal was sacrificed at 24 hrs. and the tissue ultimately processed with benzidine dihydrochloride (BDHC) or tetramethyl benzidine (TMB). Retrogradely labeled cells in the subdivisions of the inferior colliculus were counted and the percentage of total labeled cells was calculated for the pericentral nucleus, the dorsomedial division of the central nucleus, and the ventrolateral division of the central nucleus.

Of the cells labeled after injection of HRP into the caudal third of medial geniculate, 79% were localized in the pericentral and dorsomedial divisions, while only 21% were localized in the ventrolateral division. Of the cells labeled after injection into the rostral third of medial geniculate, only 23% were localized in the pericentral and dorsomedial divisions, while 77% were localized in the ventrolateral divisions.

These results demonstrate two parallel pathways from the inferior colliculus to medial geniculate, one originating in the pericentral and dorsomedial divisions, and a second originating in the ventrolateral division. A more complete interpretation of the injection sites described above suggests that the pathway originating in the pericentral and dorsomedial divisions terminates primarily in the caudal tip of medial geniculate while the pathway originating in the ventrolateral division terminates primarily in the ventral division of medial geniculate.

(Supported by NIH postdoctoral fellowship NS-05584.)

3 RESPONSES OF MEDIAL GENICULATE NEURONS TO VOCALIZATIONS IN SQUIRREL MONKEY. Garrett E. Alexander, David Symmes and John D. Newman
Laboratory of Developmental Neurobiology, NICHD, Bethesda, Maryland 20014.

The responses of 269 medial geniculate (MG) neurons in unanesthetized squirrel monkeys to tape recorded species-specific vocalizations is described. We have examined in considerable detail the degree to which the vocalization response is predictable from or follows similar dynamic constraints as the response to clicks, tone bursts, and noise. Analysis of aural dominance, response timing, response direction, and rate-level functions suggest that in the MG nucleus vocal and artificial stimuli are processed by similar neural mechanisms. Frequency tuning curves of neurons obtained from tone burst stimulation are less relevant to vocal responses, but still contribute to predictability to some extent. These conclusions are illustrated by a number of examples from our sample, and represent an initial attempt at parametric comparison of neuronal responses to artificial and species-specific auditory stimuli.

4 AUDITORY CORTICAL FIELDS OF CAT: DIRECT DEMONSTRATION OF RECIPROCITY BETWEEN FIELDS; BANDED CORTICO-CORTICAL CONNECTIVITY; SIMILARITY OF PROJECTION ONTO STRIATUM. Richard A. Andersen
Coleman Lab., Univ. of Calif., San Francisco, Ca. 94143.

Partial maps of one or more auditory fields were made using microelectrode recording techniques. Microinjections of orthograde (tritiated leucine or proline) and anterograde (HRP) tracers were made at physiologically defined loci. In some experiments, both tracers were introduced at the same loci.

Restricted AI injections produced, in the frontal plane, a radial, banded pattern of alternating heavy and light labeling for both tracers in the contralateral AI. The banded patterns of the two tracers appear to be directly superimposeable on one another in the tangential plane, suggesting the existence of reciprocal connections between the binaural response-specific bands within the two fields.

Multiple injections along a single "isofrequency lamina" in AI produced one reciprocal, banded focus of label in the contralateral field. Two single injections, one at a high best frequency and one at a low best frequency position, produced one rostral and one caudal focus of banded label in the contralateral AI. This topography of projection is consistent with a homotypic connectivity of the cochlear representation of the two AI fields. AI also projects in the form of a banded reciprocal array to at least the ipsilateral anterior auditory field (AAF).

Preliminary results indicate that AII projects to at least four different ipsilateral cortical regions as well as to the contralateral AII. All of the projections are reciprocal. Three of the ipsilateral projections are in the form of bands.

These experiments indicate that reciprocal connectivity and the vertical organization of connections within bands are important rules of structure for the auditory cortex.

AI, AII and AAF all project onto the striatum in a remarkably similar pattern. With single injections in any of these fields, there is a single restricted lamina of terminals in the caudal putamen that extends across the dorsal to ventral dimension of the nucleus and occupies an intermediate position along the mediolateral axis. There is a very light and diffuse termination in the tail of the caudate nucleus. Thus, these three fields appear to converge on the same regions of the striatum.

Supported by NIH Grant NS-10414.

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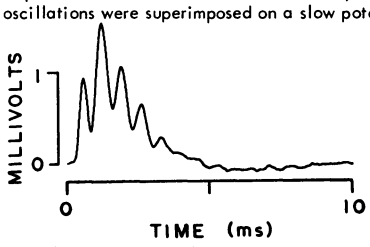
5 DEPENDENCE OF INTRACELLULAR RESPONSES ON SOUND STIMULUS PARAMETERS IN HAIR CELLS AND SUPPORTING CELLS OF THE ALLIGATOR LIZARD COCHLEA. Keld Baden-Kristensen* and Thomas F. Weiss. Eaton-Peabody Laboratory of Auditory Physiology, Massachusetts Eye and Ear Infirmary, Boston, MA 02114.

Electric responses to sound were recorded with micropipets from hair cells and supporting cells which were distinguished by electrophysiological criteria (Weiss et al., J. Acoust. Soc. Am. 55: 606, 1974). All cells with click response magnitudes exceeding 2mV peak to peak (and up to 13mV) were inferred to be hair cells. Stable resting potentials and stable response magnitudes were obtained for intervals as long as ½ hour for hair cells and 3 hours for supporting cells.

Click responses of hair cells were independent of repetition rate from 10 to 150 clicks/sec. At low click levels, response waveforms were oscillatory and symmetric about the baseline, and response amplitude increased linearly with increasing click level. At higher click levels, response waveforms became less oscillatory and more asymmetric, i.e. oscillations were superimposed on a slow potential (see figure), and the response amplitude saturated as level increased. The slow potential generally had a positive polarity. However, immediately after cell penetration, some cells were temporarily depolarized, and the slow potential could be temporarily negative without a concomitant change in the polarity of the oscillatory component. Thus the two components exhibit a degree of independence. In a few cases, the fundamental component of the response to tones was measured as a function of frequency for constant sound-pressure level at the tympanic membrane. These frequency-response curves showed sharp tuning at low levels and broader tuning at higher levels. At low stimulus levels, the best frequency of the frequency response was equal to the frequency of the oscillations in the click response.



Responses of supporting cells had a click-rate insensitive component, presumed to be of hair cell origin and a longer latency, rate-sensitive component, presumed to be of postsynaptic (neural) origin. Frequency responses were relatively broadly tuned, often exhibiting two best frequencies.

6 RESPONSE CHARACTERISTICS OF SUPERIOR OLIVARY COMPLEX NEURONS IN THE DECEREBRATE CAT. William E. Brownell, Paul B. Manis*, Louis A. Ritz, and James W. Fleshman*. Dept's. of Neuroscience and Surgery (ENT), University of Florida, Gainesville, Fla., 32610.

Anesthetics have been demonstrated to alter single unit activity in auditory nuclei peripheral and central to the superior olivary complex (SOC). Previous single-unit investigations of the SOC have been carried out in anesthetized animals. This study examines the discharge characteristics of SOC neurons in unanesthetized, decerebrate cats. Features such as tonotopic organization, shapes of excitatory tuning curves and the shapes of interspike interval histograms resemble those previously reported. However, most units display more spontaneous activity than has been reported in studies of the SOC of barbiturate anesthetized animals. This difference is particularly pronounced in the lateral superior olive (LSO) which is usually described as having little or no spontaneous activity. The mean spontaneous rate for LSO units in our sample is six spikes/sec (range 0 - 27). The mean activity for units anatomically localized to the medial nucleus of the trapezoid body is 18 spikes/sec (range 0 - 74), while units in the medial superior olive have a mean spontaneous activity of about six spikes/sec (range 0 - 11). All ipsilaterally excited and contralaterally inhibited cells (regardless of anatomical localization) have displayed some spontaneous activity (1.3 - 14 spikes/sec). The presence of spontaneous activity has permitted the demonstration of inhibitory sidebands for some LSO neurons. The significance of these observations for binaural interaction in the SOC will be discussed. (Supported by USPHS grant 1 R01 NS12209).

7 CYTOARCHITECTURE OF THE DORSAL COCHLEAR NUCLEUS IN THE TWO YEAR OLD MOUSE C57BL/6. Robert H. Browner and Alice M. Baruch, Department of Anatomy, New York Medical College, Valhalla, NY 10595.

The cytoarchitecture of the dorsal cochlear nucleus (DCN) was analyzed in 10 animals. Brains were stained in Cresyl Violet or impregnated in the Golgi-Kopsch technique (120-160 um). The DCN at its most medial aspect is tucked caudal to the inferior cerebellar peduncle. It sweeps rostrolateral to overlie the posterior ventral cochlear nucleus (PVCN) to the entrance of the eighth nerve. The rostrocaudal extent of DCN is 831 um, while dorsoventrally it is 505 um and 1380 um thick.

The DCN is divided into three regions: superficial molecular, fusiform and granular cell layers. The molecular layer is 84 um thick and contains few ovoid cells. The fusiform layer is at the interface of the molecular and granular layers. The granular layer is the central region containing (small) ovoid and (larger) multipolar cells. The multipolar cells tend to concentrate in the dorsal and ventral portions of this layer.

The long axes of the fusiform cells (30 x 9 um) are perpendicular to the cross-sectioned surface of the DCN, except dorsally where they are parallel to the surface. These cells are not uniformly distributed in the dorsoventral axis but accumulate intermittently. They have eccentric nuclei and homogeneous Nissl substance. There are two stout dendritic trunks from both ends, with few dendritic spines and 2^{o} branches. The dendritic field is at least 228 um dorsoventral by 101 um mediolateral. The Nissl substance of the multipolar cells (16 um) is densely stained and evenly distributed and a central nucleus is characteristic. Three to five main dendritic trunks radiate in all directions. The dendrites have varicosities, elongate spines and do not enter the center of DCN, but run parallel to the surface. A dendritic field is 236 um dorsoventral by 230 um mediolateral. The ovoid cells (9 um) have a large nucleus surrounded by a thin rim of cytoplasm. This is the most numerous cell type. It has two dendritic trunks, 2^{o} branches and spines on distal segments. The dendrites enter or run parallel to the molecular layer. The field is 100 um mediolateral, 83 um dorsoventral and 164 um rostrocaudal. The cells appear to be organized in columns from deep regions to the periphery. (Grant #'s - 1-78-61N-A-PS002r; 41-979-1).

8 PROJECTIONS OF THE COCHLEAR NUCLEUS AND SUPERIOR OLIVARY COMPLEX TO THE INFERIOR COLLICULUS OF THE RAT. J. Timothy Cannon* and Glenn J. Giesler, Jr. (SPON: J.C. Liebeskind). Dept. Psych., UCLA, Los Angeles, CA 90024.

Golgi preparations, retrograde and anterograde degeneration procedures and other classic anatomical techniques have provided valuable information pertaining to the sources of afferents from lower brainstem auditory relays to the inferior colliculus (IC) of the rat. With the advent of the powerful retrograde cell marker horseradish peroxidase (HRP), however, it has become possible to re-examine these connections with the intent of better defining their cells of origin in terms of location and cellular morphology. For that purpose, pressure or iontophoretic injections of HRP were made into the IC and the surrounding mesencephalon of the rat. After 17 to 48 hours the animals were sacrificed and their brains processed for the presence of HRP using either o-dianisidine or tetramethyl benzidine. Unilateral IC injections of HRP labeled approximately equal numbers of cells bilaterally within the lateral superior olivary n. In contrast, cells of the medial superior olivary n. and superior paraolivary n. were primarily labeled ipsilaterally. HRP-positive cells were only rarely encountered in the medial n. of the trapezoid body. IC injections produced large concentrations of HRP-positive cells throughout the dorsal and ventral subdivisions of the contralateral cochlear n. (CN) with only sparse labeling ipsilaterally. The occurrence of dense reaction products in many instances allowed the identification of the pyramidal cells of the dorsal and most cell types of the ventral CN as contributors of afferents to the IC (Figure). IC injections of HRP also produced labeling within the cells of the contralateral acoustic nerve n.

Supported by NS 05702 and NS 07628.

9 EFFECTS OF PUTATIVE AMINO ACID NEUROTRANSMITTERS ON RESPONSE PATTERNS OF NEURONS IN THE COCHLEAR NUCLEI. D.M. Caspary and D.C. Havey*. Division of Neurobiology, Department of Medical Sciences, Southern Illinois University School of Medicine, Springfield, IL 62708.

GABA, glycine, and L-aspartate have been proposed as agents which may govern transmission in the cochlear nuclei (CN) (Godfrey et al., J. Histochem. Cytochem. 25, 1977; Wenthold and Gulley, Brain Res. 138, 1977). The present study examined changes in auditory evoked and spontaneous single unit activity in chinchilla CN induced by iontophoretically-applied amino acids. In all cases, poststimulus time histograms (PSTH's) and interspike interval histograms (ISIH's), at best-frequency, were obtained before, during, and subsequent to the iontophoretic application of the putative neurotransmitter or antagonist being tested. Histological reconstruction of electrode tracts was carried out on all preparations, and patterns of PSTH's and ISIH's were used to aid in the localization of the recordings. As previously reported (Caspary and Havey, Neurosci. Abs. 5, 1977), the application of glycine onto dorsal cochlear nucleus (DCN) neurons displaying a pauser or build-up type pattern resulted in a profound inhibition of the response with a selective effect on the early portion of the response. The application of GABA also resulted in inhibition of activity in DCN neurons with many of these neurons sensitive to both GABA and glycine. The relative effectiveness of the two substances was evaluated by comparing the degree of inhibition resulting from the application of these agents onto the same neuron from different barrels of the same electrode. Results in DCN neurons indicate that glycine inhibition occurred at lower dose levels than those for GABA. In ventral cochlear nucleus (VCN), glycine and GABA significantly reduced the level of spontaneous activity to a greater extent than that of driven activity. Thus, complete inhibition of spontaneous activity and near-threshold driven activity was observed with many VCN neurons with the application of relatively small amounts (0-50 nA) of GABA and glycine. However, few neurons could be totally inhibited when driven at moderate intensities (20-40 dB above best-frequency threshold) without the use of larger amounts (50-150 nA). All CN neurons inhibited by GABA and glycine required increased amounts of these substances to achieve similar levels of inhibition at higher stimulus intensities. Glycine effects in both DCN and VCN could frequently be reversed by concurrent application of strychnine. Application of the putative afferent neurotransmitter L-aspartate onto VCN neurons often resulted in excitation, primarily of spontaneous activity. These data support the hypothesis that glycine, GABA and aspartate may be neurotransmitters of the CN. (Supported in part by SIUMS Research Fund)

10 DEVELOPMENT OF THE EMBRYONIC CHICK'S STATOACOUSTIC GANGLION. G. M. Cohen and C. D. Fermin*. Department of Biological Sciences, Florida Institute of Technology, Melbourne, Florida 32901.

In embryonic White Leghorn chicks, myelination of the statoacoustic neurons first begins on the neurites and then advances proximally to the perikarya (somata). By the 6th day, when this study began, processes from several adjacent Schwann cells have formed loose boundaries around large groups of neurites; at this age the naked bipolar neurons are packed closely together and are not readily distinguishable from Schwann cells except by relative position or by tracing the neurites back to their perikaryal origins. By the 8th day, the extraneous coats covering the external surfaces of Schwann cells are more distinct than their counterparts covering the inner surfaces closest to the neurites. The Schwann cells clutch the neurites more tightly as they extend processes between groups of neurites to divide them into successively smaller bundles. By the 11th day, the neurites, now termed dendrites and which have penetrated the basilar membrane earlier, innervate the hair cells. By the 13th day, the first compact myelin sheaths consisting of 2-6 layers surround some dendrites and within 2 days compact myelin begins to appear regularly; by the 21st day (hatching) 12-18 layers surround many dendrites, though the rates of myelination vary considerably among dendrites in adjacent bundles. By comparison, perikaryal compact myelin first appears by the 18th day; the larger perikaryal girths (about 15-30x) may account for the lag. Perikaryonal myelination requires the cooperation of several satellite cells and the myelin regularly alternates from compact to loose around each perikaryon during this period. By the 14th day after hatching, compactly myelinated perikarya, which are usually as thickly layered as the dendritic, greatly outnumber the loosely myelinated. Each bipolar neuron is filled with a large, eccentrically positioned nucleus that contains prominent nucleolar complexes. The nucleus, though continuing to enlarge during development, occupies a progressively smaller proportion of perikaryonal volume. Until the 14th day of incubation, perikaryonal cytoplasm is relatively pale centrally but is denser peripherally because of abundant free ribosomes, rough endoplasmic reticula, and numerous small mitochondria. Thereafter, orgenelles become more evenly distributed except that the perinuclear cytoplasm remains denser. To date only one type of bipolar neuron, the granular, has been identified. Once Schwann cells have committed themselves to dendritic segments, collagen fibrils ply between adjacent fibers. Numerous capillaries of different sizes thread through these groups of bipolar neurons, frequently contacting satellite and Schwann cells. (Supported in part by NIH Grant No. 1-508-RR09032-01).

11 ORGANIZATION OF THALAMIC PROJECTIONS TO AUDITORY CORTEX IN RAT. J. Coleman, W.E. Wells*, B. Crumpler*, D. Marsh* and W.J. Clerici*. Depts. Psychol. and Physiol., Univ. of South Carolina, Columbia, SC 29208.

A fundamental understanding of the nature of auditory thalamocortical pathways exists for certain mammalian species. Although similarities appear in organization of the auditory thalamus, organization of auditory areas of cortex seems to vary in monkey, cat and squirrel. Because of the limited number of species chosen for investigation, the study of other mammals may further reveal a particular pattern of generalized thalamo-cortical organization of the auditory pathways. In the present investigation retrograde transport of horseradish peroxidase (HRP) was used to study thalamic projections to auditory cortex of the albino rat. Micro-iontophoretic HRP injections made into auditory cortex were followed by 24 hour survival and HRP histochemical processing.

The primary auditory cortex (AI) of rat was identified by Nissl and myelin stains and found to be located within the boundaries of Krieg's area 41. HRP injections of AI resulted in a restricted band of labelled cells confined to the ventral division of the medial geniculate nucleus (GMv). Injections of various portions of AI revealed a topographical projection from GMv to AI. In addition to the core projection from GMv other subdivisions of the auditory thalamus project to at least three auditory belt areas. HRP injections of cortex immediately caudal to AI resulted in labelled cells in the dorsal division of GM and in Po. Restricted injections in a cortical belt located lateral and caudal to AI produced labelled cells concentrated in the caudal division of GM; labelled cells in a thalamic region below GMv also appeared. An additional belt area rostral to AI extending medially and laterally was identified. Injections of this area resulted in thalamic label chiefly located in the magnocellular division of GM and in Po with more sparse labelling of the dorsal division of GM. Cases involving cortex medial to this area resulted in labelling of the lateral posterior nucleus, while injection into more rostral cortex labelled cells of Po and CIN. This study demonstrates the presence of several subdivisions of auditory cortex which receive overlapping or individual projections from different ascending thalamic pathways. Organization of auditory thalamo-cortical pathways in rat appear to fit a basic mammalian plan of restricted projections (of GMv) to auditory core cortex, and belt areas receiving input from other thalamic divisions.

12 RESPONSE LATENCY OF VERTEBRATE HAIR CELLS: KINETICS OF AN IN VITRO MICROPHONIC. D. P. Corey* and A. J. Hudspeth* (SPON: M. Konishi). Div. Biol., Cal. Inst. Technol., Pasadena, CA 91125.

The delay with which a sensory receptor responds to a stimulus provides information about the steps involved in transduction of the stimulus into an electrical signal. The latency of response of hair cells, the primary receptor cells of the vertebrate acousticо-lateralis system, is not precisely known, in part because stimuli reach the cells through complex mechanical linkages with unknown delays, and in part because analysis of the extracellular receptor potential (the microphonic) is confused by complex current paths in the organs. We have investigated the response latency of hair cells with an in vitro system which largely circumvents these difficulties.

The sensory epithelium of the bullfrog sacculus, containing some 3000 hair cells, was removed and mounted as a partition separating two chambers both ionically and electrically. The potential difference across the epithelium was measured with an electrode in each chamber; a second pair was used to pass current to determine the passive electrical properties of the epithelium. The in vitro microphonic potential recorded in response to stimulation was the product of the net transduction current across the epithelium and the epithelial impedance.

Hair cells were stimulated en masse by moving the overlying otolithic membrane, into which the hair bundles insert, with a piezoelectrically driven stimulus probe. By monitoring with a photodiode the projected image of either the probe or the membrane, we could measure within 5 μs and 0.01 μm the stimulus delivered to the hair cells.

A fast pulse stimulus (200 μs duration, 0.4 μm amplitude) evokes a response with a rapid (∿100 μs) rising phase and an exponential decay. Because the time constant of the decay (350 μs) exactly matches the RC time constant of the epithelium, we believe the response waveform is largely determined by the passive electrical properties of the epithelium. After correction for the epithelial capacitance with an active filter (transfer function: $V_o = V_i + \tau dV_i/dt$), the response waveform closely mimics that of the stimulus, with slight broadening and a distinct delay of 40-50 μs. This short latency, and its modest sensitivity to temperature (Q_{10} = ∿2.5), suggest that there is not a complex series of processes intervening between the receipt of a mechanical stimulus and the resulting membrane conductance change. The short latency excludes, for instance, intermediate messengers diffusing over distances of greater than about 0.2 μm.

13 AUDITORY CORTEX LESIONS AND BRIEF TONE AUDIOMETRY IN CATS: EVIDENCE FOR DISASSOCIATION BETWEEN DETECTION AND DISCRIMINATION ABILITIES. Jerry Cranford, Dept. Otorhinolaryngology, Baylor College of Medicine, Houston, Texas, 77030.

In recent years, a number of investigators beginning with Gershuni, Baru, and Karaseva (1967) have provided evidence that the auditory cortex has a critical role in both the detection and discrimination of brief sounds. Dogs and humans with lesions of the neocortical auditory centers have been reported to exhibit significantly elevated detection thresholds for signals which are shorter than 16 msec. in duration. In tests of frequency discrimination, the same subjects also exhibit severe deficits whenever the tonal signals are shortened to less than 20-40 msec. in length.

In contrast to the Russian findings, we recently obtained evidence in our laboratory that cats with large bilateral auditory cortex lesions are unimpaired in their ability to detect 1- and 16-KHz tone pulses of 16 msec. duration or less (Cranford & Igarashi, 1977). In more recent unpublished experiments, we have been further investigating the auditory decorticate cats' ability to discriminate changes in the frequency of brief 1-KHz tone pulses. In support of the Russian findings, these new experiments have revealed that operated cats, while exhibiting normal difference limens for tones of 100 msec. duration, have significantly elevated thresholds for discriminating tones of 8 and 2 msec. duration. Of more significance is the finding that, with further testing, the same operated cats which exhibit elevated discrimination thresholds for brief tones can be shown to have normal detection thresholds. Thus, it appears that the presence of intact auditory cortex, rather than being essential for detecting the occurrence of brief sounds, may be more important for recognizing the nature of such sounds.

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Gershuni, G. V., et al. Neurosci. Trans. 17 (1967) 370-382

Research supported by a grant from The Deafness Research Foundation and NINCDS grants NS 11812 and NS 10940.

14 PHASE-LOCKING IN GOLDFISH 8th NERVE FIBERS: RELATION TO FREQUENCY DISCRIMINATION CAPACITIES. Richard R. Fay* (SPON: John Trimble). Loyola Univ. of Chicago, Chicago, Il. 60626.

The otolithic ears of fishes appear to be incapable of the degree of mechanical frequency analysis performed by the mammalian cochlea. Yet, behavioral frequency discrimination thresholds (Fay, JCPP, 73: 175, 1970) fall within the range of mammalian variation below 1000 Hz. This suggests that information about sound frequency is coded in the temporal structure of neural activity rather than in the across-fiber distribution of discharge rates. This hypothesis is evaluated for the goldfish by comparing the accuracy of phase-locking in single saccular neurons with the accuracy with which behavioral discriminations are made (the just-noticable-difference for stimulus period, ΔP).

Phase-locking accuracy was measured in over 60 saccular neurons as the standard deviations (sd) of period histograms obtained for tonal stimuli at the frequencies and amplitudes used in the behavioral study. For frequencies between 70 and 1000 Hz, log sd decreased approximately linearly with log frequency, with a mean period histogram sd of about 100 microsec at 1000 Hz. The behavioral ΔP values fall within the range of the smallest neural sd values obtained at each frequency.

A simple temporal coding hypothesis holds that stimulus period lengths are estimated by the measurement of the temporal intervals between nerve impulses. The discrimination problem may be viewed as a decision as to whether two samples of neural intervals are drawn from the same, or two different underlying distributions. Assuming the means of these distributions to be equal to the periods of the signals to be discriminated, and that the distributions' variance is completely determinec by the phase-locking variance, then one would expect threshold-like behavior to occur when the difference between the means (ΔP) is approximately equal to the distributions' sd; that is, when $d' = \Delta P/sd = 1$. The results show that this is indeed the case for the smallest sd values at each of the frequencies studied. Thus, peripheral phase-locking variance accounts well for the errors made in behavioral frequency discrimination, under the assumption that the decisions are based upon the small percentage of neurons transmitting the best information.

15 DESCENDING PROJECTIONS FROM AUDITORY CORTEX TO THE THALAMUS AND TECTUM OF GALAGO SENEGALENSIS. D. Fitzpatrick*, I. T. Diamond and D. Raczkowski*. Dept. of Psychol., Duke Univ., Durham, N. C. 27706.

We have investigated the descending projections from the auditory cortex of the lesser Galago by examining the anterograde transport of tritiated amino acids from the various subdivisions of the cortex to the thalamus and midbrain and by identifying labeled cells in the cortex following electrophoretic injections of HRP in the auditory thalamus and midbrain. The two methods complement each other so that it is possible to draw conclusions which are not revealed by one method alone.

Regarding the descending pathways from cortex to thalamus, each cortical subdivision has a unique pattern of projections and at the same time all cortical subdivisions share a common target. Concerning the different patterns, auditory koniocortex (Ak) projects in a topographic manner upon the ventral division of the medial geniculate body (GMv). The cortical regions surrounding Ak project to subdivisions other than GMv. The cortical area lateral to Ak projects heavily onto the dorsal division and to a lesser extent to Po. The cortex situated on the lateral bank of the sylvian fissure, medial to Ak, projects to a region of small cells situated rostral to the magnocellular division and medial to GMv. The cortex ventral to Ak projects to the caudal part of the medial geniculate body. The cortical area occupying the most dorsal extent of the temporal lobe has, as its main target, the suprageniculate nucleus. In addition to these distinctive patterns of projection, all subdivisions of the auditory cortex project diffusely to the magnocellular division of the medial geniculate body. The results from our HRP experiments suggest that the cortical projection to the magnocellular division may arise from layer V neurons. In contrast, the corticofugal pathways to the remainder of the auditory thalamus originate in layer VI.

Regarding the descending pathways from cortex to the midbrain a distinctive difference between the projections of Ak and the surrounding belt regions emerges. Auditory koniocortex projects to the central nucleus of the inferior colliculus and the terminations are distributed in a laminar fashion. On the other hand, the descending projections from the belt regions terminate in the pericentral nucleus of the inferior colliculus and in the deep layers of the superior colliculus. All of these corticotectal pathways originate from layer V of auditory cortex.

In summary, we have demonstrated that Ak and the surrounding belt regions stand in contrast to each other with regard to their pattern of descending projections to the thalamus and midbrain and that each cortical region is reciprocally related with its source of ascending afferent input. (Supported by NIMH grant MH-4849 and NIMH fellowship MH05964.)

16 CAT COCHLEAR NERVE FIBER SPIKE DISCHARGES: RECOVERY CHARACTERISTICS AS A FUNCTION OF INTER-SPIKE TIME. Roger P. Gaumond*, Charles E. Molnar*, and Duck O. Kim. Dept. Physiol. and Biophysics, Sch. of Med., Washington U., St. Louis MO 63110.

We have investigated the recovery of spike discharge probability of cat cochlear nerve fibers as a function of interspike time τ by collecting histograms of the intervals between spikes with and without single tone stimulation. Estimates of $\phi(\tau)$, the hazard function (Cox, Renewal Theory, J. Wiley, 1962), were formed by dividing the number of intervals of length τ by the number of intervals of length greater than or equal to τ. In most fibers, $\phi(\tau)$ for spontaneous discharges increases rapidly between τ of 1 and 4 msec, and continues to increase more slowly from 4 to 40 msec. Observed differences among fibers include a tendency of some fibers to show an "early peak" in $\phi(\tau)$ at .75 to 1.0 msec, while others never responded until at least 2.0 msec. These observations are generally consistent with Gray (Biophys. J. 7,759,1967).

Hazard functions were estimated for spike discharge responses to high frequency (>5 kHz) acoustic stimulation of sufficient intensity to cause at least a doubling of discharge rate over spontaneous rate. For discharges in response to such tones, $\phi(\tau)$ was nearly equal to $\phi(\tau)$ for spontaneous discharges multiplied by a scale factor. A Markov chain model has been developed which is consistent with this observation. An extended version of the model predicts cochlear nerve fiber discharge patterns in response to single low frequency (<5 kHz) tones. These predictions will be compared with experimental results.

17 TIME CONSTANTS OF AUDITORY ADAPTATION - ELECTRICAL STIMULATION VS ACOUSTIC STIMULATION. Chi-ming Huang.* (SPON: L. E. White). Dept. of Physiology, College of Medicine, Univ. South Alabama, Mobile, AL 36688.

Amplitudes of auditory evoked potentials change readily with repetition rate and duration of acoustic stimulation. A quantitative model was proposed to explain such changes. Electrical stimulation of the acoustic nerve was compared with acoustic stimulation in order to separate the effect due to refractory periods of auditory periphery and central elements. The model included a decrementing process due to the stimulus and a spontaneous recovery process; both having exponential time course. Results indicated that during acoustic stimulation the time constants were 10-15 and 100-120 msec for the decrementing and recovery processes, respectively. Interactions of these time constants determine the amount of response decrement in the steady state. The response decrement may be up to 90% as the stimulus rate was varied from 1 to 200/sec and the stimulus duration was varied from 1 to 1000 msec, taking care that temporal overlap between stimuli did not occur. During electrical stimulation of the acoustic nerve, the time constant for spontaneous recovery shortened and the amount of response decrement was smaller relative to the results from acoustic stimulation. It was concluded that mechanisms at the hair-cell and acoustic nerve junction may be chiefly responsible for the observed results.

18 IN VITRO ELECTROPHYSIOLOGICAL ANALYSIS OF N. MAGNOCELLULARIS AND N. LAMINARIS OF THE CHICKEN. Hunter Jackson, John T. Hackett, and Edwin W Rubel. Depts. of Physiology and Otolaryngology, Univ. of Virginia, Charlottesville, VA. 22901.

We have recently developed an in vitro preparation of the chicken brain stem, including first, second, and third order elements of the auditory system; these are the auditory (8th) nerve, n. magnocellularis (NM), and n. laminaris (NL), respectively. The system is stimulated by means of bipolar surface electrodes positioned on the 8th nerve stumps. Using micropipettes filled with potassium citrate or horseradish peroxidase (HRP) solution, we have recorded field and single cell potentials in NM and NL. The findings summarized below were obtained from late embryos, aged 17-19 days.

Field potentials recorded from NM and NL in response to 8th nerve stimulation are characterized by three distinct negativities. The first negativity (N1) is recorded from a circumscribed area comprising NM. N1 is evoked by ipsilateral nerve stimulation only, and reflects postsynaptic currents in NM elicited by 8th nerve input. The second negativity (N2) is recorded from the dorsal neuropil region of NL. Like N1, N2 is evoked only by ipsilateral 8th nerve stimulation. The latency of N2 is 3-5ms longer than that of N1; this is consonant with the view that the 8th nerve does not project directly to NL, and that N2 results from synaptic activation of dorsal NL dendrites by ipsilateral NM neurons. The final negativity (N3) is recorded at the level of the ventral neuropil of NL. N3 is evoked by contralateral nerve stimulation only and reflects the synaptic input to ventral NL dendrites from the contralateral NM.

We have also undertaken an analysis of the postsynaptic potentials generated by 8th nerve stimulation. All postsynaptic potentials thus far recorded from NM were excitatory. This has also been true for NL units regardless of whether the ipsi- or contralateral nerve was stimulated. Two types of unitary EPSPs have been recorded from NM; the first has a 2-3ms rise-time and 5-7ms duration, whereas the second has a rise-time of about 5ms and a duration of about 50ms. It is possible that these two types of EPSPs correspond to the two morphologically separable types of nerve terminals seen in NM.

In contrast to NM, NL neurons show graded EPSPs with increasing intensity of stimulation. We have traced terminal arborizations of individual NM cells following intracellular injections of HRP and found that axons from single NM neurons contact a number of adjacent NL cells. Taken with the graded EPSPs recorded from NL neurons, this indicates that terminals from several NM neurons converge on individual NL cells. (Supported by NSF grant #BNS 78-04074, the Deafness Research Foundation, and NINCDS RCDA #NS00305-01 to EWR, and RSDA 5K02 DA 00009 from NIDA to JTH.)

19 COCHLEAR INJECTIONS OF LABELLED ASPARTATE OR GLUTAMATE PRODUCE LOCALIZED GRAINS IN AUTORADIOGRAPHS. Eileen S. Kane, Dept.Anat., Univ. of Mass. Med. Sch., Worcester, Ma 01605.

Small volumes (0.5-0.7 µl) of ^{3}H-labelled L-aspartate or L-glutamate (New Eng. Nuc.,Boston) diluted in sterile saline (final conc. 30-50 µCi/µl), were injected unilaterally into cochleas of adult cats. After survival periods of 3-30 hrs, cats were perfusion-fixed, brains immediately removed and blocked, and frozen sections of stems cut (30 µm), mounted and routinely prepared for LM autoradiography. Dense grains were localized in the ipsilateral cochlear nucleus in patterns identical to those after ^{3}H-leu injections (1977, Am. J. Anat., 150:641). Heaviest grains were found in the posteroventral (PVCN) and anteroventral (AVCN) cochlear nucleus subdivisions, notably in and around the nerve root, and in the deep dorsal (DCN) cochlear nucleus into the fusiform cell layer (FCL). Sparsest grains occurred in the granule cell caps and in the DCN molecular layer. Evidence of transneuronal transport appeared with survival times over 24 hours for both ^{3}H-asp and ^{3}H-glu. Non-specific label (over unrelated stem nuclei) was heavy after 24-hr survivals. Both amino acids produced significantly higher average grain counts in zones of the ipsilateral cochlear nucleus known to receive heaviest primary inputs. For example, average counts in experimental OCA were about 70/100 µm^2 for asp and about 110/100 µm^2 for glu while average counts in the contralateral OCA were about 30/100 µm^2 for both ^{3}H-asp and ^{3}H-glu. Liquid scintillation counts of both cochlear nerve roots were significantly higher for the injected vs. the uninjected sides (a 10:1 ratio for ^{3}H-asp, a 7:1 ratio for ^{3}H-glu). Our results strongly support neurochemical findings from other labs that both aspartate and glutamate may be the neurotransmitters (or their precursors) used by primary auditory fibers.

Supported by Univ. of Mass. Med. Sch., Deafness Res. Fdn. and USPHS Grants NS 14260 and NS 00290 (RCDA) to ESK.

20 LOCALIZATION OF SOUNDS IN SPACE BY RATS WITH BILATERAL LESIONS OF AUDITORY CORTEX. Jack B. Kelly, Psychol. Dept., Carleton University, Ottawa, Ontario, K1S 5B6.

In cats, dogs and monkeys, the ability to localize an auditory stimulus in space is dependent upon auditory cortex. In these animals bilateral lesions of auditory cortex result in profound deficits in localization, even though little impairment is found in absolute sensitivity to sounds or ability to discriminate along other auditory dimensions such as frequency and intensity. Heffner and Masterton (1975) have shown in monkeys that deficits in sound localization are not due simply to an inability to discriminate between left and right but, rather, are related to the response demands of the task. In other words, in order to demonstrate a deficit it is necessary to test animals in a maze which requires a spatial response to either left or right stimuli presented briefly at the onset of each trial. In a task of this sort, monkeys with bilateral lesions of auditory cortex fail to perform above a chance level even though the same animals can discriminate left from right in a non-spatial task. We have attempted to replicate this result using albino rats as subjects. The animals were trained to localize single clicks in a two-choice spatial maze with loudspeakers separated by 180° azimuth. Further testing was then done with speakers separated by 60°. Difficulty was experienced by some animals following cortical lesions, but this did not correspond with the extent or location of the damage. More significant was the fact that most animals with large bilateral lesions of auditory cortex were still capable of high levels of performance with speaker separations of 180° and 60°. This behavior was clearly based upon the location of the sound rather than other qualitative differences between loudspeakers because responses dropped to chance when the speaker angles were reduced to 12°. Therefore, in contrast to results with monkeys, rats with bilateral lesions of auditory cortex are still able to localize sounds in space. Furthermore, because of the close similarity of testing procedures used in studies of sound localization, it seems likely that our results reflect a species difference in cortical dependency of auditory localization.

Supported by the National Research Council of Canada.

21 EFFECT OF INTER-AURAL TIME DELAY ON AUDITORY CORTEX UNIT RESPONSES. L.M. Kitzes* (SPON: J.E. Swett). Dept. of Anatomy, College of Medicine, U.C.I., Irvine, Calif., 92717.

Auditory cortex has been implicated by behavioral research in the lateralization of a sound source. The sensitivity of single neurons in primary auditory cortex of cat to inter-aural time of stimulation was examined.

The pinnae of Halothane anesthetized cats were removed and an ear piece sealed in each external auditory meatus. A Beyer DT-48 earphone was coupled to each ear piece by a short plastic tube. Pt-Ir electrodes were advanced into primary auditory cortex using a hydraulic microdrive controlled from outside a double-wall acoustic chamber. Fifty msec best frequency tone bursts (rise/fall time 5 msec) presented at moderate stimulus levels relative to unit threshold were used. Stimulus level was equal at the two ears.

Inter-aural time differences between the contralateral stimulus leading by 20 msec and the ipsilateral stimulus leading by 50 msec were explored. The most common effect observed is a reduced discharge rate when stimulation of the ipsilateral ear precedes contralateral stimulation. The reduction is observed whether or not ipsilateral stimulation alone evokes discharge activity. When ipsilateral stimulation alone does not evoke discharges, there frequently is no apparent effect of such stimulation unless it precedes contralateral stimulation. Ipsilateral suppression frequently asymptotes at contralateral delay values of 3 to 15 msec and may be effective throughout the duration of the ipsilateral signal. The pattern of the contralaterally evoked response normally remains unchanged while the evoked discharge rate varies as a function of inter-aural delay.

While other mechanisms are clearly possible, the relevance of the long inter-aural time delay functions to the lateralization of a sound source could derive from intensity differences at the two tympanic windoes due to the presence of the head in the sound path to the distal ear. This difference approaches 10 dB for high frequency signals. At near threshold levels mean response latency of AVCN cells decreases by several msec with each 10 dB intensity increase (Kitzes et al, J. Neurophysiol., in press). The latency difference between responses of the two AVCN cell populations excited by a distant sound source, arising from an inter-aural intensity difference, could result in central delay functions similar to the inter-aural delay functions presently under study.

22 AUDITORY RECEPTIVE FIELDS: CENTER-SURROUND ORGANIZATION. Eric I. Knudsen and M. Konishi. Div. of Biology, 216-76 Beckman Laboratories,California Inst. of Technology, Pasadena CA 91125

The spatial receptive fields of specialized auditory units in the midbrain of the barn owl (Tyto alba) are subdivided into separate excitatory and inhibitory areas similar to the center-surround receptive field organization described for other sensory systems. These specialized units were located in the lateral and anterior portions of the midbrain auditory nucleus (mesencephalicus lateralis dorsalis, MLD), the avian homologue of the inferior colliculus. Units in this region respond only to sounds originating from a restricted area of space (receptive field), and are arranged according to the location of their receptive fields so as to form a physiological map of auditory space. The inhibitory areas of these units were mapped under free-field conditions by using two movable sound sources; one source positioned inside a unit's receptive field to drive the unit; while a second source, positioned at various locations outside its field, tested for inhibitory effects.

Noise bursts presented outside of a unit's receptive field inhibited these units. The strength of the inhibition depended upon the location of the source, and the intensity and spectral content of the sound stimulus. Inhibition increased as the source moved in from the periphery and approached the borders of a unit's receptive field. As the source entered the unit's field, the effect of the noise changed from inhibitory to neutral or excitatory within a few degrees. Increasing noise levels resulted in stronger inhibition and an expansion of the unit's inhibitory area toward the periphery. Tone bursts also inhibited these units, but the effect was weaker. The range of sound frequencies that contributed to inhibition was wide, and changed with sound source location. Thus frequency-response curves measured using different speaker locations were often qualitatively different: inhibitory frequencies at one location being neutral or excitatory at another.

The fact that the auditory system has created center-surround receptive fields based on functional properties of the input, and independent of the topography of the sensory surface, argues strongly for the importance of center-surround organization in spatial analysis of sensory input. Work supported by an NIH postdoctoral fellowship (1 F32 NS05529-01) to E.I.K.

23 INTERAURAL INTERACTIONS IN MONKEY AUDITORY CORTEX. Alan D. Legatt, Joseph Arezzo*, and Herbert G. Vaughan, Jr. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

The effects of stimulus laterality on auditory evoked responses provide evidence about neural mechanisms related to sound localization and dichotic intensity effects. We examined gross potential fields and multiple unit activity (MUA) within primary auditory cortex in the alert monkey; these data supplement the literature on stimulus laterality effects in subcortical structures.

100 μsec 95 dBSPL clicks were delivered monaurally and binaurally at a rate of 2/sec. Three-dimensional field mapping delineated primary auditory cortex on the basis of potential gradients, polarity inversions, and MUA maxima. In the region of the largest evoked potentials, the initial cortical response to a contralateral click was surface positive, with a peak latency of 11 msec. When the click was delivered ipsilaterally, this initial positive wave occurred .5 msec earlier, and was reduced in amplitude by almost 50%. The initial positive component following binaural stimulation was double-peaked, and intermediate in amplitude between the responses to ipsi and contra monaural clicks. When the contra click was held at 95 dB and the ipsi click increased from 0 to 105 dB, the amplitude of the initial positive component decreased systematically. In the converse situation, the wave amplitude increased as the contra click was increased from 0 to 105 dB. MUA recorded in this region showed a phasic increase, whose peak latency ranged from 8 to 12 msec at different depths. The effect of stimulus laterality was similar to the pattern for the P11 component: The MUA response to an ipsi click was smaller than that to a contra click, and the response to a binaural click was intermediate in amplitude.

Longer-latency gross potential components displayed different patterns of interaural interaction. The waveform in the region from 20-35 msec is different for ipsi and contra clicks, while a negative peak at approximately 50 msec is unchanged between these two stimulus conditions. The negativity following a binaural stimulus retains the same latency, but its amplitude is greater than that of the monaural response by about 50%. No increases in MUA corresponding to any of the later evoked potential components were observed at any of the recording sites in the mapping.

To further investigate the mechanisms of sound localization, delays of up to 1000 msec were introduced between clicks of equal intensity presented to the two ears. When the contra click led, the amplitude of the initial cortical component was larger than that of the response to simultaneous clicks, while the initial component amplitude was decreased when the ipsi stimulus led.

(This work was supported by grants GM-7288, MH-06418, and MH-06723 from the USPHS.)

24 BINAURAL RESPONSE-SPECIFIC BANDS WITHIN AI IN THE CAT: SPECIALIZATION WITHIN ISOFREQUENCY CONTOURS. John C. Middlebrooks*, Robert W. Dykes, and Michael M. Merzenich* (SPON: R.L. Snyder). Coleman Mem. Lab., UCSF, San Francisco, CA 94143.

The spatial distribution of neurons with different response properties has been studied within the three dimensions of AI in the cat. This work confirms and extends the observations of Imig and Adrián (Br.Res.138:241, 1977). Vertical and topological distributions of neurons falling within characteristic binaural response classes have been mapped by recording the responses of single units and small clusters of units to dichotic stimulation. Nearly all units and clusters (98.4%) were excited by stimulation of the contralateral ear with tones near their characteristic frequencies. Simultaneous stimulation of the ipsilateral ear facilitated ("excitatory/excitatory" or "EE" neurons; 57.7% of recordings), inhibited ("excitatory/inhibitory" or "EI"; 34.3%), or did not alter ("EO";6.4%) the response to contralateral stimulation. A few neurons (1.6%) were driven by stimulation of the ipsilateral ear and strongly inhibited by stimulation of the contralateral ear. Within any given penetration, neurons studied fell within the same binaural response class (e.g., EE, or EI, or EO, or IE) in most of 800 penetrations introduced perpendicular to the cortical surface. Long electrode penetrations made parallel to isofrequency contours traversed the mediolateral extent of AI through the middle layers of the cortex (24 penetrations in 6 cortices). A sharp segregation of units by binaural response class was observed in these penetrations. Regions in which only EE responses were recorded were 200 to 3400μ wide. Regions of EI responses ranged from 100 to 2000μ across. These regions of uniform response to binaural stimulation formed vertical units of organization that were elongated in the rostrocaudal dimension and, thus, intersected the lines of re-representation of the cochlear sensory epithelium. In most cases, there were three prominent, continuous bands of EI units separated by two broad bands of EE units. In some cats, the bands of uniform binaural response types were apparently not continuous, although regions were still elongated along rostrocaudal axes. These results suggest that AI, a single auditory cortical field containing a continuous representation of the cochlear sensory epithelium, is spatially subdivisible into functionally distinct response-specific bands apparently specialized for the coding of unique aspects of binaural stimuli.

(supported by NIH grant NS10414 and Hearing Research Incorporated)

25 NEURONS IN THE INFERIOR COLLICULUS OF THE CAT WITH ASCENDING PROJECTIONS. D. L. Oliver and D. K. Morest, Dept. of Anatomy, University of Connecticut Health Center, Farmington, CT 06032.

The inferior colliculus is the major source of ascending auditory afferents to the medial geniculate body. To identify the neurons which contribute to this projection, we made injections of horseradish peroxidase (HRP) in the medial geniculate body. After 1-2 days' survival, the midbrain was prepared for light and electron microscopy and reacted for HRP with diaminobenzidine, Hanker-Yates mixture, or tetramethyl benzidine (TMB). Most HRP-labeled cells were found ipsilaterally in all major divisions of the inferior colliculus (central nucleus, lateral zone, and dorsal cortex); but some were scattered contralaterally. The present report centers on the ipsilateral central nucleus (partes medialis et centralis). Most frequently labeled were medium-sized neurons with oval somas, 14-23μm in diameter. These cells include the medium-sized disc-shaped neurons, the principal type that forms the dendritic layers of the central nucleus in Golgi preparations. Next in frequency were labeled medium-large cells (23-28μm) resembling the large disc-shaped neurons in Golgi impregnations. Both cell types are also characterized by dispersed Nissl substance, seen best in unosmicated tissue embedded in water soluble plastic and stained with toludine blue. Less frequently labeled were large neurons in excess of 30μm. Some of the labeled cells could be medium-to-large stellate neurons with dendrities crossing the laminae. Our best evidence for labeled stellate neurons comes from TMB-incubated material that reveals HRP in primary and secondary dendrites oriented across the laminae. A small number of labeled neurons also contain distinctly clumped Nissl bodies which may correspond to stellate cells with thalamic projections. Least frequently labeled with HRP were small cells. Some of these could correspond to the neurons observed in the Golgi material to have an axon with extensive local arborizations.

In electron micrographs, we identified the two most commonly labeled cell types. Both lack stacked cisterns of rough endoplasmic reticulum. The medium disc-shaped cell is distinguished from the large disc-shaped cell; the large cells display abundant axosomatic synapses and an irregular nuclear envelope.

Thus, several different types of neurons project from the central nucleus to the medial geniculate body. Possibly these are represented by the varieties of ascending axonal endings observed in the medial geniculate body in Golgi and electron microscopic preparations (Morest, JCN 162, 1975: Winer & Morest, this volume).

(Supported by USPHS grants 1 F32 NS05485 and 5 R01 NS14347.)

26 EFFECTS OF AROUSAL AND OF AUDITORY CORTEX COOLING ON GENICULATE SPIKE PATTERNS. Steven S. Orman and Gordon L. Humphrey. Dept. Physiol. and Research Resources Ctr., Univ. Il., Med. Ctr., Chicago, IL. 60680

Spontaneous and sound-evoked medial geniculate (MG) single spike discharges were recorded in chronically implanted, unanesthetized, paralyzed cats. Comparisons were made between activity during high-voltage ("h") and low-voltage ("l") ECoG periods (i.e. during different states of cortical arousal) and between periods of normal auditory cortex temperature and cooled ("c") auditory cortex (i.e., during normal and reduced corticogeniculate activity).

Both spontaneous arousal shifts and cortical cooling led to consistent MG activity changes. Chief among these were: a) Both "h" and "c" states reduced the spontaneous rate of discharge significantly for almost all cells examined. Both conditions caused a significant increase in long intervals but also tended to lead to a higher percentage of extremely short intervals. Thus, cells became more "bursty". b) Moderately short intervals with a relatively steady (and overall higher) discharge rate characterized "l" states with normal cortex temperature. c) Both "c" and "h" states caused modified responsiveness to sounds, i.e., increases or decreases in the long-latency components of the responses. While post-stimulus reverberations were generally absent in this unanesthetized preparation, subdued reverberations were sometimes seen during "h" and "c" periods. d) With respect to effects of cortical temperature and arousal states, no distinctions were obvious between ventral and dorsal divison cells.

Our spontaneous discharge rate data suggest that descending fibers from the auditory cortex are predominantly excitatory and of the subtle modulatory variety. Since the effect on MG neural activity of "h" (cortical non-arousal) was much the same as the effect of "c" (reduced corticogeniculate discharge), one consequence of reduced arousal seems to be a reduction of this cortical modulating discharge to the MG. Increased burst activity and reverberation during sleep and during cortical cooling may be "released" among relay and local circuit elements as a result of decreased cortical input. These may be less extreme examples of the same phenomena that produce bursting and enhanced reverberation in MG in the presence of barbiturate anesthesia.

27 EVIDENCE FOR TRANSNEURONAL DEGENERATION IN THE GUINEA PIG AUDITORY SYSTEM. John Persing, Oswald Steward, and Edwin W. Rubel. Departments of Neurosurgery, Physiology and Otolaryngology, Univ. of Virginia, Charlottesville, Virginia 22901.

Young adult guinea pigs underwent unilateral surgical ablation of the organ of Corti. Following post-lesion survival periods ranging form 5 to 28 days, the midbrain and brainstem were processed by reduced silver impregnation methods. Additionally , unilateral HRPinjections were placed in the round window and individual coils of the cochlea to visualize the afferent and efferent connections of the organ of Corti. Lesions of the cochlea resulted in dense degeneration throughout the ipsilateral cochlear nucleus. Moreover, terminal degeneration was also present in the superior olivary complex bilaterally at 5 days post lesion, slightly increased in intensity at 10 days, and thereafter, decreasing in intensity, such that at 28 days no argyrophilic fragments were identifiable. Ipsilaterally, degenerating terminals were apparent in the lateral superior olive(LSO), the lateral trapezoid nucleus (LTN), and the lateral aspect of the medial superior olive(MSO); contralaterally, degenerating terminals were present in the medial trapezoid nucleus(MTN), dorsomedial(DMPO) and ventromedial(VMPO) periolivary nuclei, and the medial aspect of MSO. Because terminal degeneration was seen beyond the cochlear nuclei in the olivary complex, and, because direct projections to the olivary complex in primates were recently proposed(Carpenter et al, J Comp Neurol 179 517, 1978), HRP was injected into the cochlea to examine the orthograde and retrograde transport of this enzyme in the cochlear nucleus and olivary complex. In all cases, dense accumulations of reaction product were observed in segments of the cochlear nucleus. These injections also resulted in retrograde HRP labeling of large numbers of cells, fibers and less well defined pericellular HRP reaction product ipsilaterally in LSO and LTN,and, contralaterally fewer cells,fibers and pericellular reaction product in MTN and ventral trapezoid nucleus(VTN), DMPO and lateral lemniscal nuclei (LLN). Anterograde HRP terminal field labeling, however, was not seen in LTN, VTN, MSO, LLN or IC. In conclusion, silver impregnation methods demonstrated terminal degeneration similar to that seen with isolated lesions of VCN, and retrograde HRP cellular labeling described the origin of the olivocochlear bundle. Although alternative interpretations of these observations are possible (direct cochlear nerve to olivary complex projections and/or degeneration associated with associated with olivocochlear bundle collaterals) we believe the pattern of terminal degeneration and the apparent absence of orthograde terminal field HRP labeling of the olivary complex indicate that lesions of the cochlea result in transneuronal degeneration of VCN terminals in the olivary complex.

Supported by Grant #1R01 NS12333 to OS; Deafness Res.Found. to EWR.

28 TONOTOPIC ORGANIZATION AND RESPONSE PATTERNS TO FREQUENCY MODULATED SIGNALS IN THE INFERIOR COLLICULUS OF HORSESHOE BATS. George Pollak and Gerd Schuller*. Fachbereich Biologie, Frankfurt Univ., FRG.

Horseshoe bats (Rhinolophus ferrumequinum) emit sounds having a long constant frequency (10-100 msec) portion that ranges in frequency from about 77-86 kHz during Doppler-shift compensation behavior. These bats are thought to utilize the frequency and amplitude modulation patterns superimposed on the echo CF by the beating wings of an insect for prey identification. Neurons tuned between 77-86 kHz show a number of special adaptations that include sharp tuningwith typical Q10 dB values of 100-200. In the present experiment the responses to sinusoidally frequency modulated (SFM) signals were studied. Most high frequency neurons responded to tone bursts with a phasic-on or tonic pattern but responded to SFM signals with firings that were tightly synchronized to the modulating envelope. Modulation heights as small as + 50-100 HZ evoked synchronized firings in some units. Synchronized firings were evoked even at very low signal intensities (0-40 dB SPL) in the majority of cells. Some units were unresponsive to CF signals yet fired briskly and in a highly synchronized fashion to SFM sounds. These units apparently are specialized for detecting the FM modulation patterns in the echo CF component.

A disproportionately large volume of the colliculus is devoted to processing the CF component. About 60% of the units are tuned to frequencies between 77-86 kHz. Moreover, these high frequency cells have an unusual tonotopic arrangement being organized in the anterior-posterior axis in contrast to the lower frequencies (10-70 kHz) whose tonotopic arrangement is in the familiar dorsal-ventral plane. In a given penetration units in the initial 500-700μ of tissue had progressively higher best frequencies (BF) but in the remaining 1300-1500μ the BFs were nearly constant, assuming values of from 77-86 kHz depending upon the penetration site.

The unusual tonotopic arrangement and disproportionate amount of neural space for the frequency range of the CF component results in a spacial segregation of activity evoked be the emitted cry and Doppler-shifted echo while the specialized response features to SFM sounds suggests that these cells can encode important target features required for prey identification.

29 THE EFFECT OF SIMULATED SONIC BOOMS ON THE INNER EAR STRUCTURE AND FUNCTION. Stanislav Reinis, Chris Tsaros* and James W. Featherstone*. Dept. Psychology, Univ. of Waterloo, Waterloo, Ont. and the Univ. of Toronto Institute of Aerospace Studies, Toronto, Ont., Canada.

Repeated simulated sonic booms of medium intensity, about 100 Pa, cause bleeding into the basal turn of the cochlea of C57BL/6J mice and chinchillas. Single superbooms of 200 to 500 Pa have the same effect. High frequency sounds are perceived in the basal turn of the cochlea. Experimental animals exposed to simulated sonic booms therefore show a permanent threshold shift to tone with the frequency above 20KHz. Similar experiments have been replicated with Rhesus monkeys.

30 ALTERATIONS IN THE STRIA VASCULARIS OF THE GUINEA PIG COCHLEA AFTER TREATMENT WITH KANAMYCIN/ETHACRYNIC ACID AND ETHACRYNIC ACID ALONE. N. J. Russell*, K. E. Fox* and R. F. Brummett*. (SPON: C. J. Russell) University of Oregon Health Sciences Center, Portland, Oregon 97201.

Previously we reported that injection of a single 400 mg/kg dose of kanamycin (KAN) s.c. followed 2 hrs later by a single 40 mg/kg dose of ethacrynic acid (EA) i.v. resulted in the rapid destruction of outer hair cells (OHC) (Fed. Proc. 36: 412, 1977). Early OHC damage, visible within 2-1/2 hrs after EA administration, was accompanied by swelling of the stria vascularis (SV) which was greater than that seen after treatment with EA alone. Since a functional relationship exists between the SV and the OHCs, it is possible that the SV is the primary site of KAN/EA interaction. To investigate this we examined the SV by electron microscopy (EM) at 5 periods post-EA (1/3, 2, 2-1/2, 4, 6 and 24 hrs). In each series guinea pigs were given either KAN/EA, saline (SAL)/EA, KAN/SAL or SAL/SAL. Cochlear function was monitored continuously by recording the AC cochlear potential generated by a 7 kHz tone and determining the DC endocochlear potential prior to cochlear fixation.

EM studies of tissue fixed at 20 min (1/3 hr) post-EA show SV swelling of similar severity after KAN/EA and SAL/EA. In each case, extracellular fluid had accumulated around the intermediate cells and the marginal cells were pushed outward causing the stria to bulge into the endolymphatic space. Numerous large vesicles were seen along the luminal surface of the marginal cells. At this time the AC and DC potentials had dropped to low levels. By 2 to 2-1/2 hrs post-EA, the SVs of the KAN/EA animals were significantly more swollen than those of the SAL/EA animals although vesicles were more prevalent in the marginal cells of the latter. Areas where the SV had ruptured were seen 2 hrs after KAN/EA. Dying cells were evident within these areas and in the nearby endolymphatic space. Although the AC potential was low at these times, the DC potential had returned to normal levels. By 4 to 6 hrs, SV recovery had begun whereas the DC potential had decreased and OHC loss was evident. Most SV swelling was gone by 24 hrs. However, the SV of the KAN/EA animal was thinner than normal and degenerating cells were occasionally seen indicating that prolonged edema causes considerable loss of SV cells. These results indicate that KAN adds to the swelling caused by EA either directly or by impeding the recovery process. (Supported by a grant from NINCDS 5R01 NS 12808-02.)

31 DEVELOPMENT OF BRAINSTEM AUDITORY POTENTIALS IN KITTENS. Carl Shipley*, David Geary*, Robert Norman and Jennifer Buchwald. Department of Physiology, MRRC, BRI, UCLA, CA 90024.

Brainstem auditory evoked potentials (BAEP) were recorded from ten kittens (three litters) over the first two months of life.The kittens were lightly anesthetized with Ketamine during recording sessions.Click stimuli were presented at rates of 1, 10, 50 and 100 per second.Various active-reference electrode configurations were studied.It was found that click stimuli evoked activity from all locations near the skull as referenced to a front paw.The pattern of development of the BAEP recorded at the vertex, bulla, or tongue referenced to the paw was found to be generally similar although there were consistent differences in the appearance of some of the potentials.The waves were observed as early as 7 to 8 days after birth and were present in all kittens by day 10.At this early age, all of the waves were extremely sensitive to click repetition rates faster than 1/sec.With increasing age, all waves showed a systematic decrease in latency across development which was reflected in both a decrease in the latency of the first wave as well as a non-linear decrease in the interpeak intervals between the first wave and successive waves.Several changes in the form of the waves appeared across development.As it emerged, wave 2 had two distinct components which tended to merge as the animals became older.Wave 3 also showed two separate components in the young kittens which appeared during the second week.Waves 4 and 5 appeared later than waves 1-3 and were very sensitive to stimulus repetition rate in younger animals.This sensitivity was particularly pronounced in wave 5 which was generally absent at rates of 50 or 100/sec during the first month of life.Marked increases in latency occurred in all waves when repetition rates were increased from 1/sec to 100/sec; these latency effects diminished to approximately adult values by 6 weeks.These results are interpreted in terms of possible maturational sequences in the brainstem auditory nuclei.(Supported by NIH Grant MH-24344 and HD-05958.)

32 EFFECTS OF PERILYMPH COLLECTED DURING AUDITORY FATIGUE ON COCHLEAR ELECTROPHYSIOLOGY. M. Tachibana*, C. H. Norris, W. F. Sewell* and P. S. Guth. Depts. of Pharmacology and Otolaryngology, Tulane Univ. Med. Sch., New Orleans, LA 70112.

Previously we reported the release of auditory nerve activation substance (ANAS) into perilymph during sound stimulation (Soc. Neurosci. III, 11, 1977). Recently, experiments designed to increase the output of ANAS were carried out using more intense sound stimuli during the perilymphatic collection periods. In these experiments, compound VIIIth nerve action potentials and cochlear microphonics were measured. The more intense auditory stimuli caused changes in these potentials reminiscent of auditory fatigue. Perilymph, collected during periods of auditory fatigue, when infused back into the guinea pig cochlea during silence caused changes in cochlear potentials identical with those seen during auditory fatigue. Such cochlear electrical changes are not seen with perilymph collected during quiet periods. These results suggest that ANAS and/or another substance released during sound stimulation may be responsible for the changes in cochlear electrical activity seen during auditory fatigue. These results may point to a chemical basis for auditory fatigue and such related phenomena as temporary threshold shift. (Supported by NS # 11647, The Veterans Administration Research Service and The John A. Hartford Foundation).

33 AFFERENT AUDITORY PROJECTIONS TO THE INFERIOR COLLICULUS OF BUSH BABY (GALAGO SENEGALENSIS). G. C. Thompson. Dept. of Psychology, Duke University, Durham, N. C. 27706.

The inferior colliculus of bush baby can be divided, based on its cytoarchitecture, into a central nucleus which is surrounded dorsally, medially and caudally by a pericentral nucleus and surrounded laterally and rostrally by an external nucleus. The central nucleus can be further subdivided into dorsomedial and ventrolateral divisions. To discover the cells of origin of the afferent projections to these subdivisions, horseradish peroxidase was electrophoretically injected into the inferior colliculus of 20 bush babies. Each animal was sacrificed at 24 hrs. and the tissue processed with either diaminobenzidine (DAB), catechol/p-phenylendiamine, benzidine dihydrochloride (BDHC), or tetramethylbenzidine (TMB). Labeled cells were counted in each central auditory nucleus and then converted to a percentage of the total number of labeled cells found in each case.

Considering the inferior colliculus as a whole, the results indicate that it receives bilateral input from the dorsal and ventral cochlear nuclei, lateral superior olives, medial superior olives, and dorsal nuclei of the lateral lemniscus. However, the ipsilateral projection from the cochlear nucleus is not nearly as strong as the contralateral projection, while the opposite is true for the projection from the medial superior olives (MSO). In fact, since the contralateral projection of MSO is revealed only by the more sensitive processing techniques, it probably represents collateral connections from the ipsilateral pathway. Exclusively unilateral pathways arrive at the inferior colliculus, one originating in the ipsilateral ventral nucleus of the lateral lemniscus and another originating in the opposite inferior colliculus. The final major auditory center to contribute afferents to the inferior colliculus is layer V of auditory cortex.

Turning now to the subdivisions of inferior colliculus, two major points stand out. First, the superior olivary complex projects mainly, if not exclusively, to the ventrolateral division of central nucleus, but not to the dorsomedial division. Second, auditory cortex projects mainly, if not exclusively, to the pericentral nucleus and dorsomedial division of central nucleus, but not to the ventrolateral division. These mutually exclusive projection areas suggest that interaction between third order auditory nuclei (which are probably sensory in nature) and sixth order auditory nuclei (which are probably motor in nature) is accomplished via complex interneuronal connections within the inferior colliculus itself.

(Supported by NIH postdoctoral fellowship NS-05584.)

34 PATTERNS OF SYNAPTIC ORGANIZATION IN THE COCHLEAR NUCLEUS OF THE CAT. L.P. Tolbert and D.K. Morest. Dept. Neurobiology, Harvard Med. Sch., Boston, MA, and Dept. Anat., U.Conn., Farmington, CT.

The cochlear nucleus comprises distinct cell groups, whose synaptic connections form the basis for all further integration in the auditory system. The posterior subdivision of the anteroventral cochlear nucleus of the cat, as visualized with the Nissl stain, contains two types of neurons. Globular neurons are characterized by dispersed Nissl substance and a small number of thin dendritic trunks; multipolar neurons have large Nissl bodies and many large dendritic trunks. We have previously demonstrated that globular cells correspond to the bushy cells identified in Golgi impregnations and that these are the cells which form the large synaptic calyces of Held in the medial nucleus of the trapezoid body (Neurosci. Abstr. III:12). Adams (personal communication) has shown that multipolar cells send their axons to the inferior colliculus. It was therefore of interest to investigate the types and sources of synaptic input to these two cell types.

It is possible to identify the two cell types in the electron microscope, on the basis of the established correspondence between Nissl substance and rough endoplasmic reticulum. Globular cell bodies are found to receive numerous synaptic inputs; 85% of the surface of the cell body is closely apposed to synaptic terminals. In contrast, multipolar cell bodies are almost entirely wrapped by thin glial sheets; synaptic terminals contact less than 15% of the cell body surface and tend to cluster at the bases of the dendrites. Synaptic terminals are of three kinds, types 1, 2, and 3, which contain 58 nm round, 52 nm round, and pleiomorphic synaptic vesicles, respectively. Terminals of all three kinds synapse on both types of cells. However, only globular cell bodies receive the largest of the type 1 terminals, which, on the basis of sheer size, correspond with endbulbs, seen in Golgi impregnations to arise from cochlear nerve axons. To determine which other terminals are of cochlear origin, we examined cochlear nuclei from cats surviving 1, 2, and 4 days after unilateral cochlear ablation. Cochlear ablation leads to degeneration of type 1, but not type 2 or type 3, terminals.

We conclude that globular cells, which are known to project to the medial trapezoid nucleus, receive heavy input to their cell bodies from the cochlear nerve. Multipolar cells, which project to the inferior colliculus, receive very little cochlear nerve input to their cell bodies; presumably the synaptic organization of their dendrites plays a significant role in determining the properties of the projection to the inferior colliculus. (Supported by PHS grants NS 06115, NS 13126, GM 00406, MH 14275, and NS 14347.)

35 DUAL OLIVOCOCHLEAR BUNDLES: DIFFERENTIAL ORIGINS AND TERMINATIONS IN THE CAT. W. Bruce Warr and John J. Guinan, Jr.* The Boys Town Institute for Communication Disorders in Children, Omaha, NE,68131 and Eaton-Peabody Laboratory of Auditory Physiology, Massachusetts Eye & Ear Infirmary, Boston, MA 02114.

The axonal projections of olivocochlear neurons, which provide an efferent innervation to the organ of Corti, were investigated by the technique of anterograde axonal transport of radiolabelled protein. Ten adult cats received injections of 0.5-12.0 uCi of ^{35}S-methionine in aqueous volumes of 0.02-0.08ul in various locations within the superior olivary complex. Following post-injection times of 11-36-hrs, the animals were perfused with an aldehyde mixture, the cochleas post-fixed in OsO_4, decalcified in EDTA, embedded in soft Araldite, serially sectioned at 3-10u, and mounted for autoradiography with Kodak NTB-2 emulsion. Injection sites were confirmed in autoradiographs of frozen sections. Projections to the cochlea were quantified by counting the number of silver grains in 12 x 12u boxes under the inner hair cells and under each of the three rows of outer hair cells and by plotting these data versus the graphically reconstructed distance along the organ of Corti. Our findings show that the olivocochlear bundle (OCB) can be divided into two parts: 1) One component which originates from cell bodies near the lateral superior olivary nucleus (LSO) and projects to a region underlying the inner hair cells of both cochleas, and 2) a second component which originates in the cell groups medial and ventral to the medial superior olivary nucleus (MSO) and terminates predominantly, if not exclusively, in the region beneath the outer hair cells of both cochleas. In cases analyzed so far, injections within or near the LSO produced grain counts which reflect strongly preponderant ipsilateral projections, the distributions of which were not necessarily symmetrical in the two cochleas. In contrast, injections in the region medial to the MSO produced counts which reflect predominantly crossed projections, the distributions of which appeared to be bilaterally symmetrical. Our data complement the available experimental observations with the electron microscope which show that the outer hair cells receive a major efferent innervation from the crossed OCB, and that the afferent dendrites of spiral ganglion cells, located beneath the inner hair cells, receive a major efferent innervation from the uncrossed OCB. However, our division of the OCB, which encompasses both zone of origin and site of termination, appears to provide a more functionally meaningful basis of classification than the previous scheme which was based upon whether the axons were crossed or uncrossed. (Supported by PHS Grant No.'s NS07720 and NS13126).

36 COCHLEAR NUCLEAR PROJECTIONS FROM OUTER HAIR CELLS. Molly Webster* and Douglas B. Webster. Kresge Hear. Res. Lab., Sch. Med., LSU, New Orleans, LA 70119.

The left bony cochlear shell was surgically removed in one-month-old guinea pigs, causing degeneration of the organ of Corti and gradual loss of most spiral ganglion neurons. There is good evidence that the lost neurons innervated inner hair cells, and that the few remaining after long-term organ of Corti loss innervated outer hair cells. At about one year of age a second surgery removed or destroyed the modiolus, thus destroying the axons of the residual few spiral ganglion neurons. Six days later the animals were sacrificed and the projection of the residual neurons from outer hair cells to brainstem was charted by the Fink-Heimer method and compared with appropriate controls. The ears were serially sectioned and stained with Luxol fast blue-cresyl violet to demonstrate neurons and myelinated fibers.

In one animal the initial surgery destroyed almost the entire nerve, and the second surgery missed the remainder. In this ear a few healthy neurons remain; the brain shows no degenerative debris and therefore serves as an additional control. The left ears of six other animals show the combined effects of the two surgeries; where portions of the spiral ganglion remain, the residual few neurons and their fibers are degenerating.

Degenerative debris was found in the following cellular regions of the cochlear nucleus: central region of dorsal cochlear nucleus, and multipolar, octopus, globular, small spherical, and large spherical cell regions of ventral cochlear nucleus. The debris is much less than that found after severing the cochlear nerve or destroying the organ of Corti by removing the bony shell (the first surgery done here). Nevertheless, this experiment shows that neurons innervating outer hair cells project basically in the same manner as does the entire cochlear nerve. The significance in the central nervous system of the functional differences between the two populations of hair cells therefore lies elsewhere than in their areas of projection. (Supported by Grants NS-12510 and NS-11647.)

37 CYTOARCHITECTURE OF THE GUINEA PIG COCHLEAR NUCLEUS. D. B. Wexler* and R. L. Gulley. (SPON: J. A. Paterson). Department of Anatomy, Case Western Reserve University, Cleveland, Ohio.

The cochlear nucleus of the guinea pig was studied in frozen and paraffin sections stained with thionin or by the Protargol technique to obtain information about its organization for biochemical and experimental studies. These data were correlated with radioautographs showing the distribution of primary auditory terminals labelled by perilymphatic perfusion with H^3-proline and leucine. The rostral anteroventral cochlear nucleus (AVCN) has a homogenous population of spheroidal neurons. Dorsally, these neurons are larger and more rounded than in the remainder of the region. The smallest and most densely packed spheroidal cells are found at the rostral tip. In autoradiographs, spheroidal neurons are encircled by dense accumulations of silver grains which presumably are labelled end bulbs of Held. Caudally, the spheroidal neurons are replaced dorsally by small and medium-sized neurons. These neurons are round or multipolar and are interspersed with scattered giant cells. The rostral extent of these neurons is greater medially than laterally. Ventrally, the caudal AVCN has many globular cells, which are often aligned along the ascending branch of the auditory nerve. In radioautographs, the globular neurons are surrounded by punctate accumulations of silver grains. The soma of some of the other, less numerous, neuronal types in the caudal AVCN also receive primary auditory input. Similar cell types including the globular cells, are seen within the nerve root, and in the ventral portion of the posteroventral cochlear nucleus (PVCN). The distribution of silver grains around the soma of these neurons also is similar to that seen in the globular cell region of the caudal AVCN. The anterodorsal part of PVCN has a heterogeneous neuronal population including many rounded multipolar neurons. The density of labelling around these neurons is variable. At the caudal-most aspect of the PVCN, octopus cells are interspersed with other neuronal types typical of the PVCN. In the laminar structure of the dorsal cochlear nucleus (DCN), pyramidal cells are arranged radially in the granule cell layer. Irregularly-shaped giant neurons are found within the granule cell layer and central region of the DCN. Small neurons, which frequently are round, are found throughout the DCN. The density of silver grains over the DCN is light, and it is confined to the neuropil of the granule cell layer and central region. The pyramidal neurons have scattered clusters of silver grains only along their basal dendrites. The cytoarchitectural organization of the guinea pig cochlear nucleus most closely resembles that of the rat.

(Supported by NIH grant NS-1388901 to RLG.)

38 NEURONAL ORGANIZATION AND AFFERENT CONNECTIONS OF THE MOUSE INFERIOR COLLICULUS. F.H. Willard*, J.M. Hudson* and D.K. Ryugo. Depts. Anat., Univ. Vermont, Burlington, VT 05401, and Harvard Med. School, Boston, MA 02115.

The neuronal organization of the inferior colliculus(IC) of the albino mouse was examined in Golgi, Nissl and fiber-stained material. Two major components were identified: (1) a core area or central nucleus(CN) which is composed of disc-shaped neurons whose dendrites generate a laminar arrangement, parallel to the incoming fibers of the lateral lemniscus; and (2) a cellular rind which surrounds this core and is composed of small, medium and large multipolar neurons. On the basis of cell size, cell density and dendritic organization, this rind may be further subdivided into an external nucleus, pericentral nucleus, dorsomedial nucleus and interstitial nucleus of the IC commissure.

The afferent connections of the IC were determined using the techniques of retrograde transport of horseradish peroxidase(HRP) and anterograde degeneration. Following discrete injections of HRP into CN, only ascending projections were revealed by the presence of HRP-labelled cells: (1) contralateral and topographical projections from CN and the cochlear nucleus(AVCN,PVCN and DCN); (2) bilateral projections from the lateral superior olive, dorsal nucleus of the lateral lemniscus and IC rind; and (3) ipsilateral projections from the medial superior olive and ventral nucleus of the lateral lemniscus.

Disregarding minor connectivity differences between subdivisions of the rind, HRP injections restricted to the rind demonstrated ascending projections similar to those of CN with two important exceptions: (1) AVCN does not project to the rind and (2) the rind receives a heavy projection from the dorsal column nuclei. In addition, the rind receives descending ipsilateral projections from the deep layers of the superior colliculus, the nuclei of the brachium of the IC, the dorsal and medial divisions of the medial geniculate body and layer V of auditory cortex.

The data demonstrate that the IC core(CN) receives only ascending auditory projections and projections from the surrounding rind; in contrast, the IC rind receives both ascending and descending projections from cell populations associated with auditory, somatic and visual functions. These observations are consistent with the notion of "lemniscal-line" and "lemniscal-adjunct" pathways(Graybiel, Brain Beh. Evol. 6:363, 1972) traversing the midbrain. (Supported by U.Vt. GRS Grant PSH 5429-40 and NIH Grant NS 13126)

39 SENSITIVITY OF MOUSE ACOUSTIC STARTLE BEHAVIOR AND INFERIOR COLLICULUS MULTIPLE-UNIT RESPONSES TO STIMULUS RISE-TIME AND REPETITION RATE. James F. Willott, Gregory P. Urban* and Allan Shnerson*. Dept. Psychol., Northern Illinois University, DeKalb, IL. 60115.

Various studies have implicated the inferior colliculus (IC) in the acoustic startle response. One might predict, therefore, that some neurons in the IC and the startle behavior would respond in a related manner to various acoustic parameters. To test this prediction, the influence of stimulus repetition rate and rise-time were evaluated at 3 tone frequencies (5,10,&20 kHz) in both behavioral and electrophysiological experiments with inbred C57BL/6J mice.

In behavioral experiments, mice were tested for the startle response using a stabilimeter. At the 3 frequencies used, 80 dB SPL tones (200ms duration) were effective in eliciting the startle; 5ms rise-times were more effective than 20ms rise-times; 1 per sec stimuli were less effective than stimuli presented at variable intervals (VI) of 15-30 sec. Latencies to response onset were typically 10-12ms when stimuli had 5ms rise-times and 15-17ms for 20ms rise-time stimuli.

Multiple-unit activity was recorded in the ventrolateral (central) nucleus of the IC (ICVL) and in the pericentral and external nuclei (ICP-ICX) in 22 tranquilized mice. Responses having latencies sufficiently short to mediate the startle response were analyzed (4-15ms for 5ms rise-times; 4-20ms for 20ms rise-times). Stimulus manipulations similar to those used in behavioral experiments revealed that some populations of neurons were sensitive to rise-time and repetition of 80 dB tones in ways appearing to parallel the startle behavior. Using conservative response criteria 14% of unit clusters in ICVL responded better (more discharges per 10 stimuli) to VI stimuli than to 1/sec stimuli; For clusters in ICP-ICX, 42% responded better to VI stimuli; No clusters responded better to 1/sec stimuli. In ICVL, 5% of clusters responded better to 5ms rise-times than to 20ms rise-times, while 44% of ICP-ICX clusters responded better to 5ms rise-times. In some cases, frequency influenced sensitivity to one or both parameters.

The data indicate that ICVL neurons are considerably less sensitive than ICP-ICX neurons to two acoustic parameters influencing startle behavior. The ICP and/or ICX appear well-suited to mediate the startle response.

40 MORPHOLOGY OF NEURONS AND AXONS IN THE DORSAL NUCLEUS OF THE MEDIAL GENICULATE BODY OF THE CAT: STUDY WITH THE GOLGI METHOD. J. A. Winer and D. K. Morest. Department of Anatomy, University of Connecticut Health Center, Farmington, Connecticut 06032

The dorsal nucleus is one of the largest subdivisions of the medial geniculate body, but its structure and function are much less known than that of the ventral nucleus, which has an input from the inferior colliculus and projects to the primary auditory cortex. The dorsal nucleus receives axons from the midbrain tegmentum and projects to the non-primary auditory cortex. Both nuclei receive cortico-thalamic inputs but differ in their intrinsic structure, as seen in material from cats 6-41 days old impregnated by the rapid Golgi method. The ventral nucleus is layered and contains only disc-shaped principal neurons and Golgi type II cells; in contrast, the dorsal nucleus contains at least four neuronal types (A-D) and six types of axonal endings (1-6), without obvious lamination: (A) is a large principal neuron with a spherical soma and dendritic field, and a recurved axon without collaterals on the initial part; (B) is an irregularly shaped neuron found caudodorsally; (C) is a somewhat elongated cell--the axon bifurcates, one branch ramifying among the parent cell's dendrites, while the other branch arborizes in the adjacent neuropil (it is uncertain if this cell projects beyond the dorsal nucleus); (D) is a small Golgi type II cell, drumstick-shaped, with a small dendritic field usually polarized away from the axon which arborizes repeatedly to form 15-60 branches. The axonal endings consist of (1) thin axons resembling bundles of ivy tendrils with terminal twig-like collaterals; (2) very fine axons, with a relatively straight trajectory, which bifurcate and form short terminal side-branches; (3) axons of the small Golgi type II cell; (4) slender, undulating axons with few branches ending in a burst of claw-like terminals; (5) axons with extensive collateral systems which, running in parallel bundles, ramify at right angles; (6) and axons of type C cells.

The small Golgi type II cells resemble their counterparts in the ventral nucleus but have many more axonal branches; type 1 axons are like certain fibers ascending from the inferior colliculus to the ventral nucleus; type 2 axons correspond to the cortico-geniculate fibers: these findings suggest some parallels in synaptic organization. Superimposed on this common background, the additional types of cells and axons in the dorsal nucleus evince a more complex synaptic organization, perhaps related to more heterogeneous sources of input from the midbrain tegmentum and outputs to the auditory cortex.

Supported by USPHS grants 1 F32 NS05485 and 5 R01 NS14347.

41 TEMPORAL ASPECTS OF RESPONSES OF AUDITORY-NERVE FIBERS TO STEADY-STATE VOWELS. E. D. Young* and M. B. Sachs* (SPON: N. K. Woolf), Dept. of Biomedical Engineering, Johns Hopkins School of Medicine, Baltimore, MD 21205.

To evaluate the way in which features of the spectra of multi-component stimuli are represented in the discharge patterns of auditory-nerve fibers, responses to steady-state synthetic vowels with spectra characteristic of /a/, /ɛ/, /u/, and /I/ were recorded. Large numbers of fibers (as many as 350) were studied in each cat and responses to one vowel were recorded at a number of sound pressure levels. In this way, it was possible to estimate the response of the whole population of auditory-nerve fibers to each vowel. Plots of average discharge rate versus fibers' best frequency showed separate peaks at the resonances in the vowel's spectra (formants) only at low levels (below 60-70 dBSPL) and only for vowels with widely spaced first and second formants. At high levels, the spread of tuning curves, rate saturation, and two-tone suppression combined to eliminate such peaks (Sachs and Young, J. Acoust. Soc. Am. 63, suppl. 1, S76, 1978). Thus there are no direct "place" cues for formant location which survive changes in sound level.

In this paper, we will present data on periodicity-based cues, which are one alternative. The temporal structure of the responses of auditory-nerve fibers to synthetic vowels is largely dominated by the components at the formant frequencies. For example, auditory nerve fibers responding to /a/ are locked to the first formant or second formant, depending upon the fiber's best frequency; the first two formants of this vowel are closely spaced (768 and 1152 Hz.) and separate peaks corresponding to the formants are not seen in rate versus best frequency plots at any sound level. At high sound levels, locking to the first formant predominates at the expense of the higher formants, but even so, there seems to be better representation of the characteristics of the spectra of the vowels in the periodicity information than in the place information.

42 TECTORIAL MEMBRANE AS A POSSIBLE MECHANISM FOR SHARP COCHLEAR FREQUENCY SELECTIVITY AND TWO-TONE SUPPRESSION. J. J. Zwislocki and E. J. Kletsky*. Institute for Sensory Research, Syracuse University, Syracuse, NY 13210.

Recent recordings from cochlear inner hair cells have indicated that the frequency selectivity seen in basilar-membrane (BM) vibration is sharpened mechanically at or distally to the hair cells. Excitation of the hair cells is believed to result from radial deflection of their stereocilia as a result of shear motion between the tectorial membrane (TM) and the reticular lamina (RL) in which the cells are embedded. Since the endolymph in the narrow gap between TM and RL must participate in the shear motion, the mechanism should be effective even if the stereocilia were not attached to TM. We were able to show analytically that the magnitude of the shear motion at any given cochlear location must depend on the mechanical coupling of TM to the organ of Corti and to the spiral limbus as well as on the longitudinal coupling within TM and the wavelength on BM. According to an analytical solution and computer and network simulations, TM is nearly completely entrained by the organ of Corti, and the shear motion practically vanishes, in cochlear regions where the BM waves are long compared to the longitudinal space constant of TM. This is true provided TM mass is negligible and the TM coupling to the spiral limbus is substantially weaker than to the organ of Corti. Around the vibration maximum of BM, where the waves are relatively short, the shear motion is preserved. The model distribution of shear-motion magnitude leads to a dramatic sharpening of frequency selectivity below and above the vibration maximum. The sharpening is sufficient to completely account for the tuning curves found in cochlear-nerve fibers and inner hair cells.

When, in the network simulation, the coupling between the organ of Corti and TM is made to increase with the relative radial displacement between TM and RL, two-tone suppression arises below and above the frequency of the vibration maximum. The frequency location of the two suppression maxima relative to the best excitation frequency is consistent with the available experimental data. The suppression effect is accompanied by strong distortions of the wave form of the shear motion. Whether this distortion is reflected in the BM motion or not must depend on the impedance relationships between BM and TM.

AUTONOMIC FUNCTION

43 BLOCKADE OF HYPOTHALAMIC-INDUCED INHIBITION OF VAGAL BRADYCARDIA BY γ-AMINOBUTYRIC ACID (GABA) ANTAGONISTS. Susan M. Barman, Craig A. Johnston* and Gerard L. Gebber. Dept. of Pharmacol., Michigan State Univ., East Lansing, MI 48824.

Gebber and Snyder (Am. J. Physiol. 218: 124, 1970) have previously demonstrated that baroreceptor induced vagal bradycardia is inhibited upon electrical stimulation of the lateral hypothalamus. The present study describes the effects of the GABA antagonists, bicuculline and picrotoxin, on this phenomenon. Experiments were performed on unanesthetized, high spinal (C_1 transected) cats which were artificially respired and paralyzed with decamethonium. Phenylephrine was infused intravenously (10-20 µg/min) to maintain blood pressure at a level (>100 mmHg) sufficient to reflexly activate the cardiac vagus. Stimulation (15 v; 1 msec; 5-50 Hz) of the lateral hypothalamus (A9, L2, HO to H-4) increased heart rate under these conditions. This effect can be attributed solely to inhibition of vagal bradycardia. The increase in heart rate produced by hypothalamic stimulation was blocked in a dose-related fashion by bicuculline (0.5-2 mg/kg, i.v.) or by picrotoxin (0.5-2 mg/kg, i.v.). Blockade of hypothalamic-induced effects was accompanied by a statistically significant decrease in basal heart rate. Bicuculline or picrotoxin also blocked the increase in heart rate produced by stimulation of the medullary reticular formation in high spinal cats which were decerebrated at the midcollicular level. Basal heart rate was not changed in these experiments. In contrast to the effects produced by the GABA antagonists, convulsive doses of strychnine (0.1-0.7 mg/kg, i.v.) or pentylenetetrazol (5-50 mg/kg, i.v.) failed to influence inhibition of vagal bradycardia produced by hypothalamic stimulation. These results indicate that the inhibitory effect of hypothalamic stimulation on vagal bradycardia is blocked in the brain stem by agents which interfere with GABAergic transmission. Moreover, the ability of bicuculline and picrotoxin to lower basal heart rate in high spinal cats which were not decerebrated suggests that the hypothalamic inhibitory system is tonically active. (Supported by PHS Grant HL13187.)

44 A SPINAL SYMPATHO-INHIBITORY ACTION OF CHLORPROMAZINE IN THE CAT. Patricia J. Bernthal* and Michael C. Koss. Dept. Pharmacology, Univ. Okla. Health Sciences Center, Oklahoma City, Oklahoma 73190

Chlorpromazine (CPZ) has been shown to depress several autonomic systems (Schallek & Zabransky, Arch. int. Pharm. Ther., 1966, 161, 126; Sigg et al., Neuropharmacology, 1971, 10, 621), probably by a central mode of action (Wang et al., J. Pharmacol. Exp. Therap., 1964, 144, 186). We have reported that CPZ has a dose-dependent inhibitory action on the sympathetic-cholinergic electrodermal response (EDR) evoked by either central brainstem (Davison & Koss, Neuropharmacology, 1976, 15, 197) or reflex (Bernthal & Koss, Neuroscience Abstr., 1977, 3, 17) stimulation. An observed lack of effect of CPZ on the peripherally evoked EDR (Davison & Koss, 1976) supports a central site of action of this agent. The present studies were undertaken to investigate the possibility that CPZ acts at the level of the spinal cord. Six cats were anesthetized with 36 mg/kg pentobarbital. After the cats were paralyzed with gallamine and artificially respired, the spinal cord was cut at the level of C_1. CPZ (0.03, 0.10, 0.30, 1.0, 3.0 mg/kg, cumulative doses) was administered intravenously while eliciting EDRs by stimulating the spinal cord at the level of C_3 with a submaximal frequency. CPZ depressed the electrodermal response in a dose-dependent fashion. The mean ED_{50} was approximately 0.1 mg/kg. This same dose had no effect in four cats when the EDR was elicited by stimulating the sympathetic preganglionic fibers which innervate the sweat glands. The present results suggest that CPZ acts at the level of the spinal cord to depress centrally evoked sympathetic responses. (Supported by USPHS Grant MH 25792 and a grant from the American Heart Association - Oklahoma Affiliate).

45 ANATOMICAL, PHYSIOLOGICAL AND BEHAVIORAL EVIDENCE FOR MEDULLARY RAPHE INHIBITION OF SYMPATHETIC PREGANGLIONIC NEURONS. J.B. Cabot, J.M. Wild*, D.H. Cohen, Dept. Physiol., Univ. of Virginia School of Medicine, Charlottesville, VA 22901.

A series of studies on the pigeon (Columba livia) has established a direct influence of the medullary raphe on sympathetic preganglionic neurons (SPNs) and demonstrated the importance of this pathway in the requlation of cardiovascular function.

First, the following anatomical results documented an avian raphe-spinal system that includes a direct projection upon the SPNs: (a) dense labelling of raphe neurons throughout their rostrocaudal medullary extent after injections of horseradish peroxidase in brachial, thoracic and lumbar spinal cord, (b) the presence of silver grains overlying SPNs subsequent to ^{3}H-proline injections in the medullary raphe, and (c) light degeneration within the sympathetic preganglionic cell column after lesions of the raphe.

Second, electrophysiological experiments indicated that stimulation of the rostroventral medullary raphe inhibits the discharge of SPNs which were identified by antidromic activation and collision. The inhibition begins within 10 msec of stimulus onset, is maximal by 30 msec, and shows temporal summation. On the basis of such evidence, the conduction velocity of this pathway is conservatively estimated at 4-12 m/sec. More direct estimates of conduction velocity were obtained in experiments where the sympathetic preganglionic cell column was stimulated to antidromically activate raphe neurons; these neurons also satisfied the collision criterion. All identified neurons to date have been localized to the ventral division of the medullary raphe. Raphe-spinal axons in these studies conducted at 6-23 m/sec.

Third, physiological and behavioral experiments indicated the importance of this system in cardiovascular control. Electrical stimulation of the rostroventral medullary raphe elicited significant depressor responses, with systolic and diastolic pressures decreasing 20.5±0.85 (mean±S.E.) and 28.1±1.19 mmHg respectively; response latency was 0.86±0.07 sec. The behavioral effects of raphe lesions were then assessed in a classical conditioning paradigm which reliably produces conditioned cardioacceleration and in a control sensitization paradigm. The raphe lesions markedly enhanced cardioacceleratory responses in both conditioning and sensitization paradigms, suggesting that interruption of the inhibitory raphe projection to the SPNs compromised an important pathway for limiting cardioacceleration in response to exteroceptive stimulation.

(Supported by NSF grant BNS-75-20537 to D.H. Cohen. J.B. Cabot and J.M. Wild were supported by a grant from the Alfred P. Sloan Foundation to the University of Virginia Neuroscience Program.)

46 THE ROLE OF CENTRAL ADRENERGIC PATHWAYS IN THE CONTROL OF ARTERIAL PRESSURE IN NORMOTENSIVE RATS.

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In studies of central regulation of arterial pressure related to cardiovascular effects, several areas were stimulated with adrenergic and adrenolytic substances, and dual responses, both hypertensive and hypotensive, were observed (De Jong et al, 1975). The hypothalamus has been studied by several investigators, and has been found to play an important role in the central regulation of arterial pressure. An important question revolves around the relationship between the hypothalamic structures and their α and β adrenergic receptors in the regulation of arterial pressure. The following experimental conditions were used to study the participation of hypothalamic α and β adrenergic receptors in this regulation. Rats with cannulae implanted into the hypothalamus were injected with 20 nmol noradrenaline, alone, or preceded by administration of phentolamine and propranolol in a 3- fold molar relationship in respect to noradrenaline (60 nmol).

Adrenergic stimulation of the middle hypothalamus showed two types of distinct responses: hypertension induced by noradrenaline (Mean of 18 rats= 35 mmHg, SE ± 7 from a baseline of 90 mmHg) and blocked by the β -adrenolytic agent propranolol, but not by the α -adrenolytic agent phentolamine, the other response was hypotensive (Mean of 16 rats= 31 mmHg, SE ± 5 from a baseline of 98 mmHg), and blocked by phentolamine.

These results lead us to postulate the existance of two adrenergic centers in the middle hypothalamus participating in the control of arterial pressure, the α pathway controlling hypotension and the β pathway controlling hypertension. In the lateral hypothalamic area, instead, hypertension would be determined by α receptors (Mean of 16 rats= 32 mmHg, SE ± 6 from a baseline of 102 mmHg), and hypotension by β receptors (Mean of 20 rats= 41 mmHg, SE ± 8 from a baseline of 95 mmHg).

Supported by Grant FAPESP

47 CLONIDINE-INDUCED HYPOTENSION AND BRADYCARDIA IN CATS: THE ROLE OF MEDIAL MEDULLARY RETICULAR α-ADRENOCEPTORS AND VAGUS NERVE. Yih Huey Chen and Samuel H.H. Chan. Dept. Life Sci., Indiana State Univ., Terre Haute, IN 47809.

Clonidine-induced hypotension and bradycardia have been attributed to its activation of postsynaptic α-adrenoceptors in the medulla, resulting in a modification of the autonomic cardiovascular outflows. Based on microinjection and lesion experiments, Chan and Koo (Neuropharmacol., in press) have recently identified in the rat that the medial medullary reticular formation (MMRF) may be a critical bulbar site that is related to such clonidine actions. The present study further investigates the roles played by the α-adrenoceptors in the MMRF and vagus nerve in the clonidine-elicited cardiovascular effects.

Cats (2-4 kg) lightly anesthetized with pentobarbital sodium (35 mg/kg, i.p.) were used in the present study. Tracheotomy, cannulation of left carotid artery and left femoral vein were routinely performed for the facilitation of ventilation, measurement of arterial blood pressure and injection of drugs. The animal was thereafter paralyzed with Flaxedil (4 mg/kg, i.v.) and artificially respired. The head of the animal was placed on a stereotaxic apparatus followed by an appropriate craniotomy and tentoriotomy. Local injection was administered by means of a 27-gauge syringe needle which is connected to a microinjection-device. EKG was recorded for the determination of heart rate.

Intravenous injection of clonidine (10 μg/kg) produced an initial, transient rise in arterial blood pressure, followed by a prolonged hypotension lasting 35 min. Significant bradycardia was also observed for 60 min postinjection. In animals pretreated with haloperidol, an α-adrenoceptor blocking agent, which was microinjected (10 μg/kg, 1 μl) into the bilateral MMRF, systemic injection of clonidine (10 μg/kg) could only elicite the initial vasopression with no vasodepression and bradycardia, implicating the presence and participation of α-adrenoceptors in this brain stem area in the central action of clonidine. In cats receiving bilateral vagotomy, the injection of clonidine at the same dose again induced only the initial hypertension without subsequent hypotension and bradycardia, indicating that the vagus nerve is also involved in the control of the latter cardiovascular events.

It is concluded that clonidine may excite the neurons in the MMRF by activating the α-adrenoceptors, which in turn facilitate the vagal outflow to the heart, resulting in clonidine-induced hypotension and bradycardia.

(We acknowledge the generous supply of clonidine HCl from Boehringer Ingelheim and haloperidol from Janssen Pharmaceutica used in the present study.)

48 A MORPHOLOGICAL STUDY OF THE CANINE AREA POSTREMA AND ADJACENT MEDULLA. C.L. Chernicky,* K.L. Barnes, J.P. Conomy, and C.M. Ferrario. Division of Research and Dept. of Neurology, Cleveland Clinic Foundation, Cleveland, Ohio, 44106.

Sustained arterial pressor responses can be elicited by either electrical stimulation or delivery of angiotensin into the canine area postrema (AP), implying a function in cardiovascular control for this structure. Conversely, electrical stimulation of the solitary tract nucleus (NTS) immediately ventrolateral to the AP evokes depressor responses. The anatomic proximity of these two structures suggests the possibility of functional interaction between them. Since anatomic study of the canine AP has been relatively neglected in comparison to studies of its function, we have undertaken a morphological study of the dog's area postrema.

Successful Golgi impregnations of the brainstem were achieved on 8 adult mongrel dogs according to the methods of Ramon-Moliner (Stain Technol. 33: 19, 1958; Stain Technol. 39: 65, 1964). Sections were cut serially at 50-150μ in the coronal, sagittal, or horizontal plane. For orientation purposes alternate sections were counterstained with cresyl violet. The Bodian silver technique (Bodian, Anat. Rec. 65: 89, 1936), counterstained with cresyl violet, was done on 10μ serial sections to demonstrate axonal elements and Nissl substance. Compared to other structures in the dog brainstem, the AP is rather sparsely populated with neurons. In Golgi sections the architecture of the canine AP can be subdivided into 3 regions: the periventricular mantle zone, an intermediate medullary layer, and a junctional region at the boundary between AP and NTS. In the mantle layer some of the neuronal cell bodies are located at the ventricular surface, while others slightly more ventral have dendrites which appose the ventricular surface. These complex stellate cells adjacent to the ventricular surface appear to be unique to the canine AP. The mantle zone also contains glialoid cells, whose neural or glial character cannot be determined from Golgi preparations. In the intermediate medullary region small stellate cells extend short processes to the mantle and junctional layers. Small neurons which are abundant in the junctional zone have delicate processes extending dorsally into the intermediate medullary layer and ventrally to the NTS. In addition the dog AP contains modified ependyma, glialoid cells, a network of fine axons, and densely packed capillaries.

Previous studies from this laboratory have indicated that the AP pressor response is mediated by a neural mechanism originating within the AP. The architecture of the canine AP described in this study provides support for the existence of such a neural pathway. Supported by grants from NIH, HL-6635; American Heart, #76 646; and the Reinberger Foundation.

49 EFFECTS OF UNCARINE A, CLONIDINE AND 5-(4'-CHLORO) BUTYLPICOLINIC ACID (FD 008) ON BLOOD PRESSURE AND SYMPATHETIC ACTIVITY IN UNANESTHETIZED SPONTANEOUSLY HYPERTENSIVE RATS. C.C. Chiueh, S.M. Nespor* and C.C. Chang.* Geron. Res. Ctr., NIA, Balto. City Hos., Balto., Md. 21224 and Pharmacol. Inst., Col. Human Med., Natl. Taiwan Univ., Taipei, Taiwan

Chronic indwelling tail arterial cannulae (Chiueh and Kopin, J. Pharmacol. Exp. Ther. 205: 148, 1978) were used for monitoring of blood pressure and withdrawal of blood samples from the conscious, spontaneously hypertensive (SHR) rats. Arterial blood samples (0.5 ml) were taken before and after administration of uncarine A, an alkaloid obtained from Uncaria formosana and Uncaria rhynchophylla which have been used in Chinese herbal medicine, clonidine, or FD 008 and assayed radioenzymatically for plasma norepinephrine (NE) and epinephrine (EPI). The plasma levels of catecholamines are found to be a good index of the sympathetic medullary activity in SHR rats (Chiueh, et al., Neuroscience Abstract 3: 18, 1977). As it had been previously shown, both clonidine (10 - 40 μg/kg) and FD 008 (10 - 100 mg/kg) effectively decreased systolic and diastolic blood pressure in the conscious SHR rats. Uncarine A (2 - 40 mg/kg) produced a long-lasting hypotensive effect in SHR rats but not in normotensive control rats. The hypotensive effect of uncarine A lasted for 20 - 48 hr after a single administration of this drug not only in SHR rats but also in renal hypertensive rats. The central inhibitory effect of clonidine on sympathetic tone was supported by the observation of a decrease in plasma levels of NE (0.61 ±0.08 - 0.32 ±0.06 ng/ml) and heart rate (325 ±17 - 265 ±26 beats/min) after intraventricular administration of this agent (5 μg). FD 008 (100 mg/kg) caused an increase in heart rate (340 ±12 - 488 ±8 beats/min) and a concomitant increase in plasma levels of NE (0.64 ±0.09 - 3.91 ±0.38 ng/ml), EPI (0.49 ±0.05 - 9.10 ±1.14 ng/ml) and dopamine (0.08 ± 0.02 - 0.30 ±0.03 ng/ml). Thus, the notion that the hypotensive effect of FD 008 is attributable to the decrease in NE release from the sympathetic nerve needs to be reconsidered. After 2 mg/kg of uncarine A, the blood pressure of SHR (174 ± 10/158 ± 10 mmHg) decreased to normotensive range (131 ± 10/118 ± 11 mmHg) without significant effect on heart ratesor plasma catecholamines. A high dose of uncarine A, 40 mg/kg, decreased the blood pressure to less than 100 mmHg and hence increased heart rates slightly to 380 ± 15 beats/min and plasma levels of NE and EPI to 1.41 ± 0.04 and 2.99 ± 0.98 ng/ml, respectively. The hypotensive mechanism of uncarine A should be studied further since the present results rationalize the use of Uncaria genus for the treatment of essential hypertension in Chinese herbal medicine.

50 PARAVENTRICULAR AND SUPRAOPTIC, HYPOTHALAMIC NUCLEI: WHAT ARE THEIR ROLES IN CENTRAL REGULATION OF HEART RATE? J. Ciriello and F. R. Calaresu, Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

Recent anatomical studies have demonstrated that the paraventricular nucleus (PAH) projects directly to "cardiovascular" sites in the medulla (Brain Res. 117: 305, 1976) and receives a direct projection from the nucleus tractus solitarius (NTS) (Anat. Rec. 187: 693, 1977). In addition, units in the supraoptic nucleus (SON) have been shown to alter their discharge rate during selective excitation of baro- and chemoreceptors (Brain Res. 126: 551, 1977). To investigate the role of these hypothalamic nuclei in the regulation of heart rate, three series of experiments were done in chloralosed, paralyzed and artificially ventilated cats. In the first series, the response of 185 units in the PAH and the SON to electrical stimulation of the carotid sinus nerve (CSN) was recorded. The firing frequency of 57% (106/185) of these units was altered: 48 were excited (mean latency, 27 ± 2 ms), 40 responded with excitation (mean latency, 31 ± 2 ms) followed by inhibition, and 18 were inhibited (mean latency, 72 ± 11 ms). In the second series, histologically verified sites in PAH and SON were stimulated (80 Hz, 0.2 ms, 50-150 μA) and were shown to elicit marked increases in heart rate. The response to stimulation of the SON was due to vagal inhibition, as it could be elicited in cats with spinal transection at C2 and was abolished by either the administration of atropine or bilateral vagotomy in these spinal animals. On the other hand, the response to stimulation of the PAH was shown to be due to both vagal inhibition and sympathetic excitation, as the response could be elicited in either spinal cats or in bilaterally vagotomized animals. In the final series, to investigate the effect of stimulation of PAH and SON on the bradycardia elicited by stimulation of the CSN, NTS, nucleus ambiguus (AMB) and external cuneate nucleus (ECN), the PAH or SON was stimulated simultaneously with one of these structures in spinal animals. Electrical stimulation of either the PAH or SON abolished the reflex vagal bradycardia to stimulation of either the ipsilateral or contralateral CSN. However, stimulation of these hypothalamic nuclei did not significantly alter the magnitude of the bradycardia elicited by stimulation of either the ipsilateral or contralateral NTS, AMB and ECN. These results indicate that the PAH and SON are hypothalamic areas which a) receive inputs from cardiovascular afferents, b) play an important role in the integration of cardiovascular reflexes, c) exert inhibitory effects on reflex vagal bradycardia through crossed and uncrossed descending pathways, and d) receive information from the cardiovascular system which may be integrated to produce responses concerned with homeostasis of body fluids. (Supported by MRC of Canada)

51 REGIONAL CEREBRAL BLOOD FLOW IN MIGRAINE SUBJECTS DURING SELF-REGULATION OF SKIN TEMPERATURE. James L. Claghorn, Roy J. Mathew*, John W. Largen*, Ken Dobbins*, John S. Meyer*. TRIMS, Houston, Tx. 77030

A study was undertaken to determine the effect of biofeedback induced skin temperature changes on migraine headache activity and regional cerebral blood flow. The results of earlier studies suggest that the biofeedback technique maybe effective in significantly reducing the frequency, intensity, and duration of migraine attacks. A typical classic migraine attack is characterized by a biphasic pattern of vasomotor behavior. The prodrome stage involves the reduction of intracranial blood flow followed by a reactive dilatation of the intracranial and extracranial arteries. It has been hypothesized that hand-warming through biofeedback training may result in a decrease in sympathetic outflow, thereby, interrupting the vasomotor pattern of change in a migraine headache.

Earlier research in our own laboratory, utilizing normal subjects, indicated that the mean cerebral blood flow for both "hand-warming" and "hand-cooling" groups tended to remain unchanged or shift in similar directions though the subjects self-regulated their skin temperature in significantly opposite directions. The present study was undertaken to determine if a similar pattern held true of migraineurs as well.

Twelve right-handed female volunteers, aged 27-52, were selected on the basis of the following characteristics: manifesting either classic or common migraine and having a minimum of two migraine attacks per month. Each volunteer was randomly assigned to either a hand-warming or a hand-cooling temperature biofeedback group and given extensive training in skin temperature self-regulation in a series of 12-15 sessions.

Each of the migraine subjects were subsequently given two measures of regional cerebral blood flow (rCBF) utilizing the non-invasive ^{133}Xe inhalation technique. One rCBF run was given during a relaxation/steady-state condition and a second while subjects were attempting to manipulate their skin temperature in the trained direction.

Extensive records of headache activity were made pre-, during, and post-training. Data will be presented comparing regional intracerebral and extracerebral blood flow measures between relaxation/steady-state and skin temperature self-regulation runs. Comparisons between hand-cooling and hand-warming groups will be made as well as correlations between migraine headache activity, skin temperature control and changes in regional cerebral blood flow.

52 CENTRAL BLOOD PRESSURE REGULATION : POSSIBLE ROLE OF OPIOIDS. Bernard DELBARRE, Danielle SENON*, Monique DUFRAISSE*, Monique DUPONT*. Lab.Chir.Exp.,Fac.Méd. 37032 TOURS CEDEX, FRANCE.

Previous work suggests that cAMP and drugs known to change adenylate-cyclase activity may be involved in the central blood pressure (BP) regulation (SENON, Thesis Poitiers 609, 1976). The possibility that cyclic AMP and cyclic GMP may interact with opioids and prostaglandins is suggested by numerous reports.

In this regard we have investigated the action of these drugs in chloralose-anesthetized (AN) and unanesthetized (UN) cats.
Injection of morphine (20 $\mu g.kg^{-1}$), in the third ventricle of UN cats, induces excitation and a short lasting increase of BP. The same dose of methionine-enkephalin, a pentapeptide which exert analgesic action by interacting with naturally occuring "opiate receptors" induces an increase of BP, and an important raise of heart rate (HR), in the unrestrained cats, but this drug, at the same dose, is ineffective in the third ventricle of AN cats.
A more stable D.alanine derivative of Met.Enkephalin namely D.Ala Met.Enkephalin, at a dose of 20 $\mu g.kg^{-1}$ increases BP, HR and produces important respiratory disturbs ten minutes after its administration in the IIIe ventricle of AN cats.
In the AN cats, PGE_1, at the doses of 2 $\mu g.kg^{-1}$ and 100 $\mu g.kg^{-1}$, induces hypotension, and for the highest dose, a simultaneous raise of HR and respiratory rate.
PGF_2 (1 $\mu g.kg^{-1}$) and arachidonic acid (100 $\mu g.kg^{-1}$) are ineffective on the BP. Baclofen, a structural derivative of GABA, induces a fall of BP, HR and evokes Mayer waves twenty minutes after administration of 20 $\mu g.kg^{-1}$ in AN cats.
Substance P may have dual action in brain, releasing endorphines at very low doses and directly exciting neuronal activity in nociceptive pathways at higher doses (R.C. FREDERICKSON and al., Science 199, 1359, 1978). Injection of 1 $\mu g.kg^{-1}$ of this drug in the third ventricle of UN cats induces a slight increase of BP, and no significative modification of HR, while in AN cats, Substance P (10 $\mu g.kg^{-1}$) produces no significative increase of BP and HR.

PGE_1 and baclofen induce an hypotension. PGE_1 does not affect the synthesis of cAMP (HAVEMANN and al., Naunyn Schmied.Arch. Pharmacol., 302, 103-106, 1978). The analgesic activity of baclofen is different from that of morphine since baclofen analgesia is not antagonized by naloxone (LEVY and PROUDFIT, J.P.E.T., 202, 437, 1977). Morphine and enkephalin as cAMP increase BP and HR. It is known that these drugs induce an increase of cAMP synthesis. These findings add further support to the hypothesis that cAMP may participate in the modulation of central blood pressure regulation.

53 CHANGES IN HIPPOCAMPAL RHYTHMIC SLOW ACTIVITY DURING INSTRUMENTAL CARDIOVASCULAR CONDITIONING IN THE RHESUS MONKEY. Bernard T. Engel*, and J. A. Joseph. NIA, Gerontology Research Center, Baltimore, MD 21224.

Analyses among 4-8Hz electrical activity of the hippocampus (theta, T), heart rate (HR), systolic (S) and diastolic (D) blood pressure and gross bodily movement (M) were carried out during experiments in which three monkeys were operantly conditioned to slow HR, to speed HR or during control periods. Each experiment consisted of a 512 sec. baseline during which all responses were continuously monitored (except M which was time-sampled), and a 2048 sec. performance session during which HR speeding was signaled by a green light, HR slowing by a red light. Correct performance was signaled by a white light, and incorrect performance was negatively reinforced by a 10 ma., .05 sec. shock to the tail on an FI, 8 sec. schedule. Control periods were unsignaled and unreinforced. Each experimental period was divided in 16, 128 sec. blocks and 20 samples were taken from each period as follows: speeding, blocks in which the animal increased HR >4 beats/min from the previous block; and blocks in which the animal speeded less than 4 beats/min from the previous block; slowing in which the animal decreased HR >4 beats/min; and in which the animal decreased HR <4 beats/ min. Equal numbers of blocks were taken from control sessions to match each set of experimental samples. Correlational analyses (Kendall's coefficient of concordance, W; Spearman's rank order correlation, R) showed that there was a high degree of consistency among animals in each response measure across experimental conditions (Ws: HR=.98, S=.92, D=.92, T=.61, M=.81). T was inversely related to each of the other measures during contingent HR slowing when decreases >4 beats/min were examined--i.e., T increased between blocks--but was positively related to the other measures during control periods when HR decreased >4 beats/ min. T also was positively correlated to the other measures during contingent and non-contingent speeding. For example, while HR and M are always highly correlated (R=.87 for all animals) correlations between HR and T and M are significantly increased if the contingent slow condition >4 beats/min is removed (e.g., monkey HR T with slow R=.57, without R=.89 TM R=.45, R=.91 respectively). These results suggest that T reflects both motor and success/failure related components. During contingent speeding the success/failure correlation is masked by the concomitant motor activity, however, during contingency slowing this correlation is revealed because motor activity is reduced while T is increased.

54 A NEW ASCENDING SPINAL CARDIOVASCULAR PATHWAY. A. I. Faden and T. P. Jacobs*, Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20012.

Previous work in this laboratory has demonstrated that increases in blood pressure, heart rate and cardiac contractility may be seen after electrical stimulation of the zona intermedia in the lower thoracic and upper lumbar cat spinal cord and that sites of maximal cardiovascular response (SMCR) can be histologically localized to the intermediolateral nucleus (ILN) (Faden et al, Exp. Neurol. in press; Faden and Woods, Neurology 28:322, 1978). On the other hand, evidence from peripheral nerve stimulation has shown that inotropic and chronotropic responses can only be elicited from the first five thoracic ventral roots. One hypothesis which reconciles the findings from peripheral and central nervous system stimulation is that axons of preganglionic sympathetic neurons may traverse long distances intraspinally before exiting through distant ventral roots. That the changes in heart rate and cardiac contractility are mediated through an ascending intraspinal pathway was shown by making spinal cord transections or hemisections two to four segments rostral to a lower thoracic or upper lumbar SMCR. Such lesions completely blocked the chronotropic and inotropic responses elicited from these more caudal segments. In order to localize this pathway, after SMCR had been identified in the lower thoracic region a second SMCR was located ipsilaterally two or three segments rostrally. Just lateral to this rostral site a progressively enlarging electrolytic lesion was made until the cardioacceleratory response from the more caudal site had been markedly reduced or abolished. The location of the rostral electrode was chosen to correspond to Bok's intermediolateral fasciculus. In two animals the rostral lesions completely blocked the cardioacceleratory response and these lesions involved the white matter immediately adjacent to the ILN. Two other lesions resulted in a marked reduction but not ablation of the cardioacceleratory response; interestingly these lesions spared small areas of white matter adjacent to the ILN. In all animals the superficial white matter of the dorsolateral funiculus as well as the region of the apex of the dorsal horn were spared. These findings suggest the existence of an ascending intraspinal cardiovascular pathway which is located in the deep white matter adjoining the ILN. The recent anatomic demonstration of an intraspinal sympathetic preganglionic pathway (ISPP) (Faden and Petras, Brain Res. 144: 358-362, 1978) with a suspected localization corresponding to that of the cardiovascular pathway described in these experiments, strongly suggests that the ascending cardiovascular pathway and the ISPP are the same.

55 LOCALIZATION OF CARDIAC VAGAL PREGANGLIONIC SOMA. G. Steven Geis and Robert D. Wurster. Department of Physiology, Loyola University of Chicago, Stritch School of Medicine, Maywood, Illinois 60153.

Horseradish peroxidase (HRP) was injected subepicardially in anesthetized cats for retrograde labeling of cardiac vagal preganglionic soma. The injections were made in 3 groups of animals: (1) after left cervical vagotomy (LCV), (2) after right cervical vagotomy (RCV) and (3) after combined left cervical vagotomy and section of the right cranial and caudal cardiac vagal branches (LCV-CCX). After a 48 hr survival period the cats were reanesthetized, perfused and fixed. The brain stems were removed, cut in 40 µm serial cross-sections and developed with 3,3'-diaminobenzidine tetrahydrochloride.

Approximately 350 labeled soma were identified in brain stems of cats with LCV and RCV. Distribution and morphological characteristics of the cell bodies were common among the animals. The soma were ipsilateral to the intact vagus, extending 2.8 mm caudal and 4.8 mm rostral to the obex. Distinct aggregations of cell bodies were apparent in the nucleus ambiguus (NA), the dorsal motor nucleus of the vagus (DMNX) and an intermediate zone (IZ) between the NA and DMNX. In each animal about 78% of the soma were in the NA while 17% and 5% were located in the DMNX and IZ, respectively. Approximately 72% were fusiform and 28% were round in shape. Fusiform soma had an average long axis of 28.27 µm and an average short axis of 16.96 µm. The round cell bodies demonstrated a diameter of 22.29 µm. About 27 soma were labeled in each LCV-CCX cat. It is therefore concluded that 6.7% of the identified cells in LCV and RCV animals were associated with non-cardiac tissue or the result of HRP uptake by axons traveling to the heart by vagal branches other than the cranial and caudal cardiac vagal branches.

The data demonstrate the presence of cardiac vagal preganglionic soma in the NA, DMNX and IZ. The cell bodies are predominately fusiform in shape and give rise to axons exiting the ipsilateral brain stem. The separate populations of labeled cells suggest the possibility that different cardiac vagal nuclei modulate different myocardial functions. (Supported by NIH grant HL08682.)

56 EFFECTS OF DIENCEPHALIC AND BRAINSTEM LESIONS ON HAEMOPOIETIC STEM CELLS

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A functional axis between the central nervous system and bone marrow has been postulated based upon several types of evidence. Haemopoietic cells possess receptors for certain neurotransmitter substances, nerve endings not associated with blood vessels are present in bone marrow and haemopoietic cells exhibit a light responsive circadian rhythm. To test for the existence of a brain-bone marrow axis, C57BL/6J and Swiss Webster mice received bilateral electrolytic lesions (300 µA anodal current for ten seconds) of the anterior hypothalamus, posterior hypothalamus, locus coeruleus or cerebellum. A standard in vivo technique was used to assess the number of haemopoietic stem cells. Six weeks after lesioning, the animals were exposed to a sub-lethal dose of radiation which stimulated the formation of colony forming units on the surface of the spleen (CFU-S). Only those subjects that had received locus coeruleus lesions had significantly reduced numbers of CFU-S when compared with control values. Macro-autoradiography following the injection of Fe^{59} revealed many of these colonies to be erythrocytic. An in vitro clonal assay was used to assess the number of granulocytic progenitor cells. Bone marrow cells seeded into either soft agar or methyl cellulose produced significantly reduced numbers of granulocyte macrophage colonies (GM-CFC) when the donor animals had received locus coeruleus lesions. This reduction was more pronounced in animals with bilateral lesions than in animals with unilateral lesions. Preliminary evidence suggests that this effect can be reversed by administering amphetamines. Animals with locus coeruleus lesions were also found to have lower peripheral blood white cell counts when compared with controls, however, the lesions had no effect on the differential cell count. Haemaglutinating antibody titers five days after the injection of sheep red blood cells were not affected by the locus coeruleus lesions.

These data suggest an interaction between the nucleus locus coeruleus and haemopoietic stem cells in the bone marrow. The mechanism by which this interaction is able to occur is currently under investigation.

This is Tumor Biology publication number 127

57 PUTATIVE SHORT-LATENCY BAROSENSORY CIRCUIT LINKING THE BARORECEPTORS, CAUDAL DIENCEPHALON, AND DORSAL VAGAL NUCLEUS IN RABBITS. ROBERT B. HAMILTON, JEFFREY H. WALLACH, GUY K. PETRIK,* AND NEIL SCHNEIDERMAN. Dept. of Psychology, Univ. of Miami, Coral Gables, Florida 33124.

Electrical stimulation (10 sec trains, 100 pulses/sec, 0.5 msec pulse duration, <180 µA) of specific areas within anterior hypothalamus, lateral hypothalamus, rostral zona incerta (at the thalamus-hypothalamic border), and lateral zona incerta of the subthalamic region in barbiturate anesthetized rabbits elicited primary bradycardia (M= -119 bpm; SD= ±10) accompanied by only a small decrease in mean arterial pressure (M= -6.0mm Hg; SD= ±8.3). These responses were abolished by bilateral vagotomy, but were not significantly attenuated by unilateral section. Single-pulse aortic nerve (AN) stimulation (pulse duration 0.1 msec) activated neurons, thereby defined as barosensory-sensitive, in the above areas. Electrical stimulation (10 sec train duration, 100 pulses/sec, 0.1 msec pulse duration < 35 µA) was used to identify the cardioinhibitory region of the dorsal vagal nucleus (DVN) of the medulla. Single-pulse stimulation of this region activated units in the rostral and lateral zona incerta at latencies under 10 msec. These same units were also activated by single-pulse AN stimulation at latencies as short as 4 msec. Stimulation of bradycardia-sensitive sites in the rostral and lateral zona incerta in the same region where barosensory-sensitive units were found, resulted in the activation of neurons in the bradycardia-sensitive area of DVN, which were also activated by AN stimulation. Mean onset latency of the response of these DVN units to zona incerta stimulation was 6 msec (SD= ±1 msec) (Kaufman, Hamilton, Wallach, Petrik, & Schneiderman, in preparation).

The present data suggest that the time period for barosensory information to reach the caudal diencephalon, and for information from this region to project back to the cardioinhibitory region of DVN may be as short as 15 msec or less. Our findings are also consistent with the possibility that a feedback circuit may exist linking the cardioinhibitory region of DVN with the zona incerta (Supported by NSF Grant BMS 75-10967 and by grants from the Heart Association of Greater Miami and Florida Heart Association).

58 INTRACELLULAR RECORDINGS FROM THE INFERIOR MESENTERIC GANGLION OF THE CAT. Yvon Julé* and Joseph H. Szurszewski. Dept. Physiol. & Biophysics, Mayo Med. Sch., Rochester, MN 55901.

Intracellular, in vitro recordings have been obtained from more than 200 cells of the inferior mesenteric ganglion (IMG) of the cat. Two types of cells were impaled: inexcitable and excitable cells. Ten percent of the cells impaled were inexcitable cells. They had a high resting membrane potential (up to 75 mV) and they never produced an action potential either to direct intracellular depolarizing current or to preganglionic nerve stimulation. In these cells, repetitive stimulation of a preganglionic nerve trunk gave rise to a slow depolarization which was frequency dependent. The inexcitable cells found in this study have properties in common with glial cells of the central nervous system. Ninety percent of the cells impaled were excitable cells. They received excitatory synaptic input from preganglionic nerve fibers and produced action potentials in response to a direct intracellular depolarizing current. Their resting membrane potential ranged from -45 to -85 mV (mean -56 mV), their input resistance ranged from 20 - 280 MΩ (mean 80 MΩ) and the membrane time constant ranged from 2 - 12 msec (mean 8 msec). Action potentials in these cells often had an overshoot of 10 to 25 mV. An afterhyperpolarization up to 20 mV and lasting 150 - 300 msec followed each action potential. In 50% of the excitable cells tested long intracellular depolarizing pulses (up to 1 sec) triggered a phasic discharge of action potentials; in 40% of the cells there was a tonic discharge. The excitable cells have properties in common with principal ganglion cells found in prevertebral ganglia of the guinea pig. Excitatory synaptic input was recorded from principal ganglion cells in response to stimulation of hypogastric, intermesenteric, inferior splanchnic and colonic nerves. More than 55% of ganglion cells received input from all extrinsic nerves. This suggests that marked convergence of central input and peripheral input occurs on ganglion cells in the IMG of the cat. (Supported by NIH Grant AM 17632.)

59 METABOLIC MAPPING OF NEURAL PATHWAYS INVOLVED IN GASTROSECRETORY RESPONSE TO INSULIN HYPOGLYCEMIA. Massako Kadekaro, Helen E. Savaki* and Louis Sokoloff. Lab. of Cerebral Metabolism, NIMH, Bethesda, MD 20014.

It has been known since 1927 that hypoglycemia induced by insulin increases gastric secretion. This response is mediated by the central nervous system inasmuch as vagotomy completely prevents its occurrence. Little is known, however, about the neural pathways involved in this response. The objective of the present studies was to attempt to identify these neural pathways by means of the recently developed [^{14}C]deoxyglucose method.

Fifteen albino Sprague-Dawley male rats weighing between 340-430 g were studied. Each animal was initially prepared by surgical construction of a chronic gastric fistula. One week after the surgery the animals were fasted for 18-24 hours and then anesthetized with urethane injected intraperitoneally at a dose of lg/kg. A femoral artery and vein were then catheterized. Basal gastric secretion was collected for 30 min after which insulin was administered intravenously at a dose of 0.05-0.3 units, depending on the initial plasma glucose concentration. Collection of gastric secretion continued for another 90 min. In control experiments saline was injected instead of insulin. Forty-five min after the injection of the insulin or saline a pulse of [^{14}C]deoxyglucose was administered at a dose of 125 μCi/kg. During the following 45 min timed arterial blood samples were drawn for assay of the plasma [^{14}C]deoxyglucose and glucose concentrations. At the end of the 45 min period the animals were decapitated, and the brains processed for quantitative autoradiography. During the experiments blood pressure, body temperature and blood gases were monitored. Local cerebral glucose utilization was quantitatively determined as previously described (Sokoloff et al., J. Neurochem. 28:897, 1977).

The results showed that in fourteen of the eighteen structures examined, including, for example, the ventromedial medial nucleus of the hypothalamus, globus pallidus, zona incerta and n. ambiguus, the glucose consumption remained unchanged over the 84-215 mg% range of plasma glucose concentrations. There were, however, significant inverse correlations between plasma glucose concentration and rate of glucose utilization in the n. solitary tract, perifornicial area, dorsal motor nucleus of the vagus and the superior olivary nuclei. With the exception of the superior olivary nuclei all the structures affected by low plasma glucose concentrations have been shown to be involved in the gastrosecretory response to the blockade of glucose utilization provoked by pharmacological doses of 2-deoxy-D-glucose (Kadekaro et al., Neuroscience Abstracts 29:21, 1977) suggesting that these same nuclei are also activated by hypoglycemia.

60 BARORECEPTORS, CATECHOLAMINES, NUCLEUS SOLITARIUS, AND THE AREA POSTREMA: A CARDIOVASCULAR CONNECTION? David M. Katz and Harvey J. Karten, Depts. of Biology (DMK) and Psychiatry (HJK), S.U.N.Y., Stony Brook, N.Y. 11794

The anterograde transport of horseradish peroxidase (HRP) was used to visualize the central projections of the aortic arch depressor nerve (DN) to the nucleus solitarius (nS) in pigeons. The distribution of DN afferents was compared with the distribution of monaminergic neurons within nS as revealed by fluorescence histochemistry. The relationship between the DN projection area and the area postrema (AP) was also examined.

HRP was applied to the proximal cut end of the aortic depressor nerve by means of a small implanted chamber fastened around the nerve stump. Following 2-3 day survival times, HRP granules were seen in neurons in the nodose ganglion and within axons in the tractus and nucleus solitarius. HRP labelling within nS was confined to the neuropil of only one nuclear subgroup, the subnucleus sulcalis dorsalis (Sd). Sd lies dorsal to the tractus solitarius and extends from 0.8 to 2.0 mm rostral to the obex. The caudal portion of Sd lies below the floor of the fourth ventricle at the point of attachment of the taenia choroidea.

The region surrounding the taenia chorcidea had previously been suggested to encompass the area postrema in birds (Moll,J. & Hilvering,C.,Proc.Konin.Neder.,54,1951). Because of the proximity of this region to Sd, we decided to more precisely define the extent of the area postrema in the pigeon using intravenous injections of HRP(Broadwell,R.D.,&Brightman,M.W.,J.Comp.Neur.,166,3,1976). This technique revealed that the rostral portion of the area postrema lies immediately adjacent to the caudal portion of Sd.

Combined glyoxylic acid/paraformaldehyde-induced fluorescence revealed that Sd is extremely rich in catecholamine-containing neurons. Except for a more extensive rostro-caudal distribution, the fluorescent neurons within Sd overlapped precisely with the DN projection area. Fluorescent fibers extended from the caudal portion of Sd into the adjacent area postrema and taenia choroidea.

These data indicate that depressor nerve afferents have a restricted distribution within the nucleus solitarius in pigeons. Our data also provide strong evidence for the involvement of catecholaminergic neurons within nS in a central cardiovascular sensory pathway. In addition, our data suggest an anatomical association between tha area postrema, which lacks a blood-brain barrier, and the DN projection area within nucleus solitarius. Functional connections between these two regions may be partly responsible for the cardiovascular effects of blood-borne substances, such as angiotensin 2, which are mediated by the central nervous system (Joy,M.D., and Lowe, R.D., Nature, 228,1970).

Grant support: NS-12078 to HJK

61 INTESTINAL BLOOD FLOW AND MOTILITY ACCOMPANYING INCREASED SYMPATHETIC OUTFLOW INDUCED BY BULBAR STIMULATION AND A SPINAL REFLEX. K. Alan Kelts, John W. Oehlert*, and Julie Johns*. Dept. of Neuro., Sch. Med., Stanford Univ., Stanford, CA 94305.

Efferent postganglionic sympathetic nerve activity may be increased by electrical stimulation of the "pressor" area in the medulla or by activation of spinal reflex arcs. The aim of this work was to assess changes in intestinal motility and blood flow associated with the increasing nerve activity during those two stimuli. Fifteen cats were anesthetized with chloroform and chloralose-urethane (40 - 80 mg/kg). A proximal jejunal loop was prepared for recording intraluminal volume (GV), efferent mesenteric nerve activity (NA), and venous blood flow (BF). A femoral artery was cannulated for blood pressure (BP). Further preparation involved craniectomy for 60 Hz electrical stimulation of the dorsomedial bulbar reticular formation (RFS) or cannulation of a more proximal jejunal loop for mechanical stimulation of an intestino-intestinal inhibitory reflex (I_3-R). Control and stimulation periods were divided into 6 sec intervals, data quantitated (including integration of NA and calculation of vascular resistance, VR), and significance of changes assessed by a 2-tailed T-test. RFS produced significant increases in NA ($p < 0.0005$) and in GV ($p = 0.001$) during the first 6 sec, and in VR ($p = 0.01$) after 10 - 18 sec. I_3-R's resulted in significant increases in NA ($p < 0.0005$) and in GV ($p = 0.001$) within 6 sec, but no significant change in BF. Therefore, both RFS and I_3-R produced increased mesenteric sympathetic efferent discharge and intestinal relaxation, but only RFS caused a significant increase in vascular resistance. These data suggest separate populations of efferent sympathetic fibers passing in mesenteric nerves to intestinal and vascular smooth muscles. Moreover, these populations may be activated independently by different stimuli.

62 PATTERNS OF SINGLE UNIT ACTIVITY IN SYMPATHETIC POSTGANGLIONIC NERVES (CARDIAC AND VERTEBRAL NERVES). Mark Kollai and Kiyomi Koizumi. Dept.of Physiol., State University of New York, Downstate Medical Center, Brooklyn, N.Y. 11203.

Activity patterns of single postganglionic fibers of inferior cardiac and vertebral nerves were analyzed in chloralose anesthetized and artificially ventilated cats. They were divisible into three categories according to firing patterns. The first group, 72% of cardiac fibers and 38% of vertebral nerve fibers showed rhythmic discharges which were clearly synchronized with phrenic activity during normal ventilation. Other fibers of this group originally showing irregular firing patterns became synchronized with phrenic activity during slight hypoventilation or chemoreceptor stimulation. Although only a few of these fibers showed activity synchronized with cardiac cycle pulses all were strongly affected by baroreceptor excitation. The second group, 38% of vertebral but none of the cardiac fibers, showed very regular discharges which were not related to phrenic nor to cardiac rhythms. Interspike intervals ranged from 200 msec. to 10 sec. and the discharge frequency was very constant in any given fiber for a considerable period of time; occasionally in some units the frequency shifted. The third category, 28% of cardiac and 24% of vertebral nerve fibers, showed irregular firing patterns which could not be made synchronous with phrenic nor cardiac rhythms by various maneuvers. They were unaffected by activation of baroreceptors but increased their activity greatly under asphyxia. It is hoped that such analyses of single postganglionic fibers will give us more information concerning the properties of postganglionic neurons as well as the function of the autonomic ganglia. (Supported by grants from USPHS #NS00847 and New York Heart Association.)

63 INHIBITION OF CARDIOPULMONARY SYMPATHETIC EFFERENT NERVE ACTIVITY DURING LUNG INFLATION IN THE CANINE. David R. Kostreva and John P. Kampine*. Depts. Physiol. and Anesthesiol., Med. College of Wisconsin and Wood VA Ctr., Milwaukee, WI 53193

Positive pressure lung inflation in open chested dogs resulted in short latency inhibition of cardiopulmonary sympathetic efferent nerve activity (CPSENA) before and after bilateral vagotomy. Mongrel dogs, 20-30 kg, were anesthetized using sodium pentobarbital, 35mg/kg i.v. The animals were intubated and placed on positive pressure ventilation. The chest was split transversely between the second and third ribs, to allow unimpeded access to both stellate ganglia and adjacent ansae subclaviae. CPSENA was recorded from the cut central end of one of the left or right ansae. The CPSENA was amplified, filtered and time averaged using a half-wave rectifier. Central venous pressure and systemic blood pressure were monitored from the femoral vein and artery respectively. Tracheal pressure (TP) was monitored from a large bore needle inserted into the endotracheal tube. The electrocardiogram (ECG) was monitored from leads placed in a lead II configuration. Averaged CPSENA, ECG and the pressures were recorded using a polygraph.

Prior to vagotomy, lung inflation to 15 mmHg TP resulted in a marked inhibition of CPSENA traversing the left and right ansae subclaviae. At the onset of expiration, CPSENA immediately returned to or momentarily exceeded pre-inflation levels. Each inflation consistantly resulted in a depression of CPSENA. Immediately following bilateral vagotomy, baseline CPSENA decreased markedly. However, within one minute post-vagotomy, each inflation continued to produce a short latency inhibition of CPSENA. The degree of inhibition of CPSENA in most animals was attenuated after vagotomy. This may have been due to the marked decrease in baseline CPSENA produced by vagotomy. Bilateral sympathectomy by sectioning the remaining intact ansae resulted in a further depression of baseline CPSENA. Lung inflation to 15 mmHg TP after sympathectomy did not result in an inhibition of CPSENA. However, in several animals the ventilator was shut off for several minutes and the animals attempted to breath spontaneously. During expansion of the chest, CPSENA was immediately depressed without lung inflation. This study suggests that either chest wall somatic afferents or central respiratory centers can inhibit CPSENA. This study has also provided some evidence indicating that some vagal and sympathetic cardiopulmonary afferents can have an excitatory effect on CPSENA while other vagal and sympathetic cardiopulmonary afferents can have an inhibitory effect on CPSENA. (Supported by Grant HL 16511 and Young Investigator Research Award HL 21042 from HLBI, and the Medical Research Service of the VA.)

64 IDENTIFICATION OF PATHWAYS IN THE ABDOMINAL PREVERTEBRAL GANGLIA WITH RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE. David L. Kreulen* and Joseph H. Szurszewski. (SPON: J. C. Lynch). Dept. Physiol. & Biophysics, Mayo Med. Sch., Rochester, MN 55901.

Electrophysiologic experiments on the abdominal prevertebral ganglia of guinea pigs indicate that there is viscerotopic organization of the postganglionic outflow of these ganglia to the colon. The celiac ganglia (CG), the superior mesenteric ganglion (SMG) and the inferior mesenteric ganglion (IMG) were dissected as a single preparation, pinned to the floor of an organ bath, and superfused with oxygenated Krebs at 37° C. Two major groups of nerve fibers leave the ganglia for the periphery: the celiac nerves and the lumbar colonic nerves. The celiac nerves project from the more cephalad CG while the lumbar colonic nerves project from the more caudad IMG. Solid horseradish peroxidase (HRP) was placed on crushed regions of these mesenteric nerve trunks 5 to 8 mm from the ganglia. After 1 - 6 hours the preparation was fixed and 42 μ thick frozen sections of all the ganglia were processed in benzedine. When HRP was placed on the lumbar colonic nerves, labelled neurons were found in the IMG, SMG and CG. However, labelling appeared more dense in the IMG than in the other ganglia. This indicates that while most of the axons in the lumbar colonic nerves have their cell bodies in the IMG some of the axons come from CG and SMG. When HRP was placed on the celiac nerves, labelled neurons were again found in all the abdominal prevertebral ganglia; however labelling was most dense in the CG and least dense in the IMG. This indicates that some of the axons in the celiac nerves are from cell bodies located in the IMG. These experiments show that while there is cephalad-caudad distribution of postganglionic outflow from the abdominal prevertebral ganglia there is some crossover of this outflow. That is, some neurons located in the cephalad ganglia pass through the caudad ganglia and out caudad nerve trunks. Also, some neurons located in the caudad ganglion pass through the cephalad ganglia and out the cephalad nerve trunks. This organization may be important for mediating reflexes between viscera in different regions of the abdomen. (Supported by Grants AM 17632 and T32 RL 7111-02.)

65 AUGMENTATION OF SYMPATHETIC VASOMOTOR AND RESPIRATORY NEURAL DISCHARGES BY A DERIVITIVE OF γ-AMINOBUTYRIC ACID. Peter M. Lalley. Dept. Physiology, Sch. Medicine, U. Wisconsin, Madison, WI 53706

A derivitive of γ-aminobutyric acid (Lioresal^{R}, Ciba-Geigy) was found in the present study to augment neural discharges recorded from sympathetic vasomotor fibers, and from intercostal motoneurons in cats anesthetized with urethane, pentobarbital or chloralose. Under control conditions, conditioning electrical stimulation of depressor fibers in the aortic nerve (AN) or cervical vagus nerve (XN) abolished the reflex discharges evoked in sympathetic nerves by single test shocks applied to the carotid sinus nerve (CSN). After injections of lioresal (0.5-2 mg/kg I.V.), CSN-evoked sympathetic discharges were markedly augmented by conditioning AN or XN stimulation. Similar effects were produced by supplemental subanesthetic doses (5-10 mg/kg I.V.) of pentobarbital or methohexital. Lioresal or the barbiturates also caused further enhancement of the post-tetanic facilitation of CSN-sympathetic reflexes which followed stimulation (30-50 Hz) of the ipsilateral or contralateral CSN. Lioresal also increased (25-50 mmHg) resting mean blood pressure in several experiments, in concert with an increase in spontaneous sympathetic neural discharges. In other experiments, intercostal expiratory motoneurons, identified by antidromic activation of axons in the internal intercostal nerves, also increased their rate of spontaneous discharge following lioresal administration. The effects on sympathetic discharges are consistent with the results of an earlier study (Lalley, 1978) in which lioresal consistently converted the hypotensive episodes evoked by CSN, AN or XN stimulation to pressor responses. The depressor responses were temporarily restored by picrotoxin or bicuculline, but not by strychnine or metrazol. The neuronal mechanisms responsible for these unexpected effects, including a possible GABA-mimetic action, are currently under investigation. (Supported by American Heart Association Grant No. 75 899.)

66 FIRING PATTERNS OF PREGANGLIONIC NEURONS AND INTERNEURONS IN THE SACRAL AUTONOMIC NUCLEUS OF THE CAT. R.J.Milne*, A.M.Booth* and W.C.deGroat, Dept. Pharmacol., Univ. of Pittsburgh, School of Medicine, Pittsburgh, PA 15261

Extracellular unit recordings were obtained from neurons in the region of the sacral autonomic nucleus of anesthetized cats. Preganglionic neurons of two types were identified by antidromic invasion in response to stimulation of the sacral ventral roots. Preganglionic neurons which were fired by rectal stimulation or during spontaneous colonic contractions exhibited slow axonal conduction velocities (less than 2 m/sec), whereas preganglionic neurons activated by distension of the urinary bladder or during spontaneous bladder contractions had fast axonal conduction velocities (3-10 m/sec). The mean firing rate of bladder preganglionic neurons increased monotonically from 0 to a maximum of 2-5 Hz when bladder pressure was raised from 0 to 30-40 cm H_2O pressure. At low bladder pressures, bursts of spikes preceded and corresponded to brief rhythmic bladder contractions. At higher pressures the interval between rhythmic contractions decreased and firing became continuous. Over the same range of bladder pressures the frequency of bladder contractions was proportional to the mean rate of firing of preganglionic neurons. The preferred interspike interval (range, 25 to 125 ms), as defined by the peak of the time interval histogram, was generally independent of the mean firing rate.

Interneurons exhibiting firing rates which were correlated with bladder activity were also encountered, generally slightly dorsal to the location of the preganglionic neurons. At zero pressure, while bladder preganglionic neurons were quiescent, these interneurons maintained a slow rhythmic pattern of firing. At higher pressures the firing rate increased linearly with bladder pressure and the firing became essentially continuous. The preferred interspike interval was similar to that of preganglionic neurons and was independent of the mean firing rate. Bladder interneurons were inhibited by antidromic activation of the ventral roots and by rectal stimulation.

In summary, preganglionic neurons in the micturition reflex pathway respond to afferent input from the bladder with bursts of activity. The frequency of firing within bursts is independent of bladder pressure, whereas the interval between bursts is directly related to bladder pressure. Excitatory input to preganglionic neurons may be mediated by sacral interneurons which also fire in conjunction with bladder contractions. Inhibition of these interneurons may be responsible in part for recurrent inhibition in the sacral outflow to the bladder as well as inhibitory interactions between intestinal and bladder reflex pathways.

67 IDENTIFICATION OF VISCERAL AFFERENTS TO THE SACRAL CORD OF THE CAT USING HORSERADISH PEROXIDASE. Charles Morgan, William C. de Groat, Irving Nadelhaft. Dept. Pharmacol., Univ. of Pittsburgh Sch. of Med. and V.A. Hospital, Pittsburgh, PA 15261

The parasympathetic preganglionic efferents to the pelvic viscera leave the sacral spinal cord and travel together in the pelvic nerve before separating to innervate the bladder, colon, and sex organs. Afferents from these organs also travel in the pelvic nerve and enter the spinal cord in dorsal roots S1,2,3. By cutting the pelvic nerve, exposing the central portion to a 25% solution of HRP and allowing 35-60 hrs. transport time, we have been able to label both the efferents and afferents from the pelvic viscera to the sacral cord. Frozen sections of spinal cord (42 μ) were processed in benzidine and examined with darkfield illumination. Preganglionic neurons of the sacral parasympathetic nucleus were observed in 7 cats. The distribution of HRP reaction product in primary afferent (PA) axons or terminals is shown on the right in Figure 1. The PA were observed primarily in the dorsal root entry zone, Lissauer's tract, the apex and lateral edge of the dorsal horn (DH) in the marginal layer often continuing into the region of the dorsal band of the SPN. Less frequently PA were also found on the medial side of the DH. The position of the SPN at the mid-S2 level is shown on the left in Figure 1.

Cells of the dorsal SPN often send their dendrites along the lateral edge of the DH possibly to meet incoming afferent fibers. In order to determine that the PA in that region were not actually preganglionic dendritic processes, the sacral ventral roots in one cat were cut before exposing the pelvic nerve to HRP. The distribution of PA was identical to that observed in the other cats.

In another cat the direct exposure of the S2 dorsal root to HRP showed, in addition to the commonly described afferent path through the medial DH, an extremely heavily labelled axonal path along the lateral DH, identical in position to the distribution of PA observed in the pelvic nerve experiments. Many afferent terminals labelled so strongly that they outlined the shapes of the cells with which they synapsed.

We conclude that pelvic visceral afferents to the sacral spinal cord reach the region of dorsal band of the parasympathetic nucleus by traveling primarily along the lateral edge of the dorsal horn.

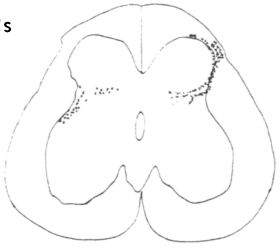


Fig. 1, Left, SPN. Right, PA.

68 BARORECEPTOR REFLEX GAIN DURING HYPOTHALAMIC ACTIVATION IN SPONTANEOUSLY HYPERTENSIVE(SHR) AND NORMOTENSIVE(WKY) RATS. S. Morrison and D. Whitehorn, Dept. of Physiology and Biophysics, Univ. of Vermont, College of Medicine, Burlington, Vt. 05401

Baroreceptor reflex mechanisms, acting at spinal and medullary levels attenuate changes in blood pressure (BP) by inhibiting sympathetic activity (SA). Stimulation of posterior hypothalamus (PH) produces sudden increases in SA and BP which are larger in the SHR than the WKY. This could be explained by a diminished baroreceptor reflex effectiveness in the SHR. To test this hypothesis we have measured the gain of the baroreceptor reflex during a step increase in hypothalamic activity.

Rats (10 WKY,8 SHR) anesthetized with alpha-Chloralose (100mg/kg) were instrumented with a femoral arterial cannula, a bipolar recording electrode on the preganglionic splanchnic nerve (SA) and a concentric bipolar stimulating electrode in the PH (AP4.5, ML .5,DV2.5). Square wave pulses (0.1 msec duration) were applied to the PH for 3 second periods at 60 or 100 Hz., at twice the intensity required to produce a discernible (5mm) change in BP. After 6 periods of stimulation at each frequency sinoaortic denervation was performed and stimulation repeated.

In the intact preparation, SA changes during stimulation exhibited three phases: an initial rapid rise beginning 65 msec and ending 150 msec after stimulation onset; a short, rapid fall (150-400 msec); and a third phase (400-2000 msec) during which SA declined more slowly. The accompanying BP increase occurred during the third phase, reaching a sustained peak at 2-2.5 seconds after stimulation onset.

After denervation the third phase was no longer present and can thus be attributed to baroreceptor reflex inhibition of SA. Denervation also resulted in a rapid increase in the level and lability of resting SA and BP. Significant differences in the extent of these increases could not be demonstrated between SHR and WKY. The SA and BP response to PH stimulation was greater in each animal after denervation, although the increase in SA during the first phase, expressed as percent of control changed only slightly and continued to be larger for SHR's.

In the intact animal, the baroreceptor reflex gain was measured as the slope of the relationship between SA(percent control) and BP during the third phase. The reflex gain was the same during 60 and 100 Hz. stimulation. The gain in the SHR ($5.58 \pm .72$) was significantly greater ($p<.01$) than that in the normotensive rats ($2.20 \pm .38$).

We conclude that the hyperresponsiveness of the SHR to PH stimulation is not due to a reduced baroreceptor reflex effectiveness. The results are expressed in a quantitative model of certain medullary and spinal components of the cardiovascular control system. (Support from Vt. Heart, 75-333).

69 THE ROLE OF THE ANTEROVENTRAL THIRD VENTRICLE (AV3V) IN DEVELOPMENT OF NEUROGENIC HYPERTENSION. Michael T. Mow*, J.R. Haywood*, A.K. Johnson, and Michael J. Brody* (SPON: W.W. Kaelber). Depts. of Pharmacology, Psychology, and Cardiovascular Center, The University of Iowa, Iowa City, Iowa 52242.

The region of periventricular tissue aroung the AV3V has been identified as the central site at which intraventricularly administered angiotensin produces its dipsogenic and pressor responses in the rat. Lesioning the AV3V region prevents the development of 1- and 2-kidney models of renal hypertension as well as DOC-salt hypertension, an experimental model with suppressed renin levels. The present experiments were conducted in an effort to determine whether the AV3V is also involved in the development of neurogenic hypertension produced by lesioning of the nucleus tractus solitarius (NTS), the brain stem region where baroreceptor afferents terminate. This form of hypertension is acute and within 4-5 hours of lesioning, rats die from pulmonary edema and heart failure. Electrolytic lesions of the NTS were performed under ether anesthesia on two groups of rats prepared 3-4 weeks earlier; one group which had received AV3V lesions and the other group which had received sham AV3V lesions. Blood pressures were recorded in the conscious state from indewlling arterial catheters and were monitored before and several hours following lesioning of the NTS. Lesioning of the NTS produced hypertension in both groups of animals. However, in the group with AV3V lesions, blood pressure rose only 40 mm Hg compared to a peak increase of 74 mm Hg in the sham lesioned group. Furthermore, the AV3V lesioned animals showed no signs of pulmonary edema. These data demonstrate that the region of the AV3V plays an important role in neurogenic hypertension. Since hypertension produced by NTS lesions is dependent upon the integrity of structures lying above the midbrain (mid-collicular decerebration will abolish and prevent NTS hypertension; Doba and Reis, Circ. Res. 32: 584, 1973), it appears that the AV3V may be a portion of long loop reflex arcs existing between the anterior hypothalamus and brainstem.

70 EFFERENT CARDIAC PROJECTIONS OF THE RIGHT STELLATE GANGLION IN THE CAT. Michael L. Niehoff* and James M. Sullivan* (SPON: P.A. Young). Dept. Anat., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Fluorescence microscopy has demonstrated extensive adrenergic periarterial plexuses around the coronary arteries. The sinoatrial and atrioventricular nodes have also been shown to be richly innervated. Using autoradiographic techniques, the present study has attempted to demonstrate the distribution of the postganglionic sympathetic cardiac fibers originating in the right stellate ganglion. A total of 500 microcuries of tritiated leucine was injected into the right stellate ganglion of the cat. After a three day postoperative survival period the animals were sacrificed. Transverse and longitudinal serial sections were made of the heart, great vessels, and trachea. The majority of the right stellate ganglionic fibers traveled between the trachea and aortic arch, and entered the cardiac plexus. Passing through the plexus these fibers coursed between the ascending aorta and the pulmonary arterial trunk to reach the left coronary artery. Periarterial and subepicardial plexuses then arose from these nerves. Most fibers formed a periarterial plexus which followed the distribution of the ventral descending branch of the left coronary artery. A few followed the circumflex artery. The conspicuous subepicardial plexus formed was most prominent in the cranial half of the left ventricle. Other fibers arising from the right stellate ganglion descended between the cranial vena cava and the right branch of the pulmonary artery. These ramified on the dorsal surface of the right atrium, and formed a large bundle which passed caudally through the interatrial septum. In the caudal part of the septum they turned ventrocranially and ascended in the septum for a short distance. They then ramified among the septal myocardial cells. In conclusion, sympathetic postganglionic fibers from the right stellate ganglion projected mainly to the left side of the heart. Periarterial and subepicardial plexuses were formed along the left ventricular myocardium. Other projections formed a subepicardial plexus in the right atrium and ramified in the interatrial septum.
(Supported in part by USPHS grant FR 05388.)

71 CONTROL OF THE RESPIRATORY CYCLE BY THE MIDBRAIN RETICULAR FORMATION. John Orem and Ralph Lydic. Dept. Physiol., Texas Tech Univ. Sch. Med., Lubbock, TX 79409.

Several studies have shown that the midbrain reticular formation (MRF) influences breathing. The MRF is essential to waking consciousness, and its role in breathing may pertain to the wakefulness stimulus for respiration. We demonstrate here that the MRF can control the respiratory cycle independent of other known control systems (the pneumotaxic centers, vagal and intercostal reflexes).

Twelve anesthetized (Nembutal, 35 mg/kg) cats (Ss) were used. Stimulation of the MRF (50 pulses, 0.5 msec, 200 cps) during expiration (E) caused a phase-switch to inspiration (I). In some cases stimulation of the same site also produced I-to-E switching. Phase-switching was easier the greater the stimulus intensity and the longer the delay after the end of I or E (if testing for E-to-I or I-to-E switching respectively). This was expressed as: Switch Factor = Log % Response (1/mA·s) where "% Responses" was the percentage of switches at a given intensity-delay combination; "mA" was the stimulus intensity in milliamps, and "s" was the delay in seconds after which the stimulus was applied. The factor was fairly constant at all intensity-delay combinations. For E-to-I, the average factor across animals was 12; I-to-E factors ranged from 6 to 14. After extensive bilateral pontine lesions (the pneumotaxic centers) the duration of I (determined from diaphragmatic e.m.g.s) increased slightly from a mean of 1.74 to 2.0 across Ss, but the duration of E greatly lengthened from a mean of 4.3 to 20.3 s. Inspiration was initiated by MRF stimulation with an average switch factor of 1.5. Stimulation of the same MRF sites produced I-to-E switching in every case (average factor = 2.0). When pontine lesions were combined with spinal cord transection at C8 and bilateral vagotomies, rhythmic breathing ceased. Inspiration or expiration, once begun, did not spontaneously terminate. However inspiration could be initiated (factor = 1.1) and terminated (factor = 0.6) by stimulation of the MRF. In these cases, MRF stimulation provided complete control of respiration.

We conclude that, although phase-switching becomes more difficult with progressive lesions, the MRF can control breathing independent of the pneumotaxic centers and intercostal and vagal reflexes.

Supported by NIH grant R01 NL 21257.

72 EFFECT OF FENFLURAMINE AND PARACHLOROPHENYLALANINE UPON CIRCADIAN BODY TEMPERATURE, OXYGEN CONSUMPTION AND ACTIVITY LEVELS IN RATS. P.E. Penn+, R.L. Gerber*, J.D. Sibonga*, and B.A. Williams*. NASA-Ames Res. Ctr., Moffett Field, CA 94035.

It has been reported that injections of the anoretic drug fenfluramine (Fen) and of p-chlorophenylalanine (pCPA) produce long term depletion of brain serotonin by different mechanisms and possibly in the process causing an initial rapid release of serotonin. Involvement of serotonin in temperature regulation has long been implicated, so the effect of Fen and pCPA on body temperature and other circadian variables was studied.

Using male rats maintained at 22°C, daily variations in body temperature (TB) and activity levels (A) were measured every minute by an inductively-powered telemetry system newly developed at Ames. Oxygen consumption (O_2) and other data were stored on a computer. Five days of control data were collected prior to injections which were made in the afternoon just before the circadian increase in the measured variables. The drugs tested were injected intraperitoneally in doses previously reported to produce significant neurochemical and behavioral effects. Fen (20 mg/kg) produced an immediate significant ($p<.05$,n=5) drop in TB, which was lowest after four hrs., when compared to the same rats' pre-injection TB (1.27°C), paired controls (1.38°C) or the same animals' TB at the same time on a previous day (1.53°C). This drop in TB was accompanied by a significant drop in O_2 of approximately 33%($p<.05$,n=5) when compared to the same three controls (1.27, 1.58 and 1.93 W/kg). Activity levels showed no consistent change. Food and water consumption were lower for 24 hrs. Before 24 hrs. had passed, all variables measured returned to normal levels and normal cycling. These initial responses were in contrast with those obtained following injections of 300 mg/kg pCPA. TB and O_2 also significantly dropped immediately following injection (TB -2.02°C,n=5;O_2 -25%). However, normal cycling did not occur for several days, with O_2 remaining at a low normal level and TB returning to and remaining at a high normal level. Food and water intake was lower during this time. Destruction of the B9 serotonergic cell bodies by Fen does not appear to have long term effects upon TB, O_2, A and food and water consumption while more extensive serotonin depletion by pCPA does. It is possible that the initial falls in the TB and O_2 were due to the immediate release of serotonin posited to occur with Fen and pCPA. The serotonin precursor tryptophan (Try) has been reported to cause an immediate, short term increase in serotonin levels and, probably, release. Injections of Try (300 mg/kg) produced significant short lasting drops in TB (2.30°C,n=3) which were lowest at two hrs. Although other causes cannot yet be ruled out, serotonin release may be responsible for the immediate short term falls in TB and O_2 seen with Fen and pCPA. (+NRC assoc. at Ames)

73 ALTERED UPTAKE OF NOREPINEPHRINE BY THE CARDIOVASCULAR SYSTEM OF SPONTANEOUSLY HYPOTENSIVE RATS.
Joon H. Rho* and Natlie Alexander* (Spon: B. Newman). Clinical Pharmacol., Dept. Med., Sch. Med., USC, Los Angeles, CA 90033.

The uptake of exogenous tritiated norepinephrine (H^3-NE) by the cardiovascular blood vessels of spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto (WKR) rats have been investigated. The isolated tissues were incubated with H^3-NE and the storage vesicles were isolated by means of sucrose density gradient centrifugation. Studies of H^3-NE incorporation into the isolated storage vesicles indicate that the portal-mesenteric vein synaptic vesicles of 12 week old SHRs take up less H^3-NE than those of WKRs (approximately 56% of the WKR), and it appears to be even less in the 36 week old SHRs. The mesenteric artery and branches to the gut wall have been processed in the same manner as the portal-mesenteric vein. In contrast to the results on the latter, the mesenteric arteries of SHRs showed an increase in uptake from 63 to 100% over that of the WKR control. A similar trend has been noted in atria. These results indicate that the neurotransmitter uptake is different on the low and high pressure sides of the circulatory system. Studies of H^3-NE incorporation into isolated storage vesicles of the tissues, thus, permit an assessment of vesicular uptake and binding mechanisms and their possible alterations in hypertension. These results will be discussed with regard to a possible defect in the neuronal membrane transport system.

74 EFFERENT PROJECTIONS OF THE PARABRACHIAL NUCLEUS. C.B. Saper and A.D. Loewy, Depts. Anat. & Neurobiol. and Medicine, Wash. Univ. Sch. Med., St. Louis, MO 63110

Interest has been focussed on the parabrachial (PB) nucleus because this region, surrounding the brachium conjunctivum in the dorsolateral pons, receives key inputs from the hypothalamus, amygdala and nucleus of the solitary tract, and is thought to be important in central autonomic regulation.

In order to study efferent projections from the PB nucleus, we made small injections of a mixture of ^3H amino acids into the region of the PB nuclei of rats and after 5 to 7 days survival, the brains and spinal cords were processed using the autoradiographic method. We have found major differences in the efferent projections from each of the subdivisions of the PB complex. The lateral PB appears to project rostrally through the contralateral medial lemniscus to the ventrobasal complex of the thalamus, lateral hypothalamic area, zona incerta, lateral preoptic area, and central nucleus of the amygdala and ipsilaterally in the central tegmental fields to reach the dorsal tip of the cerebral peduncle, and to enter the stria terminalis. From here, this bundle projects to the bed nucleus of the stria terminalis and the central nucleus of the amygdala. Descending fibers which appear to originate from the region of the Kölliker-Fuse nucleus travel in the ventrolateral medulla and along the medial edge of the spinal trigeminal nucleus. Some fibers appear to go as far as the spinal cord, traveling through the dorsal part of the lateral funiculus to innervate the intermediolateral cell column. The medial PB projects rostrally mainly through the ipsilateral central tegmental fields to enter the lateral hypothalamic and preoptic areas, dorsomedial, ventromedial and paraventricular nuclei of the hypothalamus, zona incerta, central nucleus of the amygdala and bed nucleus of the stria terminalis. Other fibers ascend through the central gray to the periventricular nuclei of the thalamus and hypothalamus. Ipsilateral fibers enter the ventral supraoptic commissure and cross to the contralateral lateral hypothalamic and preoptic areas. Descending fibers appear to travel in a similar trajectory similar to that described for axons from the lateral PB complex but none have yet been traced as far as the spinal cord. Various portions of the PB complex appear to give rise to separate portions of the total projection, as small differences in injection sites may cause certain pathways not to be labeled. We conclude that the PB complex may play an integral part in the highly interrelated central autonomic control system. Supported by USPHS grant #12751 and American Heart Association grant #77 797.

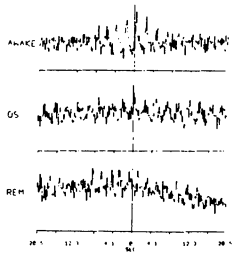
75 FUNCTIONAL CHARACTERIZATION OF SYMPATHETIC CARDIAC AFFERENT FIBERS. Jeanne L. Seagard* and John P. Kampine* (SPON: A. Sances, Jr.). Depts. Physiol. and Anesthesiol., Med. College of Wisconsin and Wood VA Ctr., Milwaukee, WI 53193

Evoked potentials were employed to functionally characterize the numbers and types of cardiac afferent fibers present in the thoracic sympathetic nerves of dogs.

Ten mongrel dogs were anesthetized with sodium pentobarbitol (35 mg/kg, i.v.) and placed on positive pressure ventilation. The second to fifth ribs along with the adjacent sternum were removed from the left side to expose the thoracic nerves. The ventrolateral (VLCN), ventromedial (VMCN), and left stellate (SCN) cardiac nerves were isolated and sectioned, with the central end retained for stimulation. The left sympathetic chain and T_2-T_4 white rami communicantes were isolated by sectioning the chain below T_4 and each white ramus at its junction with the spinal nerve. The T_2-T_4 chain and corresponding rami were placed in a nerve chamber filled with warm mineral oil for recording purposes. Each ramus was desheathed and dissected into smaller bundles of fibers. Individual bundles were sequentially placed on tungsten-carbide recording electrodes which were connected to high gain preamplifier-amplifier system in series with an Ortec averaging computer. VLCN, VMCN, and SCN were individually positioned across bipolar stimulating electrodes connected to a constant current stimulator. Supramaximal parameters of 3 Hz, 0.5 msec, and 12-15 ma resulted in the stimulation of both Aδ and C fibers. Stimulation of each nerve, while recording from T_2-T_4 white rami, evoked a greater percentage of C fiber potentials (0.31-1.8 m/sec) than Aδ fiber potentials (2.1-16.4 m/sec). Stimulation of the SCN and VMCN produced a greater number of potentials than did stimulation of the VLCN. The VLCN contained few fibers with conduction velocities greater than 2 m/sec. There did not appear to be any preferential distribution of cardiac afferent fibers arising from any ramus examined in this study (T_2-T_4). This study has demonstrated that 1) both Aδ and C fibers carry sympathetic cardiac afferent activity and 2) there is a greater percentage of C fibers than Aδ fibers involved with the transmission of this cardiac afferent activity. (Supported by Grant HL 16511 and the Medical Research Service of the VA.)

76 RESPIRATORY RELATED DISCHARGE OF PONTINE NEURONS DURING SLEEP AND WAKING STATES IN FREELY-BEHAVING CATS. G. C. Sieck* and R. M. Harper. Dept. Anat. and Brain Research Inst., Sch. Med., UCLA, Los Angeles, CA 90024.

Single neuron activity of the nucleus parabrachialis medialis (NPBM) was examined in unanesthetized, freely-behaving cats during sleep-waking states. A bundle of 10 fine wire (62 nichrome) electrodes was implanted together with electrodes for monitoring cortical, hippocampal, and lateral geniculate EEG, eye movements, and EKG. Respiratory movements were monitored using a piezoelectric strain gauge placed across the rib cage. Relationships between neuronal activity and respiratory movements during sleep-waking states were determined by cross-correlation techniques. Sleep-waking states were assessed using established criteria. Discharge rates of neurons in the NPBM fell into two categories with respect to sleep-waking states: (1) cells showing no specific relation to state charge and no relation to the respiratory cycle; (2) cells showing state-dependent changes in discharge rate which were correlated with respiration. The latter group of cells tended to have greatly increased firing rate during active sleep and comparable firing rates during quiet waking and quiet sleep. The increase in firing of NPBM cells during active sleep was not related to the phasic events of this state, and in fact firing often slowed during eye movements. A respiratory pattern of discharge in NPBM cells was present in each sleep-waking state. The degree of coupling to respiration and the phase relationship to the respiratory cycle depended upon the sleep-waking state and the cell being examined. Increased coupling was observed in active sleep in some cells; however, other cells showed a decreased coupling during both active and quiet sleep compared to the awake pattern (see figure). These findings demonstrate respiratory related discharge of neurons in the pneumotaxic center of non-vagotomized, unanesthetized cats. Alterations in the firing of these cells during different sleep-waking states may provide a basis for the varying respiratory control characteristic of these states.



Cross-Correlated Histograms Between Respiration & NPBM Neurons During Waking, Quiet Sleep, & Active Sleep

This research was supported by Grant HL 22418-01 from the National Institute of Health.

77 AN AUTORADIOGRAPHIC STUDY OF VAGAL PREGANGLIONIC FIBERS TO RAT BRONCHI, HEART, AND LOWER ESOPHAGUS. J.M. Sullivan* and N. Connors* (Spon: K.R. Smith). Dept. Anat., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Silver techniques have previously been used to demonstrate the visceral motor parasympathetic fibers that originate from the dorsal motor nucleus of the vagus. There is a minimum of information concerning the anatomical relationships of these preganglionic fibers within the bronchial and epicardiac ganglia and within the myenteric plexus of the lower part of the esophagus. The exact mode and arrangement of terminations has not yet been elucidated. The purpose of this study was to demonstrate by autoradiography the terminal branches of these preganglionic fibers on the postganglionic neurons within the bronchial and epicardiac ganglia and the myenteric plexus of the lower part of the esophagus. Twelve adult albino rats of both sexes were studied. The right dorsal vagal nuclei in six animals and the left nuclei in another six animals were injected with 250 microcuries of tritiated leucine. Following a postoperative survival period of three days, the animals were perfused with 10% buffered neutral formalin and serial sections of each lung, heart, and lower part of the esophagus were processed for autoradiography. Microscopic examination indicated a specific arrangement of preganglionic fibers within the vagus. The bronchial and heart tissue contained heavily labelled vagal fibers in close proximity to the postganglionic neurons. The bronchial ganglia containing labelled vagal axons consisted of clusters of postganglionic neurons located in close relationship to the smooth muscle down to and including the respiratory bronchioles. Epicardiac ganglia containing labelled vagal axons were located along the epicardial surface of the right atrium. The number and location of the epicardiac ganglia varied slightly in each animal. The esophageal preganglionic fibers of the vagus did not appear to be heavily labelled. Our results show a large labelled vagal preganglionic input to the bronchial and epicardiac ganglia but few labelled vagal preganglionic axons to the myenteric plexus of the lower esophagus.
(Supported by USPHS grant FR 05388.)

78 SELECTIVE PROTECTION AGAINST BETHANECHOL-INDUCED DEPLETION OF SMALL INTENSELY FLUORESCENT (SIF) CELL HISTOFLUORESCENCE IN SYMPATHETIC GANGLIA. E.J. Tsevdos*, A.O. Humbertson, Jr., A.E. Langley* and R.W. Gardier*. The Ohio State University, College of Medicine and Dentistry, Columbus, Ohio 43210

Electrical recordings of the superior cervical ganglionic surface potential in cats indicated that gallamine or pancuronium inhibit the muscarinic mediated hyperpolarization (s-IPSP) without affecting slow depolarization (S-EPSP) by selective blockade of postsynaptic cholinergic receptors, typed M_i on the ganglionic interneuron (Gardier et al., J. Pharmacol. Exp. Ther. 204:46-53, 1978; Gardier et al., Fed. Proc. submitted). Since the development of the s-IPSP theoretically results from sitmulation of dopamine receptors on the ganglion cells, the effect of haloperidol was investigated and found not to affect the s-EPSP but to intensify the s-IPSP presumably by a preeminent blockade of dopamine receptors on the interneuron.

The selective postsynaptic blockade by gallamine or pancuronium suggested from the surface potential studies was evaluated further by the histochemical fluorescent technique of Falck et al. (J. Histochem. Cytochem. 10:348-354, 1962). In vitro exposure of rat superior cervical ganglion (SCG) to bethanechol, a muscarinic stimulant, reduced fluorescence in both ganglionic neurons and interneuronal (SIF) cells. Treatment of rat SCG with gallamine (in vivo and concomitant with bethanechol in vitro) or pancuronium (in vivo) prevented the decrease in SIF cell fluorescence resulting from in vitro exposure to bethanechol. Pretreatment of the SCG with gallamine or pancuronium alone had no observable effect on either ganglionic neuronal or SIF cell fluoresence. Exposure of the SCG to haloperidol in vivo as well as during in vitro exposure with bethanechol produced a depletion of SIF cell and ganglionic neuronal fluorescence comparable to that from bethanechol alone. The failure of haloperidol to protect against histofluorescence depletion in the ganglionic cell conforms to its lack of effect on the s-EPSP. These data provide further documentation for two pharmacologically distinct muscarinic receptors (M_i and M_e) in sympathetic ganglia.

Supported by NIDR Grant DE00291, OSU Studybaker Fund and OSU Development Fund #533768.

79 SPINAL PROJECTIONS OF LEFT VENTRICULAR AFFERENTS
W. Hugh Vance and Robert M. Bowker*. Dept. of Cardiology and the Marine Biomedical Inst., University of Texas Medical Branch, Galveston, TX 77550.

The location of the cells of origin of the spinal afferents innervating the left ventricle in the area of the left anterior descending artery (LADA) have been determined in the cat and dog using the retrograde transport of the enzyme horseradish peroxidase (HRP).

Adult animals were anaesthetized with barbiturate anaesthetic and intubated with a tracheal tube. A routine thoracotomy was performed and the animal was maintained by artificial respiration throughout the surgical procedure. After the pericardial sac was opened, the LADA was identified and 15 injections consisting of 10 µl each of 1% HRP solution were injected subepicardially in the region of the LADA. After a survival period of 72 hours the animal was perfused with 0.5% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer. Dorsal root ganglia (DRG) of levels C7 through T6 were collected bilaterally and serially sectioned at 50µm. The sections were treated with tetramethyl benzidine according to the method of Hardy and Heimer (1977) in order to obtain reaction product.

Labelled neurons have been found at spinal levels C8 through T5 but predominantly at T2 and T3 with respectively 40% and 49% of the labelled cells found so far being at these levels. The labelled cells are also of relatively small size, being approximately 20µm to 40µm in diameter.

Our data is then in general agreement with but extends the classic work of Nettleship (1936) who described the cells innervating the "endocardial net at the apex of the ventricles and the wall of the coronaries" as being located at levels T1 through T5; as well as that of Ellison and Clark (1975) who injected HRP into the left and right ventricles and atria and found reaction product in the left T2 DRG.

Supported by Texas Heart Association grant #1-19511-488009.

80 RENAL NERVE RESPONSES TO ACTIVATION OF CARDIAC SYMPATHETIC AFFERENT NERVES BY CORONARY OCCLUSION AND BY TOPICAL APPLICATION OF AUTOCOIDS TO THE MYOCARDIUM. Lynne C. Weaver, Dept. of Physiology, Michigan State University, E. Lansing, MI 48824.

Activation of cardiac sympathetic afferent fibers by ventricular stretch induces reflex excitation of renal nerve activity (Weaver and Macklem: Fed. Proc. 37:743, 1978). Cardiac sympathetic afferent fibers also can be excited by coronary occlusion or by topical application of autocoids such as bradykinin, prostaglandin E_2 or histamine to the myocardial surface. Reflexes evoked by these stimuli may resemble those induced by natural activation of ventricular sympathetic afferents. Therefore, these stimuli were used to excite cardiac sympathetic afferent fibers to assess their influence on renal nerve activity and systemic blood pressure. Experiments were performed in α chloralose anesthetized, vagotomized, sino-aortic denervated cats. Occlusion of one or both branches of the left coronary artery caused myocardial ischemia and reflexly excited renal nerve activity by 23-94%. Occlusion of the left descending coronary artery appeared most effective. This increase in renal nerve activity was not observed in cats whose stellate ganglia and thoracic sympathetic chains had been removed. Coronary occlusion also caused systemic blood pressure to fall; however, equivalent decreases in blood pressure induced by hemorrhage failed to elicit similar increases in renal nerve activity. Solutions (1 ml) containing various concentrations of bradykinin (0.1-50 µg/ml), prostaglandin E_2 (0.1 µg/ml), or histamine (1-50 µg/ml) were perfused over the cardiac ventricles. After a latency of 5-10 sec., this application of bradykinin increased systemic blood pressure by as much as 90 mm Hg and increased renal nerve activity by 17-131%. The duration of the responses was 30 sec. to 3 min. Tachyphylaxis was apparent upon repeated administration of one concentration of bradykinin. Blood pressure responses were attenuated and renal nerve responses eliminated by removal of the stellate ganglia and thoracic sympathetic chains. Similar responses could not be evoked by intravenous or intramuscular administration of bradykinin. Increasing blood pressure with intravenously administered phenylephrine (equivalent to the pressor response to bradykinin) did not excite renal nerve activity. Application of prostaglandin E_2 or histamine to the ventricles also induced increases in systemic blood pressure and renal nerve activity. However, responses to these agents were less consistent than those to bradykinin. In summary, activation of cardiac sympathetic afferent fibers by coronary occlusion or by bradykinin, prostaglandin E_2 or histamine can reflexly increase renal nerve activity. This reflex may be responsible for some of the changes in kidney function associated with cardiac dysfunction. (Support: Mich. Heart Assoc. and NIH HL 21436.)

81 ACUTE CHANGES IN BODY FLUIDS AFTER BARORECEPTOR DENERVATION.
A.H. Werber* and G.D. Fink* (SPON: J. Thornburg), Dept. of Pharmacol., Mich. State Univ., E.Lansing, Mich. 48824

In 1964, Krieger reported the production of hypertension in rats by sinoaortic (SAD) or aortic baroreceptor denervation (ABD). In 1973, Guyton and co-workers reported that after SAD in dogs, although blood pressure (BP) was more variable, there was no rise in the 24 hr average level of mean arterial blood pressure (MABP). Guyton postulated that any rise in BP due to interruption of baroreceptor pathways would be transient, with restoration of BP occurring through an enhanced renal excretion of salt and water, contraction of the plasma volume and reduction in cardiac output. Nevertheless, recent results indicate that interference with baroreceptor function can result in prolonged hypertension (Jones and Hallback, 1978; Scher, 1978; Doba and Reis, 1973). Fluid volumes and renal function were not determined in these latter studies.

The present experiment was designed to examine the effects of ABD on cardiovascular and body fluid parameters in the conscious rat. Heart rate (HR), MABP, water intake (WI), urine ouptut (UO), body weight (BW), urinary sodium excretion ($U_{Na}V$), urinary potassium excretion (U_KV), plasma volume (PV), and extracellular fluid volume (EFV) were measured. Water balance (WB), a parameter derived by subtracting UO from WI was also measured. MABP was measured directly in the freely moving animal by means of an indwelling aortic catheter. PV and EFV were measured by dilution of Evan's blue and thiocyanate, respectively. WI, UO, $U_{Na}V$, U_KV were determined for 24 hr periods in rats housed individually in metabolism cages. The duration of the study was 7 days.

BP was significantly elevated 1, 2 and 3 days after ABD. HR was increased significantly 1, 2, 3 and 4 days after ABD. WI was significantly depressed on all days following ABD, with the smallest intake the day after ABD. UO was not increased after ABD, and decreased 2, 3 and 4 days after ABD. WB was reduced 1, 2 and 3 days after ABD, with the largest fall on the first day after ABD. BW was significantly lower all 5 days after ABD. $U_{Na}V$ was decreased 2, 3 and 4 days after ABD. U_KV was decreased all days after ABD. Five days after ABD, PV and EFV were 15% lower than preoperative values. There were no significant changes in any of these parameters in sham operated control rats.

These results confirm that in the rat an acute neurogenically-induced rise in BP results in contracted fluid volumes, and a tendency for blood pressure to return to control levels. However, it appears that this phenomenon is not dependent upon increased salt and water excretion by the kidney.

82 LOCALIZATION OF FROG PREGANGLIONIC SYMPATHETIC CELL BODIES USING HRP. Robert D. Wurster. Department of Physiology, Loyola University of Chicago, Stritch School of Medicine, Maywood, Illinois 60153.

On 6 bull frogs anesthetized by ice water immersion the 9th and/or 8th paravertebral ganglia were injected with 30% horseradish peroxidase solution. The frogs were returned to room temperature and allowed to survive for 48 hours. The animals were then pithed, perfused and fixed. The spinal cords were removed, sectioned and reacted according to the diaminobenzidine technique. Labeled cell bodies are located in the lateral horn about 275 to 575 µm from the lateral surface of the spinal cord and 425 to 625 µm from the dorsal surface. The cell bodies occur in clusters of 2-4 cells at about 70 µm intervals in the longitudinal direction. Interspersed between the clusters are nerve fibers communicating between the white and gray matter. These cell bodies have 2 to 3 processes and have a major diameter of 8 to 22 µm (mode of 14 µm) and a minor diameter of 8 to 16 µm (mode of 10 µm). These cell bodies are smaller and are more localized in the lateral horn than those of the cat. (Chung, et al., Brain Research 91: 126-131, 1975.) (Supported by NIH grant HL08682.)

83 IN VITRO STUDIES ON THE FUNCTIONAL ANATOMY OF SYMPATHETIC GANGLIA USING HORSERADISH PEROXIDASE. R.E. Zigmond, C.W. Bowers* and B.D. Wise*. Dept. of Pharmacol., Harvard Med. Sch., Boston, MA 02115.

We have recently used horseradish peroxidase (HRP) in vivo to study the location of neurons in the rat superior cervical ganglion (SCG) which use particular postganglionic trunks to reach their target tissues (Fed. Proc. 37: 526 (1978)). These studies showed that the ganglion can be divided into a caudal group of neurons whose axons project out the external carotid nerve and a rostral group of neurons whose axons project out the internal carotid nerve.

Since many other autonomic ganglia are located in regions of the body which are not as easily accessable as the SCG, we have developed an in vitro method to study them. A ganglion is removed from an animal together with as much of its pre- and postganglionic trunks as possible and placed in a petri dish. A small teflon ring is coated on the bottom with a layer of vacuum grease and placed over one of the cut trunks to isolate it from the ganglion cell bodies and the other trunks. The compartment created by the teflon ring is then filled with a 10% solution of HRP and the rest of the petri dish is filled with Ringer's solution. After 15 min the HRP solution is removed. The ganglion is then maintained in organ culture for about 18 h. The tissue is processed for the HRP reaction using normal procedures.

In vitro studies on the postganglionic trunks of the SCG produced the same distribution of labeled neurons in the SCG as found in vivo. Studies on the cardiac nerve of the inferior cervical ganglia demonstrate that the neurons whose axons project out this trunk are localized in a distinct region of this ganglion. Labeled neurons were observed only in the medial half of the ganglion and were particularly clustered near the exit of the cardiac nerve. These results demonstrate that sympathetic ganglia are not homogeneous structures but are made up of regions containing neurons innervating specific tissues. Our in vitro method should be useful in further studies on the anatomy of these ganglia. (Supported in part by Amer. Heart Assoc. grant #76723.)

AXONAL TRANSPORT

84 THE EFFECTS OF VARIOUS CATIONS UPON THE INCORPORATION OF PROLINE INTO THE MACROMOLECULES OF LARGE CELLS WITHIN THE DORSAL COLUMN NUCLEI OF CATS. K. J. Berkley, H.H. Molinari and D. C. Mash. Dept. Psychol., Fl. St. Univ., Tallahassee, FL 32306.

Many different amino acids appear to be incorporated nearly equally into the macromolecules of all of the different types of neurons located in the vicinity of an injection site. Proline, however, differs in that it appears to be incorporated preferentially only into the macromolecules of certain types of neurons. This pattern has so far been observed in the cerebellum (Felix and Künzle, 1974) the lateral reticular nucleus (Künzle and Cuénod, 1973) and the dorsal column nuclei (Berkley, 1975) of the cat. In these experiments, ^{3}H-proline was not incorporated as heavily by Purkinje cells of the cerebellum or by large cells of the lateral reticular n. and dorsal column n. as it was by other neurons in these regions.

The mechanisms which underly this preferential incorporation pattern are not yet clear, as the pattern seems to be unaffected by many experimental or technical manipulations (Berkley, et al. 1977). One variable which does affect this pattern, however, is the presence of calcium in the injection solution. If ^{3}H-proline is dissolved in a solution of calcium chloride and injected into the dorsal column nuclei of cats, the ^{3}H-proline appears to be incorporated by large as well as by small neurons throughout the injection site. If the ^{3}H-proline is dissolved in distilled water, or solutions of sodium-, potassium-, or magnesium chloride, however, the preferential incorporation pattern is unaffected. This ion-specific effect is consistent with the suggestion that the failure of the large cells within the dorsal column nuclei to incorporate extracellularly available proline into its macromolecules is occasioned at least in part by some active property of the membrane of the large cells.

Supported by PHS grants KO4 NS 00118, RO1 NS 11892 and NSF grant BNS 76-01393.

85 PROTEINS IN THE WAVEFRONT OF FAST AXONAL TRANSPORT. Mark A. Bisby, Division of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

L-[^{35}S]-methionine was used to label axonally transported proteins in rat sensory neurons of the L5 dorsal root ganglion (DRG). 1-2 h after injection of precursor into the DRG, in some experiments the DRG were removed. In all experiments the dorsal roots and sciatic nerves were removed 3-4 h after precursor injection and cut into segments. Some segments were prepared for scintillation counting while others were processed for SDS polyacrylamide gel electrophoresis and run on exponential slab gels.

Profiles of protein-incorporated ^{35}S activity along the length of the nerves revealed waves of activity characteristic of the rapid axonal transport of protein (Ochs: J. Physiol. 227, 627-645, 1972). Fluorographs of gels containing extracts from different regions of the wave of activity showed that the same transported polypeptides were present in all parts of the wave. However, the forward edge of the wavefront, containing the fastest transported proteins, was enriched in two polypeptides (S_1 and S_2) with apparent molecular weights (MW) of 24,300 and 20,700 daltons. Using the fluorographs as a guide, regions of gel containing specific labeled polypeptides were excised and counted. This quantitative data confirmed the results of fluorography. Slight differences were noted between the fluorographic banding patterns of gels containing dorsal root and sciatic nerve segments. The possibility that these differences arise as a result of diffusion of precursor along the dorsal roots cannot yet be eliminated.

Segments situated nearer to the cell bodies, containing more slowly transported proteins, were not enriched in polypeptides of lower MW, as would be predicted by the micro-stream hypothesis of transport (Gross & Weiss, Neurosci. Lett. 5, 15-20, 1977). On the other hand, the preferential transport of specific polypeptides at the fastest velocity may support the transport-filament hypothesis of Ochs (Ann. N.Y. Acad. Sci. 228, 202-223, 1974).

(Supported by the Medical Research Council of Canada).

86 RETROGRADE TRANSPORT OF NERVE GROWTH FACTOR TO LUMBAR SPINAL GANGLIA IN THE CHICK EMBRYO. J.K. Brunso-Bechtold and V. Hamburger, Dept. Biology, Washington University, St. Louis, Mo 63130.

Retrograde transport of nerve growth factor (NGF) has been reported in postnatal and adult rodents (Hendry, et al., Br. Res. 68:103, 1974; Stoeckel, et al., Br. Res. 89:1, 1975). Since NGF, delivered systemically, is known to produce an enlargement of spinal ganglia only in the embryo, it was of interest to find out whether retrograde transport of NGF to the spinal ganglia occurs in the embryo. ^{125}I-NGF impregnated pellets of polyacrylamide gel were implanted into the lower leg of stage 36 (10 day) chick embryos. The embryos were sacrificed 8 hours after injection and processed for routine autoradiography. Inspection of the leg shows heavy concentrations of silver grains in distal leg tissues in the region of the pellet, but virtually no grains above background at the base of the leg. Hence, there appears to be no spread of ^{125}I-NGF to the trunk by diffusion. Heavy labelling is seen within the lumbar sensory nerves along their extent to the ganglia as well as in the ganglia themselves, but the dorsal roots, ventral roots, and lateral motor columns are unlabelled. No label above background is found in any of the contralateral sensory roots or spinal ganglia. It can therefore be concluded that NGF is selectively picked up by neurons of the spinal ganglia, and transported retrogradely to the cell bodies, but not beyond them. (Supported by NIH grant NS05721 and Jerry Lewis Neuromuscular Disease Research Center grant.)

87 TRANSPERIKARYAL PASSAGE OF HORSERADISH PEROXIDASE ALONG PERIPHERAL SENSORY NERVES OF THE RAT. Thomas M. Brushart* and Marek-Marsel Mesulam. Neurological Unit, Beth Israel Hospital, Boston, Massachusetts.

Anterograde degeneration (Sprague & Ha, Prog. Brain Res., 1964) and HRP (Proshansky & Egger, Neurosci. Lett., 1977) methods for tracing the central terminations of individual somatosensory nerves require axonal interruption or HRP application proximal to the dorsal root ganglion (DRG). These studies thus reveal the central connections of individual dorsal roots rather than those of specific peripheral nerves. We now report a method which demonstrates nearly all central sensory connections after HRP application to peripheral nerves.

The proximal ends of cut rat sciatic nerves were anchored within HRP-filled microtubing (10 µL 10-20% Miles HRP). In other rats intramuscular HRP injections were made in the peroneal nerve distribution. After 24°-48° survival, the rats were perfused and the tissue reacted with tetramethyl benzidine (TMB) as described by Mesulam (J. Histochem. Cytochem., 1978).

Retrograde transport of HRP produced intense labeling of anterior horn neurons. In addition, granular HRP label was seen in DRG neurons as well as their peripheral and central processes. HRP-labeled axons could be followed into the dorsal root, the Tract of Lissauer, and the fasciculus gracilis. HRP reaction product suggestive of axon terminations was found in: (1) Rexed laminae I-IV; (2) the internal basal nucleus (medial laminae V-VI); (3) the nucleus of Clarke; (4) discrete sites in the intermediate zone and ventral horn; (5) the nucleus gracilis in the medulla.

When compared with 24° survival, 48° gave more consistent visualization of sensory connections. Few sensory connections were demonstrated with intramuscular HRP injection so that continuous exposure of cut nerves to HRP was clearly superior. Retrograde transport was not as susceptible to these manipulations. The importance of the sensitive TMB procedure was also demonstrated: motoneuron labeling was attenuated and the labeling of sensory connections abolished in matching sections processed with DAB.

Although Proshansky and Egger (op. cit.) produced exquisite axonal detail, they had to apply HRP proximal to the DRG and could only follow axons for 2.5 cm. In contrast, the present technique has resulted in trans-perikaryal passage of HRP from peripheral nerve through the DRG into the spinal cord and brainstem, and may thus be used to trace the central connections of many sensory fibers in peripheral and cranial nerves.

(Supported in part by NIH grant 09211)

88 ANTEROGRADE TRANSPORT OF HORSERADISH PEROXIDASE IN THE VISUAL SYSTEM OF THE TREE SHREW. Russell G. Carey* and Michael Conley* (SPON: M. Wolbarsht). Dept. of Psychol., Duke Univ., Durham, N.C.

The purpose of the present study was to demonstrate that the enzyme horseradish peroxidase (HRP) can be used as a sensitive anterograde tracer and compares favorably with the autoradiographic (ARG) techniques used to demonstrate the anterograde transport of amino acids. We examined the retinal projection of the tree shrew (Tupaia glis) by making intraocular injections of either 50 µl 30% HRP or 150-500 µCi of tritiated proline or a proline-leucine mixture. Survival times range from 8 hours to 6 days.

The HRP tissue was processed with benzidine dihydrochloride (BDHC), 3,3', 5,5'-tetramethyl benzidine (TMB) and 3,3' diaminobenzidine (DAB). Best results were obtained with BDHC or TMB. Little or no transport could be demonstrated with the use of DAB. Labeled terminals could be identified in contralateral visual nuclei (e.g., lateral geniculate, superior colliculus, pretectum, etc.) as early as 8 hours post injection. The optimal results, however, were obtained after 48 hour survival times. In these cases labeled axons could be traced in the optic tract to the sites of retinal termination. No evidence of transneuronal transport was apparent even with the longer survival times and multiple injections.

In terms of sensitivity every retinal terminus identifiable with the ARG method was shown with the same clarity using the HRP methods, and in certain instances the results from the HRP method were superior to those of the ARG. For example, the ipsilateral projections to the pretectal complex and medial terminal nucleus were much more distinct in the HRP cases than in the ARG material. Also, the ipsilateral "puffs" in the superior colliculus, often difficult to see in the ARG material, were well defined with HRP. Further, the anterograde transport of HRP has allowed us to confirm the contralateral retinal projection to the anterior dorsal nucleus first demonstrated by Conrad & Stumpf (Exp. Brain Res., 23, 1975).

In conclusion, these results demonstrate that anterograde transport of HRP can be used as an effective means for tracing retinofugal projections. The comparable sensitivity and the time saving advantages of this method indicate that is is a valuable alternative to the ARG and anterograde degeneration techniques. We are currently applying this method in the study of corticofugal systems in the tree shrew and these results suggest that this method may be valuable in tracing these connections as well.

(Supported by NIMH postdoctoral fellowship MH05867 (R.G.C.) and NIMH grant MH-4849 to I.T. Diamond.)

89 LIGHT AND ELECTRON MICROSCOPIC STUDIES OF THE CELLS OF ORIGIN OF THE INFRAORBITAL NERVE UTILIZING RETROGRADE AXONAL TRANSPORT OF HORSERADISH PEROXIDASE AND A NONCARCINOGENIC SUBSTITUTE FOR DIAMINOBENZIDINE. Keith A. Carson, Warner J. Lucas*, John M. Gregg* and Jacob S. Hanker. Dental Research Center, School of Dentistry, and the Neurobiology Program, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514.

Neuroanatomical studies have been greatly facilitated through the use of retrograde axonal transport of horseradish peroxidase (HRP). The technique for histochemically demonstrating HRP has recently undergone refinement, including the introduction of paraphenylenediamine-pyrocatechol (PPD-PC; Polysciences), a more specific and more sensitive noncarcinogenic substitute for diaminobenzidine (Hanker et al., Histochem. J., 9:789-792, 1977).PPD-PC proved to be readily oxidized to a dark, insoluble, osmiophilic copolymer by HRP, but unlike DAB it was only slightly active or inactive as a substrate for endogenous peroxidases, catalases, hemoproteins, and mitochondrial enzymes. Light and electron microscopy have been combined with HRP techniques to investigate the somatotopic localization and nature of the cells of origin of the infraorbital branch of the trigeminal nerve.

Following surgical exposure of the infraorbital nerve bilaterally on four Sprague Dawley rats, the main trunk was sectioned at its exit from the infraorbital foramen. Crystals of HRP (Type VI, Sigma) were applied directly to the isolated proximal stump and allowed to remain for 15 minutes. All animals were sacrificed 48 hours later and the trigeminal ganglia were sectioned, stained, and prepared for light and electron microscopy.

Light microscopic examination of the ganglia revealed the presence of many neurons filled with dark granules. Staining due to endogenous peroxidases and hemoproteins was negligible. Labeled cells had generally consistent topographical locations in all ganglia. Counts of labeled cells demonstrated that approximately 60% of cells with axonal processes in the infraorbital nerve were stained. Mapping techniques showed the labeled cells to be consistently in the middle of the ganglion just rostral to the point of separation of the mandibular branch. Dorsal sections in the horizontal plane showed labeled cells located medially while in more ventral sections labeled cells were situated laterally. Electron microscopic studies showed that the reaction product filled large membrane-bound granules and endoplasmic reticulum and showed no evidence of diffusion. In addition, these experiments demonstrated the advantages of PPD-PC for demonstrating HRP at light and ultrastructural levels and its applicability to many systems.

Supported by NIH grants DE 02668, DE 00288, 1-RO1-DE04730-01, RR05333, MH14277, and a grant to the Neurobiology Program from the Alfred P. Sloan Foundation.

90 THE CYTOSKELETON OF AXOPLASM EXAMINED BY HIGH VOLTAGE STEREO ELECTRON MICROSCOPY; A POSSIBLE VEHICLE FOR AXOPLASMIC TRANSPORT. M. H. Ellisman° and K. R. Porter+. °Dept. Neurosci., Univ. Calif., San Diego; +Dept. Molec. Cell & Devel. Biology, Univ. Col., Boulder.

Axoplasmic streaming is a dramatic example of cytoplasmic motility. Constituents of axoplasm migrate as far as 400 mm/day or at about 5 µm/second. Thin section studies have identified the major morphological elements within the axoplasm as being microtubules, neurofilaments (100 A filaments), vesicles of the smooth endoplasmic reticulum, and finally a matrix of ground substance in which the tubules, filaments, and vesicles are suspended. In the ordinary thin section image, the ground substance is comprised of wispy fragments which, in not being noticeably tied together, do not give the impression of representing more than a condensation from what might otherwise be a homogenous solution of proteins.

Using the high-voltage microscope on thick (0.5 µm) sections, we have noticed that the so-called wispy fragments are part of a three-dimensional lattice. The individual strands or trabeculae radiate into the ground substance often at right angles to the microtubules and neurofilaments. In thus reaching out, they interconnect the filamentous components of the axoplasm with each other and with vesicles of the endoplasmic reticulum, with multivesicular bodies, and the undersurface of the plasma membrane. The axoplasm is found to have the same structured ground substance as has been reported for the general cytoplasm of other cells, particularly where viewed in whole cells.

Better ways of staining the trabecular lattice to allow a more complete visualization in thick sections also are being examined. Uranyl-acetate block stains or tannic acid glutaraldehyde fixation appear to improve visualization of the matrix through an enhancement of its electron density. Thus, with improved staining of the trabecular material in sections, it should be possible to visualize, in detail, any changes in the lattice associated with motion of endoplasmic reticulum cisternae (containing, for example, transported protein) within the axoplasmic column.

[Supported by research grants from the Muscular Dystrophy Assn. to K. Porter and M. Ellisman, and from NIH to K. Porter (#RR00592).]

91 BIDIRECTIONAL AXONAL TRANSPORT OF MACROMOLECULAR (16S) ACETYLCHOLINESTERASE. Barry W. Festoff and Hugo L. Fernandez. Dept. of Neurology, University of Kansas Med. CTR. and Neurobiology Research Lab., VA Hosp. Kansas City, Mo. 64128

The restricted presence of macromolecular acetylcholinesterase (AChE) in innervated, end-plate enriched skeletal muscle regions of rat (16S) and chicken (19.5S) has suggested their role in nerve-muscle communication (see Vigny et al 27: 1347,1976). It was suggested that this AChE form was exclusively myogenic (Hall, J. Neurobiol. 4:343,1973; Vigny et al J. Neurochem. 27:1347,1976) because it could not be detected in central or peripheral nervous tissue. Recently, however, a small amount of 16S AChE was detected in rat sciatic nerve, which increased in a time-dependent fashion in the proximal stump after neurectomy (Digiamberardino and Couraud, Nature 271: 170,1978) suggesting that it moved with the rapid phase of axonal transport. In studies concerning trophic regulation of end-plate AChE, transport of 16S AChE could provide a source of the enzyme to the end-plate. For this reason evaluation of molecular forms of AChE were evaluated in control nerves, proximal, distal and between double ligatures in experimental nerves.

Sciatic nerves of Sprague-Dawley rats were ligated with fine silk thread and animals sacrificed 4,6,12,16,20,42 and 72 hrs later. Control nerves were divided into 3 equal 1 cm sections which were obtained proximal to the 1ST ligature, distal to the 2nd and between both ligatures. Nerves were homogenized in high ionic strength buffer containing 1% lubrol-WX and molecular forms of AChE separated on linear sucrose gradients. AChE (sensitive to BW 284C51) was estimated by a sensitive radioassay. Our results indicate that 16S AChE is present in control nerves representing approximately 3% of total AChE. Futhermore, this macromolecular form accumulates proximal to the 1st and distal to the 2nd ligature. No accumulation of 16S AChE occurred between the ligature. Although 10S and 4S AChE are present in blood only 10S showed proximal but not distal accumulation. 6.5S, an AChE form which may be specific to nerve, showed slight proximal and distal accumulation. Our emphasis, however, is on 16S AChE and confirms its presence in peripheral nerve. Its proximal and distal accumulation indicate its bidirectional transport. Such data shed light on trophic nerve-muscle interaction. Present experiments are concerned with estimating its transport rate and effects of agents known to block axonal transport.

(Supported by Muscular Dystrophy Assn. and the Medical Research Service of the Veterans Administration.)

92 RAPID AXONAL TRANSPORT OF [^{3}H]FUCOSE AND [^{35}S]SULFATE-LABELLED MACROMOLECULES IN RAT VISUAL SYSTEM. Jeffry F. Goodrum*, Arrel D. Toews, and Pierre Morell. Dept. of Biochem. and Biol. Sci. Res. Ctr., UNC, Chapel Hill, NC 27514

The axonal transport of [^{3}H]fucose and [^{35}S]sulfate-labelled macromolecules in retinal ganglion cells of 16 day Sprague-Dawley rats was investigated following simultaneous intraocular injection of precursor. Maximal incorporation of fucose into acid insoluble material in the retina was at 8 h, followed by a slow decline. Transported [^{3}H]fucose was in the optic nerve (ON) and tract (OT) by 2 h and in the lateral geniculate body (LGB) and superior colliculus (SC) by 3 h after injection, indicating a rapid rate of transport. Labelled fucose continued to accumulate in the SC for 8-12 hours, and began a slow decline by 24 h. More than 98% of the acid insoluble [^{3}H]fucose in the SC was in glycopeptides. Proteins in the SC were fractionated on 7.5% SDS polyacrylamide gels. There were no radioactive peaks present by 24 h that were not also present at 3 h, suggesting that there is little heterogeneity of fast transport rates for fucose-labelled material. However, over the 24 h period a 45,000 MW peak accounted for a progressively increasing fraction of radioactivity on the gel; possibly a function of its greater metabolic stability relative to other nerve terminal glycoproteins.

In contrast to fucose, incorporation of [^{35}S]sulfate into acid insoluble material in the retina was maximal at 2 h after which there was a rapid decline. The appearance of [^{35}S]sulfate in ON, OT, LGB, and SC preceded by a short time that of the [^{3}H]fucose. Consistent with the pulse labelling of the retina by sulfate, the total transported sulfate in the SC peaked by 4-6 h and was decreased by 65% at one day. At all times, acid insoluble [^{35}S]-sulfate in the SC was equally divided between glycopeptides and glycosaminoglycans, indicating these macromolecules are transported at the same rate. Fractionation of sulfate-labelled protein on gels revealed a prominent radioactive doublet between 70,000 and 80,000 MW at 3 h which was diminished at 4 h and absent at 8 h implying extremely rapid turnover of the sulfate moiety in these two proteins.

These results suggest that there is a delay of about 1 h before injected fucose becomes incorporated and available for transport in contrast to sulfate which begins transport very soon after injection. Once transport is initiated, labelled macromolecules of both species are rapidly transported at a single rate (approx. 200 mm/day). Upon arrival at nerve terminals the macromolecules turn over at different rates; of particular interest are two proteins containing sulfate moieties with a half-time of turnover of the order of hours.

Supported by U.S.P.H.S. grants NS11615 and HD03110.

93 HORSERADISH PEROXIDASE EVALUATION OF LATERAL HYPOTHALAMIC AREA INTERCONNECTIONS. R. Guevara-Aguilar, H.U. Aguilar-Baturoni, F.C. Barone and M.J. Wayner. Departamento de Fisiología, Facultad de Medicina, U.N.A.M., Mexico and Brain Research Laboratory, Syracuse University, Syracuse, New York, USA.

Horseradish peroxidase (HRP, Sigma Type VI, 4 to 40 percent) was applied in the mid-lateral hypothalamic area (LHA) of female rats. HRP was administered by two different techniques in order to cross-validate results and eliminate non-specific damage associated with large cannula used for infusions of the enzyme into the brain. In some cases, macrocannulae (250 µm) were used to infuse 0.1 µl into the LHA. In other cases the HRP was applied to the LHA by microiontophoretic ejection from glass micropipettes with tips broken to 25 µm. Positive DC current, 1.0-1.5 µA, was applied for 8 to 20 min. After 24 to 48 hr survival time, all animals were perfused intracardially with 1 percent formaldehyde and 2 percent glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. The brains were sliced in the frontal plane on a freezing microtome in 30 to 50 µm sections. Some sections were processed with DAB tetra HCl for the brown reaction, and others with BDA for the blue reaction according to the Nauta Technique. Labeled neurons were identified and photographed under the light microscope. Results were analyzed by means of mapping the results from several sequential sections onto copies of plates extracted from the König and Klippel rat brain atlas. HRP was observed to move rostrally, following the Diagonal Band of Broca and the Medial Forebrain Bundle , into the Lateral Septal Nucleus and as far as the Anterior Olfactory Nucleus. Axons containing HRP were identified at the level of the Globus Pallidus. Movement in the caudal direction was also determined. Many labeled neurons were observed in the Parafasiculis Nucleus, Posterior-Medial Thalamic Nucleus, the Ventral Thalamic Nucleus as well as the Zona Incerta.

94 ISOLATION OF RAPIDLY SYNTHESIZED CALCIUM BINDING PROTEIN FROM SYNAPTOSOMAL AND SOLUBLE FRACTIONS OF RABBIT BRAIN CORTEX AFTER TOPICAL APPLICATION OF ^{3}H-LEUCINE. Zafar Iqbal and Sidney Ochs. Dept. Physiology and Medical Biophysics, Indiana University School of Medicine, Indianapolis, IN 46202.

In our earlier work the presence of a calcium binding protein (CaBP) of 15,000 daltons was reported in cat brain synaptosomes (Iqbal & Ochs, Neurosc. Abs., 2:47, 1976). We now report the rapid labeling in situ of this protein. The incorporation of ^{3}H-leucine was studied by applying the precursor topically to the exposed parietal occipital cortex of anaesthetized rabbits. The animal and temperature of the cortex was maintained at 38°C by means of a thermoregulator device controlling a heating lamp shined through Saran wrap covering the cortex so as to prevent dehydration. Three to 3.5 hr after the application of the precursor, the cortex was removed, washed and homogenized in 0.32 M sucrose. Subcellular fractionation of the homogenate performed according to the method of Gray and Whittaker showed an incorporation of ^{3}H-leucine into proteins of all fractions with 6.9% in the synaptosomal (P_2B) and 33.6% in the soluble (105,000 xg/1h, S_3) fractions. The soluble fraction was filtered through Amicon Diaflow DM-5 membranes to remove low molecular weight components and the retentate analyzed by gel filtration chromatography on BioGel A5m columns. Using a 10 mM phosphate buffer of pH 7.5 for elution, 6.4% of the radioactivity was found to be present in the first peak (Ia) eluting with the void volume. The amount of radioactivity in the proteins of 40,-180,000 daltons (second peak, Ib) was 53.9% whereas the third peak, Ic, containing proteins of less than 20,000 daltons had 25.7% of the radioactivity. On further analysis of Ic peak proteins by electrophoresis on 10% acrylamide gels containing 0.1% SDS, among other proteins a significant amount of the 15,000 dalton protein was found to be labeled with radioactivity. This protein had earlier been found to have calcium binding properties. Similarly, when the synaptosomes or the synaptosomal soluble fraction obtained from synaptosomes after hypotonic shock was analyzed on SDS containing acrylamide gels, the protein band of 15,000 daltons was observed to be labeled with radioactivity. The protein present in the synaptosomes and the soluble fraction (S_3) show similarities to the CaBP of 15,000 daltons isolated from cat sciatic nerve which has recently been shown to be transported at a fast rate (Iqbal & Ochs, J. Neurochem., 1978 in press). Supported in part by the NIH,grant PHS RO1 NS 8706-09, & the NSF,grant BNS 75-03868-A03.

95 AXONAL PROTEIN TRANSPORT IN THE NIGROSTRIATAL AND MESOLIMBIC NEURONAL PATHWAYS IN THE RAT. Edgar T. Iwamoto. Dept. Pharmacol., Sch. Med., UCSF, San Francisco, CA 94143.

Axonal transport of tritiated protein was investigated in Sprague-Dawley rats after an injection of tritiated lysine into the ventral mesencephalic tegmentum (VMT), or into the substantia nigra (SN). Five, 60, 120 and 180 minutes after the injection of labelled lysine into the VMT, approximately 4, 35, 57 and 80 percent, respectively, of the radiolabel recovered from the midbrain injection site was present as TCA-insoluble protein; less than one percent of the label two hours after lysine injection was tritiated water. Three days after injecting 3 to 6 µCi of labelled lysine in 0.1 to 0.5 µl of artificial CSF into either the VMT or the SN, over 95 percent of the radiolabel recovered in thin-sections of the forebrain was bound to protein. VMT injections resulted in the preferential accumulation of labelled protein in known limbic projections (nucleus accumbens, olfactory tubercle, septum); less than 12 percent of forebrain radiolabel was located in the adjacent neostriatum. SN injections of labelled lysine resulted in the preferential accumulation of labelled protein principally in the nucleus caudatus-putamen. Coinjection of 1 µg of colchicine with the labelled lysine into VMT depressed recoverable forebrain radiolabelled protein by over 60 percent without altering midbrain amino acid pools. Coinjection of 1 µg of cycloheximide decreased labelled lysine incorporation into midbrain protein by over 75 percent and also diminished labelled protein recovery from limbic termina. Tritiated lysine injections in the SN of rats with chronic, 6-hydroxydopamine-induced lesions of the SN resulted in reduced recovery of radiolabelled protein from both limbic and neostriatal brain regions to less than 50 to 11 percent of control. 6-hydroxydopamine lesions of thd caudate nucleus decreased labelled protein accumulation in the neostriatum, but not in the nucleus accumbens or olfactory tubercles. Unilateral kainic acid lesions of the caudate nucleus, which presumably spare axons of passage and nerve terminals, did not appreciably alter the accumulation of radiolabelled protein in any brain region after SN injection of labelled lysine. The data demonstrate that axonal transport of protein from the VMT and SN region to both limbic and striatal termina in rat brain is sensitive to inhibitors of both microtubule assembly and protein synthesis, and may depend upon the integrity of a 6-hydroxydopamine-sensitive neuronal pathway. (Supported in part by a Research Starter Grant from the Pharmaceutical Manufacturers Association Foundation).

96 RAPID AXOPLASMIC TRANSPORT OF (U-^{14}C)PHENYLALANINE LABELED MATERIAL IN THE DORSAL SENSORY NEURON OF THE CAT. Jeffery L. Johnson and Jong-Hwan Kim*. Section on Physiology, Div. Biochem/Phys/Pharm, Univ. So. Dak. Sch. Med., Vermillion, SD 57069.

Even though the clinically important amino acid phenylalanine (PA) is necessary for normal neuronal function as evidenced by the neurological effects of PA deficiencies or excesses, very little is known about the importance of this amino acid with respect to neuronal constituents migrating via the rapid axoplasmic flow process down the axons. CNS studies (J. Neurochem. 23, 1974, 1065) at least, have suggested that analysis of such a process for phenylalanine labeled proteins would be quite fruitful using a branched neuron such as that seen in the sensory ganglia where a length of fiber can be easily separated and divided for analysis. (U-^{14}C)Phenylalanine (>480 mc/millimole) was injected into the seventh lumbar dorsal root ganglia bilaterally and at times ranging from 1-8 hrs following injection, the proximo-distal distribution of label in successive 3 mm segments was analyzed. Evident was a clear proximo-distal advancing front of labeled material peripherally (sciatic nerve) and centrally (dorsal root) moving at an average rate of 380 mm/day. Background counts distal to this front ranged from 2-20 cpm, suggestive of very little diffusion or extra-axonal spread of injected label. 5 x 10^{-3} g/ml puromycin markedly suppressed the amplitude of this advancing front and a nerve freeze resulted in a marked damming proximal to the freeze zone. This suggests that intraaxonal phenylalanine which has been incorporated into protein is a major component of the advancing front. A marked accumulation of phenylalanine labeled material in the dorsal spinal cord gray where much of the incoming dorsal root fibers terminate was noted, and this appeared to be greater than the amount advancing up the dorsal white tracts containing the ascending dorsal root fibers. A precise linear relationship between the counts in the ganglionic (perikeryal) injection site and the counts in the sciatic nerve advancing front was uncovered over the entire range of injected PA used (0.8-17 μc), suggestive of a precise relation between perikeryal protein formed and protein in the advancing sciatic plateau AND a lack of saturation of the protein synthetic mechanism by the amounts here injected. Following an early rapid flow component of high labeling in the dorsal root which was cleared from the dorsal root between 3-4 hrs after ganglionic injection, the dorsal root counts were consistently less than that seen in the sciatic nerve. Reasons for the difference will be discussed. (Supported by the Ralph W. Parsons Medical Research Fund.)

97 BRAIN STEM AND SPINAL CORD PROJECTIONS OF VAGAL SENSORY AND MOTOR FIBERS IN THE CAT USING THE TETRAMETHYL BENZIDINE (TMB) REACTION FOR HORSERADISH PEROXIDASE (HRP). Madhu Kalia and Marek-Marsel Mesulam. Dept. Physiol., Hahnemann Med. Col, Phila, PA 19102 and Harvard Neurological Unit, Beth Israel Hosp, Boston, MA 02115

In the past, investigations of the sensory and motor terminations of the vagus nerve have posed great technical difficulty since these studies were based on degeneration techniques. The recent availability of sensitive HRP methods has made it possible to study the afferent and efferent connection of the peripheral nerves and in the present study we have applied these techniques to the vagus. In adult cats the cut central end of the rt. vagus nerve at the level of the larynx was continuously immersed in 33% HRP (Type VI Sigma) for the 48 hrs; the brains perfused with 2.5% buffered glutaraldehyde and the tissue reacted with TMB (Mesulam J.Histochem. Cytochem. 26:106,1978). Uncounterstained sections were examined under dark-field illumination for HRP reaction product. During the survival period if the animals remained anesthetized (Chlorolose), the amt. of HRP reaction product visualized was significantly less than that seen in unanesthetized cats. The HRP label was seen in 3 types of structures within the brain stem: neurons, nerve fibers and patterns that seemed to be nerve terminals. Retrogradely labeled neurons were seen in: 1) Dorsal Motor Nucleus (DMN) of vagus; 2) Nucleus Ambiguus (NA); 3) Nucleus retroambigualis (NRA); 4) Isolated labeled neurons were located in what seems to be the reticular formation of the medulla. We cannot exclude the possibility that these are aberrant neurons of the DMN or NA; 5) In upper cervical segments of spinal cord labeled neurons were seen just lateral to the central canal and medially in the ventral horn. HRP labeled fibers were seen entering through the dorso-lat. side of the medulla. These fibers could be classified into two groups, one belonging to axons of labeled neurons and the other belonging to the central processes of Nodose Ganglion (NG) cells. Virtually all HRP labeled axons were directed at the DMN. Some of these axons joined labeled neurons of the DMN and some other axons looped ventrolat. to reach labeled neurons in NA. The central processes of NG neurons reached and coursed within the Tractus Solitarius. Some of these fibers seemed to form terminal patterns in the components of the Nucleus of the Tractus Solitarius (NTS) such as: lat. NTS, med. NTS and subnucleus gelatinosa of NTS. Such afferent terminals also seemed to be present within the DMN. Thus, this procedure enables the demonstration of afferent and efferent connections which characterize the peripheral components of the vagus nerve and illustrates that the transperikaryal passage of HRP across the Nodose Ganglion may be demonstrated with the use of sensitive histochemical procedures. (Support by NIH grants HL 17800, NS 09211, RCDA HL 00103 and Scottish Rite Freemasonary of North America.)

98 **AXONAL TRANSPORT OF ENZYMES REQUIRED FOR PHOSPHOLIPID SYNTHESIS.** **M. H. Kumara-Siri* and Robert M. Gould, Dept. Neurochem., Inst. Basic Res. Ment. Retard., Staten Island, NY 10314.**

Lipids and proteins used for the maintenance and integrity of neuronal processes and terminals are synthesized in the perikaryon and axonally transported to the sites of utilization in the axolemma and distant terminal regions. Previously it has been shown by autoradiographic techniques that, in addition to this mechanism, inositol lipid synthesis takes place locally in the mammalian axon (Gould, 1976, Brain Res. 117, 169-174). In the present investigation we have demonstrated that enzymes required for the synthesis of phosphatidylinositol and phosphatidylcholine are being supplied to the processes by axonal transport. Studies of the transport of the enzymes were performed with in vivo ligature paradigm using the sciatic nerves of adult male rats. Activities of CDP-diglyceride: inositol transferase (IT) and 1,2 diglyceride:choline phosphotransferase (CPT), the terminal enzymes of phosphatidylinositol and phosphatidylcholine synthesis, were optimized in homogenates of sciatic nerves using CDP-dicaproin (for IT) and 1,2 diolein (for CPT) as substrates. Specific activity for IT was 38.0 ± 2.58 (S.E.M. n=33) and that for CPT was 0.36 ± 0.031 (S.E.M. n=35) nmoles product formed per mg protein per hr. Ligatures were left for 2-72 hr periods and proximal segments (5mm) were removed at sacrifice, along with control segments (5mm), from same and contralateral side. Enzyme activities (both IT and CPT) in homogenates of proximal segments increased in a time dependent manner compared with the controls. Our results suggest that the two enzymes are axonally transported at slow rates. Build-up of IT in the proximal segment was at all times greater than that for CPT. IT activity was found to double at 40 hr after ligation, whereas at this time activity of CPT enzyme was increased approx. 60%. These results provide additional evidence for a specific need of local inositol lipid metabolism within the processes and perhaps in nerve terminal.

99 DESHEATHED SPINAL NERVE TRUNKS EXHIBIT A DIVALENT CATION REQUIREMENT FOR FAST AXONAL TRANSPORT DISTINCT FROM THAT IN GANGLIA. P.-A. Lavoie*, F. Bolen* and R. Hammerschlag. Div.of Neurosciences, City of Hope Med. Ctr., Duarte, CA 91010

The amount of [^{3}H]protein undergoing fast axonal transport in primary afferent neurons of bullfrog is depressed when ganglia but not when nerve trunks are incubated in medium with either calcium removed or cobalt added (R.H.,Dravid,& Chiu,Science 188: 273,1975; R.H. et al, J.Neurobiol. 8:439,1977). Our proposal, based on these observations, was that a calcium-dependent step occurs in the soma prior to translocation of proteins by the transport system. This has been brought into question by recent studies showing that axonal transport is blocked in desheathed mammalian nerve exposed to calcium-free medium (Ochs, Worth, & Chan, Nature 270: 748, 1977). To assess whether, in bullfrog, desheathing reveals a similar dependence of transport on calcium, a 4 mm region of sheath was removed from 8th and 9th spinal nerves. Preparations were preincubated for 5 h with ganglia in normal medium and nerve trunks in calcium-free medium containing 1 mM EGTA with or without 4 mM $MgCl_2$. Ganglia were pulse-labeled for 1 h with [^{3}H]leucine,and incubation was continued for 17 h at 18°C with nerve trunks selectively exposed to the appropriate calcium-free medium. Similarly desheathed preparations maintained in normal medium served as controls. [^{3}H]protein transported beyond the desheathed region was depressed by 53 ± 9% (n=10) in nerves exposed to calcium-free medium lacking Mg^{++}, whereas no depression of transport was detected with Mg^{++} added to the calcium-free medium (104 ± 9%, n=10). These results suggest that ongoing fast transport in axons has a divalent cation requirement that can be met by Ca^{++} or Mg^{++}. The effects of desheathing on transport were also tested in nerves incubated in medium containing 0.18 mM $CoCl_2$. Ganglia were pulse-labeled for 1 h and preparations were initially transferred to normal medium for 5 h to allow labeled fast-transported proteins to enter the nerve trunks. Ganglia were then removed and the isolated nerve trunks were placed in cobalt-containing medium for an additional 12 h at 18°C. The amount of transport through the desheathed region was similar in nerves incubated in medium with and without cobalt. These results suggest that cobalt does not compete with calcium for the axonal divalent site involved in fast transport. The divalent cation requirement for transport in axons differs from that in ganglia since, in axons, Mg^{++} can replace Ca^{++}, and Co^{++} does not act as a Ca^{++} antagonist. The original proposal that calcium is required in the soma for initiation of transport is therefore not affected by the finding of a calcium requirement for maintenance of transport in the axon.
(Supported by NSF Grant BNS75-17640, and by MRC of Canada Fellowship to P.-A.L.)

100 EFFECTS OF COLCHICINE AND CYTOCHALASIN B ON AXONAL TRANSPORT IN NORADRENERGIC NEURONS OF THE RAT BRAIN. B.E. Levin. College of Medicine and Dentistry of New Jersey and VA Hospital, East Orange, NJ 07019.

Proteins labelled with [^{3}H] leucine are transported in four waves in the ascending noradrenergic neurons of the locus coeruleus. Waves I (72-192 mm/d) and II (24-48 mm/d) correspond in rate and subcellular distribution to two waves of [^{3}H] fucose labelled glycoproteins. Norepinephrine (NE) is also transported at a rate of 48 mm/d. Waves III (13-20 mm/d) and V (1.4-2.9 mm/d) travel at intermediate and slow rates respectively. The effect of colchicine and cytochalasin B on axonal transport within these neurons was compared to that for 6-hydroxydopamine (6-OHDA), a specific toxin for catecholamine axons, by injecting 1 μl of the three drugs stereotaxically into the ascending dorsal noradrenergic bundle (DB) at various times prior to precursor injections into the locus coeruleus. Colchicine (5 μg/ μl) caused a time dependent block in net transport (correction was made for changes in precursor incorportion) of [^{3}H] proteins, glycoproteins and [^{3}H] NE comparable to that for 6-OHDA in waves I, II and V. Wave III was only partially blocked (70%) by colchicine when compared to 6-OHDA (100%). Cortical levels of NE and dopamine-B-hydroxylase (DBH), the vesicle bound glycoprotein enzyme which synthesizes NE, were decreased distal to colchicine DB lesions comparably to 6-OHDA lesions. Cytochalasin B (24 μg/ μl) significantly reduced net transport of only waves I and III and failed to alter cortical NE and DBH levels. There were no specific ultrastructural changes in microtubles or neurofilaments found in the lesioned areas by electron microscopy. The differential effects of colchicine and cytochalasin B on the four waves transported in this system suggest that the various subcellular constituents in each wave are transported by differing mechanisms. The similar effects of both drugs on transport of wave II proteins and glycoproteins and NE, as well as similar changes in cortical levels of NE and DBH, suggest that both are transported in this wave by a similar mechanism. This supports the hypothesis that both DBH and NE are transported together within the same vesicles in noradrenergic neurons.

101 RETROGRADE AXONAL TRANSPORT OF NERVE GROWTH FACTOR (NGF) IN THE CILIARY GANGLION OF THE CHICK. S.R.Max, M.Schwab*, M.Dumas* and H.Thoenen, Biocenter of the University, CH 4056 Basel, Switzerland

Retrograde axonal transport of NGF has been demonstrated in peripheral sympathetic and sensory neurons, but not in motor neurons. We have investigated the possibility that NGF is also transported in parasympathetic neurons. This was accomplished by measuring the accumulation of radioactivity in the ciliary ganglia of 1-2 day post-hatching chicks following unilateral intraocular injection of ^{125}I-NGF. The difference in radioactivity between ganglia on injected (I) and uninjected (U) sides is a measure of specific transport. We have substantiated this technique for peripheral systems in other species. To study selectivity of transport, we also tested ^{125}I-labelled tetanus toxin, wheat germ agglutinin (WGA), and cytochrome C.Tetanus toxin and WGA have been shown to be transported in all peripheral and central neurons thus far investigated. Cytochrome C, which has physical properties similar to those of NGF, is not transported in these systems. NGF was transported in the ciliary ganglion (I/U=2.2), as were tetanus toxin (I/U=2.5) and WGA (I/U=3.8). Cytochrome C was not transported (I/U=1.0). Autoradiographic studies support the biochemical evidence for selective retrograde axonal transport of NGF in the ciliary ganglion. Thus, in contrast to generally held views, NGF may have a physiological function in the parasympathetic nervous system. (Supported by the Swiss National Foundation and by the Roche Research Foundation).

102 OUABAIN INHIBITION OF FAST AXOPLASMIC TRANSPORT: Robert O'Neill,* Fred Samson and J. Alejandro Donoso. Ralph L. Smith Research Center, University of Kansas Medical Center, Kansas City, Kansas 66103. The intracellular ionic environment is probably important in fast axoplasmic transport (FAXT). Since the intracellular ion composition can be altered by inhibition of the Na-K ATPase, we have studied the effect of ouabain, which causes a Na^+ gain and K^+ loss intracellularly, on FAXT and axonal ultrastructure. Ouabain, at low concentration, has no effect on tubulin/microtubule polymerization in vitro, structures believed to be involved in FAXT. FAXT of protein in the cat cervical vagus nerve was studied in vitro. ^{3}H-leucine was microinjected into the nodose ganglion; after 2 hrs (for labelling of proteins and initiation of transport) the nerves were removed, incubated for 2.5 hr in Krebs-Ringer solution with 1, 10 or 100 μM ouabain and the FAXT character ascertained from the radio-activity in 2 mm nerve segments. In control experiments the labelled material distribution along the nerve is characterized by (a) a significant amount of label at the injection site; (b) a region of gradual decrease of label with distance from the ganglion ("saddle area") and (c) a more distal radio-active peak or "front" of transported material. Ouabain 1 μM caused a partial blockade of FAXT as evidenced by the appearance of extra peaks behind the normal front. Higher concentrations cause a correspondingly greater inhibition, that is, a filling of the saddle area. Preliminary electron microscopy revealed in unmyelinated axons prominent "vacuoles", swelling of some mitochondria and signs of intraaxonal lysis; other axonal constituents such as microtubules and neurofilaments appeared intact. These axoplasmic alterations are more extensive with the high ouabain concentrations. Myelinated axons and glial cells appear less affected. The ionic imbalance induced by ouabain may disrupt the FAXT system by a general spatial disorganization of the cytoskeleton or possibly directly by a specific ionic effect. Supported in part by NICHHD-02528, U.S.P.H.S.

103 AXONAL TRANSPORT OF ^{35}S TAURINE ALONG NEONATAL AND YOUNG ADULT RAT OPTIC AXONS. Michael Politis and Nicholas Ingoglia. Dept. Physiol. and Neurosci., N.J. Med. School, Newark, N.J. 07103

Brain concentrations of taurine are highest in early development and decrease as a function of age. Studies in this laboratory have indicated that this sulfonic amino acid is axonally transported along goldfish optic nerves. In the present experiments the axonal transport of taurine has been examined in neonatal and young adult rat optic axons. The rat optic system was chosen because a large extent of development in this system occurs postnatally, the majority of synapses in the lateral geniculate bodies being formed between 5 and 12 days after birth.

^{35}S taurine was injected into the vitreous humor of right eyes of developing (1 to 15 day old) or young adult (40 day old) rats. At various times after injection ranging from 3 hrs. to 7 days, right retinae and left and right geniculates were removed and assayed for radioactivity. Since the rat optic system is predominately crossed, left minus right geniculate (L-RLG) radioactivity was used as an index of axonally transported ^{35}S taurine activity. Results indicated that taurine was rapidly transported along both neonatal and young adult optic axons. This is in contrast to other amino acids (i.e., leucine and proline) which are not axonally transported in this system. Peak L-RLG ^{35}S activity in both neonatals and adults was reached by 24 hrs. after injection. Significant developmental variations were seen in the levels of both L-RLG and right retinal ^{35}S taurine activity 24 hrs. after injection of 1,4,7,11,15 and 40 day old rats. Autoradiographic analysis of retinae following intraocular injection of ^{3}H taurine indicated that the proportion of total retinal radioactivity in the ganglion cell layer was approximately 15% in 5,11 and 40 day old rats. Thus, total right retinal ^{35}S activity was used as an index of ^{35}S taurine uptake by retinal ganglion cells. The levels of L-RLG ^{35}S taurine activity "corrected" for retinal uptake in animals injected at 1,4,7 and 11 days after birth (prior to and during the major period of synaptogenesis in the geniculates) were 4.5, 3.1, 2.3 and 2.6 times higher, respectively, than those in the young adults. In contrast, the amount of corrected L-RLG ^{35}S activity in animals injected 15 days after birth (after synaptogenesis) were not significantly different than those in the young adults.

These data show that taurine is transported along both neonatal and young adult optic axons and that the amount of taurine transported along neonatal axons prior to and during the development of synaptic connections is several fold higher than along adult axons. It is proposed that axonally transported taurine may be acting as a stabilizer of axonal membrane activity during the formation of synaptic connections.

104 SPECIFIC AND RAPID TRANSPORT OF FREE GLYCINE IN IDENTIFIED AXONS OF APLYSIA. C.H. Price, D.J. McAdoo, and W. Farr*. Marine Biomedical Inst., Univ. Texas Medical Branch, Galveston, TX 77550.

Neurons R3-R14 in the parietovisceral ganglion (PVG) of Aplysia may be glycinergic (Price, et al., and McAdoo, et al., 1978, Brain Res., in press). Their axons travel down the branchial nerve to peripheral terminals presumed to be near the junction of the heart and efferent vein from the gill. The 12 axons are easily identified in histological sections due to their large size, characteristic granules, and extensive invagination by glial cells. Using a double-chamber apparatus, the PVG was incubated in vitro, in media containing ^{3}H-glycine, and the branchial nerve was extended into a radioactivity-free, superfusion chamber. After incubations for several hours, the nerve was either frozen in place and sliced into sequential 1 mm pieces for liquid scintillation spectrometry or fixed with 3% glutaraldehyde for autoradiography. In light microscope autoradiographs of cross-sections taken at 4 mm from the PVG, 45% of the silver grains were localized in R3-R14 axons; these axons take up less than 10% of the entire nerve area. Electron microscope autoradiographs confirmed that the silver grains in R3-R14 were inside the axons and not in surrounding glial tissue. When other ^{3}H-amino acids were used instead of glycine, the axons of R3-R14 were labeled equally to other axons. At least 85% of transported radioactivity comigrated with free glycine in thin-layer chromatographic analyses of pieces of nerve from experiments in which ganglia were incubated in media with ^{3}H-glycine for 3-24 h. Free glycine was transported down the branchial nerve at a faster rate (65 mm/day) than other amino acids (20-48 mm/day) and in greater quantities (>6 times as much). In the left pleurovisceral connective (which contains no R3-R14 axons), glycine was transported at 25 mm/day. Transport of glycine down the branchial nerve was inhibited by mercuric ions ($HgCl_2$, 1 mM), by lowered calcium (oxalic acid, 20 mM), and by the microtubule agents vinblastine (0.1 mM) and Nocodazole (10 µg/ml) but was not affected by 2,4 DNP (2 mM), NaCN (1 mM), or high K^{+} (150 mM). Ligation stopped transport; no proximal accumulation of radioactivity was observed. Glycine is transported retrogradely at a rate comparable to orthograde transport but in quantities 9 times less. In autoradiographs of nerves from retrograde experiments, silver grains were localized almost exclusively to R3-R14 axons. We have demonstrated the fast and energy-dependent axonal transport of large quantities of free glycine in identified neurons R3-R14. This specific transport, which is directly dependent on the presence of calcium and may involve microtubules, strengthens the notion that there is a non-metabolic role for glycine in these neurons, perhaps as a neurochemical messenger. This work was supported by an NIH fellowship to CHP (#1F32NS 05856) and DHEW grant #11311 to DJM.

105 DIFFERENTIAL TURNOVER OF AXONALLY TRANSPORTED GLYCOPROTEINS. James A. Ripellino* and John S. Elam, Dept. Bio. Sci., Florida State Univ., Tallahassee, FL. 32306

It has previously been shown that axonally transported glycoproteins reach maximum accumulation in the goldfish optic tectum at 25-30 h after intraocular injection of ^{3}H- fucose (Forman et al, Br. Res. 48, p. 327 (1972); Monticone & Elam, Br. Res. 100, p. 61 (1975)). This label was found to undergo subsequent turnover with a half life approximately of 20-30 days. In the present study, the distribution and turnover of axonally transported glycoproteins was assessed in the membranous and soluble subfractions of goldfish tectum at various times after intraocular injection of ^{3}H fucose. The membranous fraction (sedimentable at 100,000 g) comprised 87% of the rapidly transported label 24 h postinjection. This fraction exhibited a half-life for the disappearance of radioactivity of 20 days. In contrast, the 100,000 g soluble fraction showed a more rapid turnover, with an apparent halflife of 7 days.

Axonally transported glycoproteins associated with the membranous and soluble fractions were converted to glycopeptides (by pronase digestion) and separated into dialyzable and non-dializable fractions. At 24 h post injection the membrane derived non-dialyzable glycopeptides produce a broad peak within the included volume of Sephadex G 50. At 20 days post injection, this distribution shows an apparent increase in molecular size, suggesting more rapid turnover of the lower molecular weight chains. There was no corresponding turnover-dependent change in the size of non-dialyzable glycopeptides associated with the soluble fraction.

The results suggest that soluble glycoproteins are utilized more rapidly than membranous glycoproteins in the nerve terminals. The various sized glycopeptides from the soluble fraction appear to have similar turnover rates while glycopeptides from membranous fraction show size dependent differences in turnover. These patterns support the concept of differential utilization of various classes of glycoproteins at the nerve terminal.

106 NEUROTOXIC HEXACARBONS INTERFERE WITH NERVE FIBER GLYCOLYSIS. Mohammad I. Sabri*, Cyril L. Moore*+ and Peter S. Spencer, Neurotoxicology Unit, Albert Einstein College of Medicine, Bronx, N.Y. 10461 and +the School of Medicine at Morehouse College, Atlanta, Georgia.

Prolonged, low-level exposure to neurotoxic carbon compounds such as methyl n-butyl ketone (MBK) or its metabolite, 2,5-hexanedione (2,5HD) initially causes axonally transported materials to accumulate and form swellings on the proximal side of nodes of Ranvier in the distal region of long and large PNS myelinated fibers, and, subsequently, fiber breakdown below an axonal swelling. Speculating that anterograde axonal transport blockade might first occur at nodes of Ranvier if decreased amounts of ATP were available to drive energy-dependent axonal transport and impulse conduction, investigations commenced to determine the possible effects of hexacarbons on metabolic pathways concerned with energy production. We have focused our studies on glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphofructokinase (PFK), major control steps in glycolysis. When crystalline GAPDH or PFK was pre-incubated with MBK or 2,5HD enzyme activity was inhibited as a function of the concentration of the toxin and the period of pre-incubation. Enzyme activity was preserved by dithiothreitol, suggesting that sulfhydryl groups were vulnerable to hexacarbons. A comparable response was found when these enzymes were assayed in both PNS and CNS tissue pre-incubated with MBK or 2,5HD in vitro. Double reciprocal plots of velocities against substrate concentration revealed that GAPDH was inhibited un-competitively by 2,5HD. By contrast, 1,6 hexanediol (a neurologically inactive hexacarbon compound) and acetone failed to inhibit crystalline GAPDH activity. The specificity of hexacarbon-induced GAPDH inhibition was shown by preservation of crystalline and tissue lactic dehydrogenase acticity, even after prolonged pre-incubation with 2,5HD or MBK in vitro.

These data demonstrate that neurotoxic hexacarbon compounds interfere with energy metabolism by inhibiting key glycolytic enzymes in peripheral nerves. Because GAPDH blockade is known to result in a failure of fast axonal transport, we suggest that this mechanism may be in part responsible for the abnormalities of axonal transport seen in hexacarbon neuropathy. Furthermore, these data provide the first evidence to support our hypothesis that hexacarbons are targeted directly against enzymes required for axonal integrity, the nerve cell body perhaps being unable to meet the presumed increased axonal demand, thereby causing distal regions to suffer first from a failure and undergo degeneration.

Supported by USPHS grants, OH00535 and NS 03355 and a grant from the American Petroleum Institute.

107 A MULTIWIRE PROPORTIONAL CHAMBER USED TO DETECT AXONAL TRANSPORT OF ^{35}S-METHIONINE AND ^{32}P-PHOSPHATE LABELLED MATERIAL.

R.E. Snyder*, T.R. Nichols* and R.S. Smith. Division of Biomedical Engineering and Applied Sciences, the Department of Physics and the Department of Surgery, University of Alberta, Edmonton, Canada.

A multiwire proportional chamber was used to detect axoplasmic transport in dorsal root ganglion/sciatic nerve preparations from *Xenopus laevis*. Nerve cell bodies exposed to ^{35}S-methionine were shielded from the detector by a lead-lined compartment; the sciatic nerve ran in an unshielded chamber over the detector. In experiments with ^{32}P, the effects of secondary X-rays were avoided by loading the label into the cell bodies for several hours in a separate bath; the dorsal root ganglia and adjacent nerve roots were then removed and the sciatic nerve alone was placed in a chamber over the detector. The detector collected radiation from a series of 6 mm segments of nerve for consecutive periods of 1/2 h for the duration of experiments lasting 18-22 h. Typically, in the ^{35}S-methionine experiments, the radioactivity of each nerve segment showed an initial plateau at background followed by a rise which was linear through time. Plots of the time at which the rise in radioactivity took place against the position of each segment of the nerve yielded transport velocities of about 6.2 mm/h. Cutting the nerve proximally showed that label left each segment of nerve at the same velocity. Material labelled with ^{32}P was also transported at a similar rapid velocity. An order of magnitude less label was transported if ^{32}P-phosphate was used in conjunction with phosphate-buffered saline than if the saline were HEPES-buffered and free of cold phosphate. With HEPES buffer, the amount of ^{32}P transported was about two orders of magnitude less than the amount of ^{35}S-methionine which was transported in similar preparations.

Supported by the Medical Research Council of Canada.

108 TRANSNEURONAL TRANSFER OF AXONALLY TRANSPORTED RADIOACTIVITY DURING POST-NATAL DEVELOPMENT OF THE HAMSTER VISUAL SYSTEM. Susan C. Specht* (SPON: W.C. De Mello). Dept. Pharmacol. UPR Sch. Med., San Juan, PR 00936.

Transneuronal transfer (TT) of axonally transported protein-bound radioactivity was examined in the visual system of neonatal and adult albino hamsters. Following intraocular injection of 3H-proline, radioactive protein is axonally transported to nerve endings in the lateral geniculate body (LGB) and superior colliculus (SC). Some of the transported radioactivity is transferred to LGB cells and subsequently transported to LGB nerve endings in layer IV of visual cortex where it can be detected by scintillation counting or autoradiography. Non-specific TT from SC to overlying retrosplenial cortex was also observed.

TT was evaluated both 1 and 11 days after intraocular injection of 3H-proline by scintillation counting of visual and non-visual cortex. Early TT (1 day) was greatly enhanced during the period of eye-opening (14-16 days post-natal) and then diminished in older animals. Labeling of LGB was not significantly different. Early TT was not detected against the cortical background radioactivity in 8 and 10 day animals. Late TT (11 days) was observed in all ages, but was significantly greater in young animals. Non-specific transfer to retrosplenial cortex followed essentially the same developmental pattern. These results demonstrate that the onset of functional visual activity in the optic nerve is accompanied by a large but transient increase in TT of rapidly (or intermediately) transported radioactivity. Increases in both specific and non-specific transfer suggest that the primary event is enhanced release from optic nerve endings and not a post-synaptic event, e.g., enhanced uptake or enhanced protein synthesis, although these may play a part. The fact that TT decreases in older animals with functional visual activity supports the conclusion of Grafstein and Laureno (Exp. Neurol. 39:44, 1973) that synaptic activity per se is neither a sufficient nor necessary condition for TT. The brief increase in TT at the time of eye-opening may be related to inductive events in visual system development. (Partly supported by grant CA-16598-02).

109 SIMILAR PROTEINS ARE RAPIDLY TRANSPORTED IN DORSAL ROOT SENSORY NEURONS AND VENTRAL HORN MOTONEURONS. George C. Stone and David L. Wilson, Dept. of Physiol. and Biophys., U. of Miami Sch. of Med., Miami, Fla. 33152.

Despite histochemical and enzymatic evidence suggesting the rapid axoplasmic transport of proteins unique to specific types of neurons, the majority of electrophoretic studies to date have found no differences in rapidly-transported proteins in various neuronal systems.

Two-dimensional gel electrophoresis has allowed a higher-resolution comparison of rapid transport in dorsal root sensory neurons with that in ventral horn motoneurons in sciatic nerve. Experiments were performed in vitro in frog as described previously (Stone et al., Brain Res. 144; 287-302). Dorsal root ganglia 8 and 9, or hemisected spinal cord were selectively exposed to ^{35}S-methionine for 6 hr, while the sciatic nerve was ligated 30 mm distal to the label site. After 24 hr at 18°C label which accumulated in a 3-mm segment proximal to the ligature was subjected to two-dimensional gel electrophoresis. Comparisons were made of fluorographic patterns from dried gels. Fifty independent species of protein, including all the more abundant ones were consistently common to both dorsal root and ventral horn neurons (see figure, closed symbols), while at most 4 minor protein species were possibly transported only in ventral horn neurons (open symbols). Less counts were transported in ventral horn neurons, so the determination of transported proteins unique to dorsal root neurons was not attempted.

The analysis suggests that approximately 50 of the most abundantly-transported, independent proteins are common to these two sets of neurons which subserve very different functions. This result verifies and extends the earlier observations of others made with lower-resolution electrophoretic techniques.

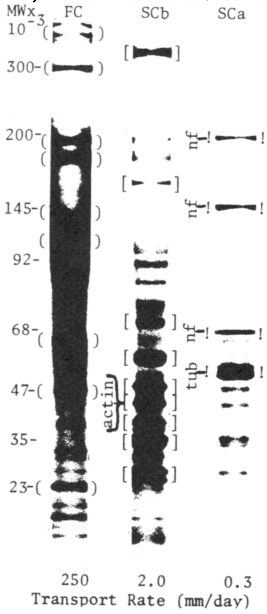
Supported by NIH grant NS12393 and a biomedical research support grant. GCS is a NIH postdoctoral trainee (NS07044).

110 COMPARISON OF FAST TRANSPORTED PROTEIN AND GLYCOPROTEIN DOWN-FLOW PATTERNS IN SENSORY AND MOTOR FIBERS OF MAMMALIAN SCIATIC NERVE. Daniel P. Stromska* and Sidney Ochs. Dept. Physiology, Indiana University School of Medicine, Indianapolis, IN 46202.

Glycoproteins and some polypeptides are considered to be carried in nerve fibers by fast axoplasmic transport, primarily to the axon terminals, while the bulk of the proteins synthesized by the soma are transported more slowly. We compared the outflow characteristics of fast transported ^{3}H-fucose and ^{3}H-glucosamine labeled glycoproteins with ^{3}H-leucine labeled proteins in the motor and sensory fibers of the cat sciatic after injecting the labeled precursors into the L7 dorsal root ganglion or L7 ventral cord. The resulting outflow patterns showed a higher amount of incorporated ^{3}H-leucine transported in the crest than of ^{3}H-fucose labeled components in the sensory fibers. The ganglion pool - nerve crest amplitude relationship (Ochs, J. Physiol. 255:249, 1976) was the same with either ^{3}H-fucose or ^{3}H-leucine as the precursor. The slope of the advancing front of ^{3}H-fucose labeled activity was shallower than that of the ^{3}H-leucine labeled proteins in the sensory fibers after 3 and 7 hours of downflow. A shallow front slope was also observed with ^{3}H-glucosamine labeled material in sensory fibers. The maintained shallow slope of the labeled fronts in the sensory fibers with distance of the ^{3}H-fucose labeled substances, suggests, that in contrast to ^{3}H-leucine labeled materials, fucose labeled glycoproteins have a different process of synthesis and export into the fibers. Glycoprotein synthesis requires transit through the Golgi apparatus, the shallower front slope found for the sensory fibers with ^{3}H-fucose compared to ^{3}H-leucine may be due to this process. The outflow patterns in the motor fibers generally show a somewhat higher amount of ^{3}H-fucose labeled material transported than ^{3}H-leucine labeled components, as judged by their crest amplitudes. In contrast to the sensory fibers, the slopes of the fronts of both ^{3}H-fucose labeled glycoproteins and ^{3}H-leucine labeled proteins were shallow in the motor fibers and this was seen after both 4 and 7 hours of downflow. This observation suggests that ^{3}H-leucine polypeptides are processed in motor neurons at a slower initial rate than in sensory neurons. The ^{3}H-leucine labeled polypeptides in the motoneurons may be more dependent on Golgi processes and/or slower polypeptide synthesis which occurs at a rate comparable to that of glycoproteins in sensory neurons. Supported in part by NIH grant PHS RO1 NS 8706-09 and NSF grant BNS 75-03868-A03.

111 AXONAL TRANSPORT IN GUINEA PIG OPTIC NEURONS: EACH COMPONENT CONSISTS OF A DISTINCT PATTERN OF PROTEINS. M. Tytell* and R. J. Lasek, Neurobiology Center and Anatomy Department, Case Western Reserve Univ., Cleveland, Ohio 44106.

When guinea pig optic neurons are pulse labeled, axonal protein transport appears as three major waves of radioactivity defined by their velocities: the fast component (FC), >250 mm/day; slow component b (SC_b), 2.0 mm/day; and slow component a (SC_a), 0.3 mm/day (Black & Lasek, Soc. Neurosci. 3:29, 1977). The hypothesis that each component contains a distinct class of proteins was tested by SDS-PAGE analysis. ^{3}H-lys and -pro were injected into the vitreous hymor of guinea pig eyes and the animals sacrificed after 6 hrs for FC, 6 days for SC_b, and 38 days for SC_a. For FC, the optic nerve was cut slightly anterior to the chiasm to cause the FC proteins to accumulate. The portion of nerve containing the wave of labeled proteins was homogenized in 8 M urea with 5% BME and 1% SDS, which solubilizes essentially all proteins. Following centrifugation at 140,000g-30 min, the labeled proteins in the supernatant were analyzed by SDS-PAGE on a 4-17.5 % gradient gel. In the Fig, the bands unique to each component are enclosed by () for FC, [] for SC_b, and !! for SC_a. The patterns are clearly different. None of the proteins are present in equal amounts in more than one component and most do not overlap at all. The few bands which appear similar in two components (80K, 92K, and 180K daltons for FC and SC_b and 35K, 43K, 50K, and 250 K daltons for SC_b and SC_a) are most likely different proteins with similar electrophoretic mobilities or small amounts of proteins trailing behind the wave. It is also possible that a few proteins are actively conveyed in more than one component. These possibilities are being explored. Since neurofilament proteins (nf) and tubulin (tub) are conveyed exclusively in SC_a, actin in SC_b and FC contains mainly membraneous proteins, each component of axonal transport appears to represent a specific set of structural and functional entities.



112 AXONAL TRANSPORT OF TRITIATED PROLINE IN THE CORTICOSPINAL TRACT OF THE RAT SPINAL CORD. H. Lee Vahlsing*, Earl R. Feringa and Ronald B. Hirschl*. Depts. Neurol. and Path., VA Hosp. and U. of Mich., Med. Ctr., Ann Arbor, MI 48105

Thirty-six female rats received injections of tritiated proline (100 µCi in 5 µl) into the sensory-motor cortex at eleven weeks of age. At time intervals of 1,2,4,6,8,16 and 24 hours and 2,4,7,14 and 21 days after injection, groups of three rats were sacrificed and formalin perfused. The spinal cords were completely removed and divided into sixteen 5 mm long segments beginning at the obex. Each segment was then analyzed for tritium content using scintillation counting technique. Radioactivity was detected in the spinal cord segments of rats sacrificed one hour after injection in amounts 5-10 times greater than that found in uninjected controls. Tritium label in segments from rats sacrificed two hours after injection showed similar low level background, but also exhibited the leading edge of a wave of tritium which passes down the spinal cord at the rate of 15-20 mm/hr. This wave, which was initially five to ten times the magnitude of background levels found at one hour, was approximately five cm in length and was skewed toward the leading edge. This wave left behind a residuum of tritium in each segment which was approximately proportional to the mass of that segment. Autoradiography on spinal cord segments revealed that most tritium was confined to the corticospinal tracts, but a minor amount was somewhat diffusely present in the dorsal and lateral horns of the gray matter. At four days after tritium injection, the leading edge of a second wave of tritium was apparent in the first few segments of spinal cord. Initially this peak was about 100 times greater than the background found in the one hour rats, but as it traveled at 3-4 mm/day, the peak decreased progressively leaving a residuum of tritium which labeled the corticospinal tracts lying ventrally in the dorsal white columns. Autoradiography revealed that this wave also frequently labeled a small tract adjacent to the ventral median fissure. This tract was most easily identified in the cervical regions. The label in the horns of the gray matter was less intense and more diffuse. Autoradiography after the injection of the sensory-motor cortex of only one cerebral hemisphere revealed only the contralateral dorsal corticospinal tract was labeled while the ventral tract was labeled only on the ipsilateral side of the cord.

113 RETROGRADE TRANSPORT OF FLUORESCENT SUBSTANCES IN THE RAT BRAIN. Derek van der Kooy, Henricus G.J.M. Kuypers[X], Coriene F. Catsman-Berrevoets[X] and Marina Bentivoglio[X]. Dept. Anat., Sch. Med., Frasmus Univ., Rotterdam, The Netherlands.

A search has been made for fluorescent substances that are retrogradely transported in axons. Evans Blue, which fluoresces red, and a mixture of Primuline and 4'-6-diamidino-2-phenylindol 2 HCL (DAPI), which fluoresces blue with golden granules, can both be shown to travel retrogradely from an injection area in the caudate-putamen back to cell bodies in the substantia nigra of the rat. Recently, Propidium Iodide, which fluoresces red- orange, has also been shown to undergo retrograde axonal transport in the nigro-striatal system. Two of these fluorescent compounds can be used to simply and effectively demonstrate double labelling of single cells. This has been shown in the mammillary bodies, after injection into two divergent axon collateral areas. Rats were injected unilaterally with 0.10-0.40 ul of 10% Evans Blue solution into the AV, AD, and AM thalamic areas, and with a similar quantity of 10% Primuline-2.5% DAPI solution into an area medial to the red nucleus where the descending mammillary body fibers travel. Many of the cells in the ipsilateral lateral mammillary nucleus and in the medial part of the ipsilateral medial mammillary nucleus were labelled both with Evans Blue and DAPI-Primuline. This double labelling technique has also been used to study the degree of collateralization of certain brainstem projections to the forebrain. After injections of Evans Blue into the frontal cortex- olfactory tubercle and DAPI-Primuline into the temporal cortex- amygdala, many double labelled cells were seen in the dorsal raphe nucleus. In contrast, separate, but intermixed, clusters of red and blue fluorescent cells were seen in the substantia nigra. Other experiments have demonstrated that a population of substantia nigra pars reticulata cells give collateral projections to both the thalamus and superior colliculus. A continuing search is in progress to find other fluorescent substances transported retrogradely in neurons, in order to improve further the double labelling methodology with the aim to investigate the axon collaterals of cells in other neural systems.

114 SUBCELLULAR LOCALIZATION OF PARTICLES INVOLVED IN THE TRANS-LOCALIZATION OF PROTEIN IN CEREBRAL TISSUE. Fredric P. White. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada. A1B 3V6

The translocation of newly synthesized protein from one subcellular site to another within brain cells has been investigated using cerebral cortex slices. In one experimental design vinblastine, an inhibitor of fast axoplasmic flow, was used in conjunction with a double label routine. In these experiments some slices were incubated with vinblastine [0.1µM], which does not inhibit protein synthesis in slices, and [^{3}H] leucine while other slices were incubated in ^{14}[C] leucine alone. The slices were homogenized together and fractionated by differential sedimentation and discontinuous sucrose gradient techniques. Comparisons of the ratios of $^{3}H/^{14}C$ for each fraction enables one to determine if any fraction is enriched for an export particle or import particle. An export particle is one in which vinblastine causes an accumulation of newly synthesized protein and an import particle is one in which vinblastine prohibits the accumulation of newly synthesized protein. This analysis has shown the presence of at least one export particle, $P_1 6$. This particle pellets in the crude nuclear fraction and has a density between 1.166 and 1.280. Light microscopy inspection of these fractions show the presence of nuclei, however, the particle of interest shows a skewed distribution towards the lighter portion of the gradient in comparison to DNA. The greatest enrichment for an import particle has been obtained from the crude mitochondrial pellet. It [$P_2 2$] has a density between 1.0592 and 1.1036, and is marked by the presence of both 5'AMPase and 2', 3' cyclic nucleotide 3'-phosphohydrolase [2',3'CNP]. Continuous sucrose gradients have shown that the 5'AMPase and 2',3'CNP activities can be separated and the import particle is skewed towards the 5'AMPase. It is believed, therefore, that 5'AMPase is a marker for this import particle. In other experiments using pulse-chase procedures $P_1 6$ was shown to decrease in specific activity during the chase period while $P_2 2$ increased in specific activity during the same period. Thus the conclusion drawn from the vinblastine experiments, that $P_1 6$ was an exporter of protein and $P_2 2$ an importer, were confirmed. Lowering the [Ca^{++}] of the incubation media inhibits the synthesis of protein by the slices, but transport is stopped only when the media is made up Ca^{++} free. It is concluded that subcellular particles can be isolated by ultra centrifugation techniques which are involved in the vinblastine and [Ca^{++}] sensitive transport of proteins. This research was supported by MRC Grant MA-5404.

BASAL GANGLIA

115 RESPONSES OF CAUDATE NEURONS IN AWAKE MONKEYS TO A VISUAL STIMULUS THAT INITIATES A MOTOR TASK. J. Wayne Aldridge, R.J. Anderson and J.T. Murphy. Dept.Physiol., Univ.of Toronto, Toronto, Ontario, Canada, M5S 1A8.

Monkeys were trained to perform a motor task involving wrist movements. The object of the task was to superimpose a square on a vertical target line displayed on a TV monitor. The monkeys achieved this through a manipulandum by flexion or extension of the wrist which corrected the horizontal position of the square. For this study experimental trials were designed to examine responses to the sudden appearance of the line and square on the TV screen, that is, to the visual cue initiating the task. Each trial consisted of a control period during which the display was blank followed by a period during which the monkey performed the task. Spikes from the caudate nucleus (Cd) were recorded with PtIr electrodes and spike times, angular velocity and angular position of the wrist were recorded by an on-line computer. Histological verification of cell locations was carried out.

Two hundred and sixteen Cd cells were examined for a visual cue response. Of this sample 100 neurons (45.6%) altered their cell firing with the appearance of the display. Fifty-two cells were inhibited, 56 were excited and 9 cells had both excitation and inhibition. The majority of the responses observed were phasic in nature. The mean latencies were 238($\pm$164) msec and 200($\pm$67) msec for excitations and inhibitions respectively. Ninety-two cells (92%) responded before the onset of movement. The mean latencies to movement were 200 msec and 261 msec for excitation and inhibition respectively.

These results demonstrate that a significant proportion of Cd neurons have responses correlated with a visual stimulus signifying the onset of a motor task. The anatomical position of the caudate may allow it to direct this information to other motor structures through the pallidal and thalamic pathways.

Supported by MRC of Canada.

116 DEMONSTRATION OF RAT CAUDATE PROJECTION NEURONS BY INTRACELLULAR HORSERADISH PEROXIDASE TECHNIQUE. G.A. Bishop, R.J. Preston* and S.T. Kitai. Dept. Ant., Mich. State Univ., E. Lansing, MI 48824

The caudate (Cd) nucleus is composed of a variety of neuronal types differentiated on the basis of somatic size and dendritic morphology. While previous studies have attempted to identify which of these various neurons are projection cells, no conclusive evidence has been reported. In this study, therefore, we have combined intracellular recording and intracellular staining techniques to (1) identify Cd projection neurons and (2) examine the physiological responses of these projection cells to stimulation of the substantia nigra (SN). Male hooded rats (200-400 g) were anesthetized with urethane(120 mg/100g). SN stimuli (0.05-0.1 msec pulses) were applied through stereotaxically positioned insulated needles. Beveled microelectrodes filled with 4% HRP in 0.5 M KCl-tris buffer were used to record from and stain (positive DC pulses 5-20nA; 1-5 min; f=5/sec) single Cd cells. Following appropriate histological processing, neurons filled with HRP were serially reconstructed with the use of a drawing tube and identified as projection neurons by following their axons out of the CD nucleus. In these neurons, SN stimuli evoked monosynaptic EPSPs at an average latency of 4.3 msec. Often the EPSPs were followed by hyperpolarization lasting 100 msec or more. The injected neurons were located in various regions of the head of the Cd nucleus. Their somata had diameters of 14-24 µm, were either spherical or elliptical in shape and gave rise to 4-9 primary dendrites. These primary dendrites branched repeatedly into secondary and tertiary branches extending 250-400 µm anterposteriorly, 300-350 µm dorsoventrally and 150-200 µm mediolaterally. The somata and primary dendrites of the projection neurons were virtually spine free while the secondary and tertiary dendrites were heavily covered with both pedunculated and sessile spines. The axon arose either directly from the soma or from a primary dendrite and gave rise to 2-4 local collaterals which branched extensively to form a fine axonal plexus within, and to some extent beyond, the dendritic domain of the parent cell. Without branching further in the Cd, the parent axon coursed ventrally and caudally out of the nucleus and into globus pallidus (GP). Some axons appeared to terminate in GP while others, often after giving rise to a beaded collateral in this nucleus, continued into the internal capsule. Several of these latter axons were traced to a point near the entopeduncular nucleus; however, the precise area of termination of these axons has not yet been observed. This study has clearly demonstrated that at least one type of projection neuron in the Cd nucleus of the rat is a medium sized spiny neuron and that this neuron receives excitatory input from the SN. (This work was supported by NIH grant NS00405.)

117 EFFECTS OF VENTRAL MESENCEPHALIC TEGMENTAL LESIONS ON LOCOMOTOR ACTIVITY IN RATS. M.G. Boyeson, G.K. Hodge, and R.T. Linn. Dept. Psychology, U. of New Mexico, Albuquerque, NM 87131.

Recently, increasing concern has been focused on the systems underlying spontaneous locomotor activity in hopes of better understanding the motoric dysfunctions respondsible for hyperactive behavior exhibited by children. The exact neuroanatomical structures and neurophysiological functions respondsible for hyperactivity remain unclear. The integrity of monoaminergic pathways of the mesolimbic and nigro-neostriatal systems, however, appear essential in maintaining normal locomotor activity. Bilateral lesions in the ventral mesencephalic tegmentum, in particular, area A10, and pars compacta of the substantia nigra have resulted in increased locomotor activity (Le Moal et al., EXP NEUROL 50: 521, 1976; Hodge and Butcher, NEUROSCIENCE ABSTRACTS 1: 196, 1975). To further delineate the substrates of spontaneous hyperactivity, bilateral radiofrequency lesions were made in the ventral mesencephalic tegmentum of rats. Locomotor activity was assessed in an open field apparatus for 15 minutes on six days prior to, and twelve days following, the operations. The lesions resulted in a significant increase in locomotor activity. Subsequent histological evaluation revealed that hyperactivity was associated with damage to area A10; such lesions, however, also involved nucleus linearis centralis, believed to be comprised of serotonergic cell bodies. In fact, lesions of nucleus linearis centralis alone were found sufficient to elicit hyperactivity. These data suggest that serotonergic as well as dopaminergic systems may contribute to the increased activity produced by lesions of the ventral mesencephalic tegmentum. (Supported by U.N.M. Research Allocation grants 020-812-270 and 020-812-402.)

118 THE EFFECTS OF STIMULATION OF THE SUBSTANTIA NIGRA ON ENDOGENOUS AND DEUTERIUM-LABELLED ACETYLCHOLINE (ACh) IN THE CAUDATE NUCLEUS OF THE CAT. S.H. Butcher and E. Garcia-Rill, Mental Retardation Res. Ctr., Sch. Med. UCLA, Los Angeles, CA. 90024

The existence of an interaction between dopamine (DA) and ACh in the caudate nucleus has been accepted for many years. However, the exact nature of this interaction or modulation of ACh activity by DA, i.e. whether DA has an excitatory or inhibitory effect on ACh, remains a controversy. In an attempt to examine this interaction in the cat caudate nucleus we employed electrical stimulation of the nigrostriatal pathway as a means of stimulating the DA input to this region. A four-pronged stimulating comb was stereotaxically introduced unilaterally into the substantia nigra in locally-anesthetized, paralyzed cats. The cortex overlying the caudate nuclei was removed by suction allowing the removal of biopsies as described by Anchors and Garcia-Rill (Brain Res., 133:183, 1977). Throughout the experimental procedure animals were infused with deuterium-labelled choline ($^2H_9$-Ch, 1µmol/kg/min). Endogenous and labelled ACh and Ch were analyzed using the gas chromatographic-mass spectrometric method of Jenden et al (Anal. Biochem. 55:438]973). In an attempt to diminish post-mortem changes in ACh, tissue samples were withdrawn into a solution of 1N formic acid/acetone (15/85 v/v) within 1 sec and quenched in liquid nitrogen within 4 sec. In four cats, biopsies from both caudate nuclei were removed before and after 5 min of unilateral stimulation of the substantia nigra (15v, 20Hz, 0.2ms ea).

Stimulation of the nigrostriatal pathway produced a 74% decrease in deuterium-labelled ACh levels, as well as a decrease in the levels of endogenous ACh levels. This decrease in the specific activity of ACh (57%) implies that stimulation of the nigrostriatal pathway had an excitatory effect, (eg. increased ACh release) on ACh neurons in the caudate nucleus. This increased release in ACh probably represents release of ACh from the newly synthesized pool.

This work was supported by NIH postdoctoral Fellowship 5F3Z NSO 5316-0Z, USPHS HD-05958, MH-7097 and NS-12324.

119 AN ELECTROPHYSIOLOGICAL STUDY OF CORTICO-CAUDATE CONNECTIONS: ROLE OF THE CENTER MEDIAN-PARAFASCICULAR NUCLEI OF THE THALAMUS. E. Cherubini*, M.S. Levine, N.A. Buchwald and C.D. Hull. Mental Retardation Research Center, Sch. Med., UCLA, Los Angeles CA 90024.

In cats, the pericruciate cortex (CX) projects heavily to the head of the caudate nucleus (CD). Electrical stimulation of CX produces an initially excitatory response in CD neurons. Some of the responses are monosynaptic but there are other routes by which stimulation of CX can influence CD neurons. For example, stimulation of CX can activate thalamic nuclei which project to the CD or may antidromically activate collaterals projecting from intralaminer thalamus to both CX and CD. There is anatomical evidence for each of these alternative routes. The present experiment was designed to determine the extent to which indirect activation of CD neurons occurs in response to CX stimulation. Extracellular spike potentials of CD neurons were evoked by electrical stimulation of precruciate CX in intact cats. Mean latencies to response and the distribution of these latencies were determined. Following these recordings electrolytic lesions of the center median-parafascicular complex of the thalamus (CM-PF), an important contributer of inputs to CD were produced in the same cats in order to interrupt some of the previously mentioned indirect CX-CD pathways. The responses of CD neurons to CX stimulation were then redetermined. Experiments were begun 1-2 hrs postlesion. Analysis of the data revealed a significant decrease in mean latencies to respond from 20 msec prelesion (6 cats, 86 units) to 12 msec postlesion (77 units). The distribution of latencies for individual units was also markedly changed. A shift from a peak latency of 20-25 msec (30% of the response) prelesion to a peak of 5-10 msec (30% of the response) postlesion occurred. In the lesioned cats less than 8% of the units responded with latencies of more than 25 msec while in intact animals more than 21% of the latencies were in this range. In addition to the shortening in latency, the postlesion responses were less variable. Average standard deviations (SD) decreased from 4 msec prelesion to 2.2 msec after the lesion. Similarly, there was a marked shift in the distribution of SDs. Before the lesion only about 10% of the response showed SDs less than 1 msec. After the lesion more than 40% of the responses were in this catagory. These results were not due to a non-specific effect of the lesions since additional control lesions in the entopeduncular nucles did not markedly alter response latencies. We conclude that in the intact cat many of the CX-CD responses may result from indirect activation of the CD through orthodromic or antidromic activation of thalamic inputs. Supported by USPHS grants MH-7097 and HD-05958.

120 THE SUBTHALAMIC NUCLEUS AND THE SUBSTANTIA NIGRA OF THE MONKEY. NEURONAL ACTIVITY IN RELATION TO MOVEMENT. Mahlon R. DeLong and Apostolos P. Georgopoulos, Dept. Physiology, The Johns Hopkins Sch. Med., Baltimore, MD 21205.

Single neurons in the subthalamic nucleus (STN) and substantia nigra pars reticulata (SNpr) and pars compacta (SNpc) were studied in the monkey during the performance of a step and a pursuit tracking task (see Georgopoulos,A.P. and DeLong,M.R., The globus pallidus of the monkey: Neuronal activity in relation to movement, this volume). 227 neurons were isolated in 32 histologically identified penetrations in two hemispheres: 123 from STN, 90 from SNpr and 24 from SNpc. Nearly all of the task-related units were activated in both the step and the pursuit tasks; detailed analysis of the relations of unit activity to the amplitude, velocity and acceleration of movement and to EMG activity is currently being done. Further observations were made by careful examination of the animal to specify the responses of the units to "passive" manipulations and to active movements that the animal made outside the context of the task. Most STN units exhibited a tonic, bursting pattern of spontaneous discharge. Many cells were strikingly modulated by active movements of individual contralateral limbs, or of the face, tongue or jaw. Most of the units related to the limbs discharged with proximal movements. Passive driving, when present, was usually weak and was related to deep structures in and around the region involved in the active movement. Movement related units were found largely in the lateral portion of STN, where units related to specific body parts were grouped together suggesting a somatotopic organization. Most units isolated from the medial part of STN were not clearly affected by movements. SNpc units had low spontaneous discharge rates and did not show clear modulation with active movements or passive manipulations. SNpr units had high spontaneous activity, similar to those of internal pallidal units. Many SNpr cells were modulated by chewing, licking or swallowing movements; only a few were related to limb movements, and a few were modulated by eye movements.

It appears that the pars reticulata of the substantia nigra and the internal segment of the globus pallidus (GPi) together form a functional entity which has been divided by the internal capsule. This is suggested by: (a) the striking similarities in the morphology of their neurons, neuropil and ultrastructure, as well as in their afferent and efferent connections, (b) the nearly identical patterns of spontaneous activity (high tonic discharge), and (c) the apparent continuation of the GPi body representation into the SNpr, with units related to licking and chewing movements located in the medial portion of GPi (Georgopoulos and DeLong, this volume) and in the lateral portion of SNpr, indicating further the arbitrariness of their separation by the internal capsule.

121 TWO FORMS OF CATALEPSY REVEALED IN DIFFERENTIAL EFFECTS OF MORPHINE VERSUS HALOPERIDOL ON POSTURAL SUPPORT MECHANISMS. Marc De Ryck*, Timothy Schallert*, and Philip Teitelbaum. Dept. Psych., Univ. of Illinois at Urbana-Champaign, Champaign, IL 61820.

Neuroleptics (e.g., haloperidol), which block dopaminergic receptors, and narcotic analgesics (e.g., morphine) produce catalepsy, i.e., active maintenance of passively imposed postures. The differences between neuroleptic and narcotic catalepsy are still poorly understood (Costall & Naylor, 1973). By electromyographic recordings from antagonistic flexor and extensor muscles in the frontpaw and hindpaw of 20 rats, we show that morphine and haloperidol have opposite effects on static support mechanisms. We analyzed an important feature of catalepsy, i.e., resistance to passively induced displacement ("negativism"). When haloperidol-treated rats (1, 5, 10 mg/kg) were pushed forward, backward, or sideways, they resisted passive displacement by enhanced bracing reactions in the limbs. Bracing was characterized by simultaneous tonic contractions in antagonistic flexor-extensor muscles of the frontpaw (biceps and triceps) and hindpaw (tibialis anterior and gastrocnemius). Synergistic activation of antagonistic limb muscles is a normal mechanism involved in static support (the positive supporting reaction of Magnus, PSR). In haloperidol-treated rats, the PSR appeared spontaneously, even in the absence of external challenges. Haloperidol-induced release of PSR was transient; it waned when haloperidol-treated rats were completely immobile in a stable posture. Thus, haloperidol causes a transient, synergistic rigidity in response to challenges to static equilibrium. Morphine (20, 30, 40 mg/kg) produced persistent rigidity in limb musculature normally antagonistic to a given limb position, i.e., extensor hypertonia during paw flexion and flexor hypertonia during paw extension. When subjected to challenges of body stabilization, morphine-treated rats failed to display bracing reactions and allowed themselves to be rolled over to their sides. Thus, morphine causes a free-running, steady-state, antagonistic rigidity insensitive to challenges to static equilibrium. In other words, whereas the PSR was released during haloperidol catalepsy, it was suppressed during morphine catalepsy. Like the PSR, cold-induced shivering in the limbs consists of synergistic contraction of antagonistic muscles. Haloperidol permitted limb shivering, but morphine abolished it. Haloperidol rats showed rapid contact or air righting from a supine position, whereas righting reflexes in morphine rats were slow or entirely inhibited. Tonic grasp was present in haloperidol rats, but absent in morphine rats. Thus, in haloperidol catalepsy, tonic postural mechanisms involved in securing and maintaining a normal stable equilibrium posture are released, whereas in morphine catalepsy these mechanisms are inhibited. Supported by NIH Grant #RO1 NS 11671.

122 ELECTRON MICROSCOPY OF GOLGI IMPREGNATED SPINY AND ASPINY NEURONS IN MONKEY NEOSTRIATUM. M. DiFiglia, P. Pasik and T. Pasik. Dept. of Neurol., Mount Sinai Sch. of Med., CUNY, New York, N.Y. 10029.

Most of the neurons in the monkey neostriatum are of medium size and, as shown in Golgi impregnations, include cells with spiny dendrites and long axons, and cells with aspiny, varicose dendrites and short axons (DiFiglia et al, 1976). Standard electron microscopic methods have provided data which permitted only a tentative correlation with the above findings (Pasik et al, 1976). Presently, however, positive identiffcation can be achieved by applying a gold-toning technique to Golgi impregnated material (Fairén et al, 1977). This procedure allows the examination of cytological detail of deimpregnated cells at the EM level. A modification of the latter method was used in the present study for the unequivocal identification of neostriatal Spiny I and Aspiny I neurons (DiFiglia et al, 1976). Golgi impregnated sections (175 μm) were gold-toned, and regions containing isolated neurons of the above types were microdissected and flat-embedded in Epon. Neurons were photographed or drawn with camera lucida, and then serial thin sections were cut and mounted on formvar-coated slot grids.

A comparison between these 2 neuronal types shows that Spiny I neurons have a relatively poorer content of perikaryal organelles. Axosomatic synapses are more numerous and are formed by various types of axon terminals, most of which contain pleomorphic vesicles and make symmetric contacts. Fewer synapses of similar nature are seen on proximal spineless portions of the dendrites. Distal branches are rich in synapses particularly on spines. Serial sections show that most axospinous complexes are surrounded by glial somata or their processes. Symmetric synapses are also present on the axon initial segment of these neurons. Aspiny I neurons are richer in most organelles: Golgi substance, mitochondria, lysosomes and rough endoplasmic reticulum. Some small stacks of the latter are also present. Axosomatic synapses are infrequent. In contrast, proximal and distal dendrites are contacted by numerous axon terminals with either small round vesicles and asymmetric junctions, or with pleomorphic vesicles and symmetric contacts. Synapses on the axon initial segment are only occasionally seen. Serial sections show that a single axon terminal can contact both a dendritic spine, most likely belonging to a spiny I neuron, and a dendrite of an aspiny I neuron. Such axonal endings can be of 2 categories: a frequently seen profile containing small round vesicles and making asymmetric contact, and another containing pleomorphic vesicles and making a symmetric contact.

These findings offer distinguishing features of the 2 most common medium size neostriatal neurons, and indicate a different distribution of inputs on these cells. Aided by NINCDS Grant NS11631.

123 THE LOCALIZATION OF ACETYLCHOLINESTERASE IN THE CORPUS STRIATUM AND SUBSTANTIA NIGRA OF THE RAT. H. C. Fibiger[1], John Lehmann[1] and L. L. Butcher[2] (SPON: J. Pinel). [1] Div. Neurological Sci., Dept. Psychiatry, University of British Columbia, Vancouver, B.C. Canada, and [2] Dept. Psychology, University of California, Los Angeles, California, U.S.A.

The distribution of acetylcholinesterase (AChE) in some extrapyramidal nuclei was examined by means of intrastriatal injections of kainic acid. Pharmacohistochemical experiments showed that AChE-containing neurons in the striatum were among those which were destroyed by kainic acid. In complementary biochemical studies, it was demonstrated that approximately 50% of the total AChE activity in the striatum was localized in these AChE-containing neurons. Intrastriatal injections of kainic acid produced a substantial decrease in the activity of the glutamic acid decarboxylase in the substantia nigra, thus demonstrating that neurons contributing to the striato- and/or pallido-nigral pathways had been lesioned. However, nigral AChE activity was not significantly reduced by the striatal kainic acid injections. Furthermore, stereotaxic injections of colchicine along the course of the striato-nigral projection failed to produce an accumulation of AChE in these fibres proximal to the injection. In contrast, injections of colchicine into the nigro-striatal projection led to a proximal accumulation of AChE in the fibres of this system, thus confirming the presence of AChE in these dopaminergic neurons. It is concluded that the striato- and pallido-nigral projections in the rat do not contain AChE. Furthermore, AChE-containing neurons in the striatum appear to be interneurons rather than the source of striatal efferents. It is suggested that these AChE-containing neurons may be striatal cholinergic interneurons.

	Corpus Striatum	
	% of control	Control value ± SEM
AChE	62.5% ± 6.0%*	43.4 ± 2.07 umole/mg prot-hr
CAT	27.5% ± 7.3%*	109.9 ± 4.01 nmole/mg prot-hr
GAD	23.3% ± 3.4%*	103.7 ± 3.71 nmole/mg prot-hr
TH	126.8% ± 5.5%*	7.88 ± .319 nmole/mg prot-hr
	Substantia Nigra	
	% of control	Control value ± SEM
AChE	92.0% ± 4.8%	10.9 ± .132 umole/mg prot-hr
CAT	104.0% ± 9.8%	16.7 ± .96 nmole/mg prot-hr
GAD	51.1% ± 3.8%*	265.0 ± 8.75 nmole/mg prot-hr
TH	93.9% ± 4.9%	5.59 ± .404 nmole/mg prot-hr

n = 12, * $p < .001$, Student's two-tailed test.

Table I. Neurotransmitter-related enzymes in corpus striatum and substantia nigra two weeks after injection of kainic acid (10 nmole) in the caudate putamen.

124 ALTERATIONS IN SPONTANEOUS NEURAL ACTIVITY IN THE CAUDATE NUCLEUS AFTER UNILATERAL NIGRO-STRIATAL LESIONS. E. Garcia-Rill, N. Buchwald, C.D. Hull, and E. Cherubini*. Mental Retardation Research Center, University of California, Los Angeles 90024.

We have shown previously, that unilateral electrolytic lesions intersecting the nigrostriatal bundle in the lateral hypothalamus (MFB lesions) of cats deplete dopamine by 90% in the ipsilateral caudate nucleus but have no statistically significant effect on spontaneous neuronal firing in that structure. In contrast, spontaneous neuronal firing in the contralateral caudate is slowed markedly by the unilateral lesions. In these earlier studies, the recordings of neuronal activity were made at least 2 weeks after the lesioning. It was possible, therefore, that significant but temporary alterations in caudate unit firing occurred earlier in the cat's post-lesion course. Accordingly, in a new series of cats, caudate neuronal firing rates and the extent of dopamine depletion were assessed at 3 and 7 days post-lesion. Statistically significant decreases (68%) in ipsilateral caudate dopamine levels occurred by 3 days. By 7 days the depletion averaged more than 90%. Spontaneous neuronal firing rates in ipsilateral and contralateral caudates were sampled simultaneously. Ipsilateral caudate firing slowed significantly at 3 days but returned to values similar to those measured in intact controls by seven days. Firing rates tended to be somewhat faster in cats tested at $\geq$ 2 weeks post-lesion but these values did not differ significantly from those tested at 7 days or from intact controls. In the caudate contralateral to the lesion, a gradual slowing of spontaneous firing occurred with post-lesion time. At 3 days post-lesion, spontaneous activity remained at intact levels, and it slowed significantly by 7 days and even more at $\geq$ 14 days. These data indicate that a long-term remodeling of spontaneous firing rates occurs following the MFB lesions. The net result of this remodeling is to produce a side-to-side asymmetry in the activity of caudate neurons. Previous studies have established 1) that an important factor in producing this asymmetry is interruption of caudate output fibers by the MFB lesion, and 2) that the asymmetry can be corrected by additional lesions of the thalamus.

Supported by USPHS HD-05958, MH-7097 and NS-12324.

125 CAUDATE UNIT ACTIVITY IN RESPONSE TO INPUT FROM NUCLEUS CENTRALIS MEDIALIS IN CATS. Edgar L. Gasteiger[1], Irma Zarco-Coronado* and Héctor Brust-Carmona. Depto. de Fisiología, Div. de Investigación, Fac. de Medicina, UNAM, México 20, D.F.

Ablation, neurotransmitter and field potential studies have shown that the ventral medial and lateral area of the head of the caudate nucleus (CN) is essential for the performance of motor conditioned responses. Stimulation-recording experiments in cats (Diez-Martinez et al., Physiol. Behav. 19: 269-276, 1977) revealed projection of the nucleus centralis medialis (NCM) to the CN. To further localize these projections we are characterizing and localizing unit activity in the head of CN (A16) in response to input from NCM in normal and in cats with sustantia nigral lesions produced by local injection of 6-hydroxydopamine. Extracellular recordings of unit discharges and their associated slow waves are recorded from stainless steel microelectrodes while stimulating NCM through concentric bipolar stainless steel electrodes. FM tape recordings are made and the activity then analyzed by use of an HP signal analyzer and a PDP 11/40 computer. Only spontaneously active units are being studied but attention is given to small "distant" units which can not be readily analyzed by computer. When using metal electrodes in flaxedilized cats free of general anesthesia, spontaneous unit activity is more plentiful than commonly reported. Preliminary findings indicate existence of a dorsal border strip of heightened activity composed of many small and a few medium unit discharges, and a second active area in the ventral medial caudate giving rise to small and medium unit discharges. Large unit activity has been recorded in the area bordering the internal capsule. Stimulation of NCM causes driving of some units in all three areas with the most common response consisting of a sequence of early firing (10-60 msec), inhibition (30-275 msec), and rebound discharge. A second frequent response is potentiation of discharge frequency which may last only during the initial period of tetanization (8-12/sec) or, in some cases, be prolonged after tetanization for more than a minute. During transient potentiation the positive slow waves show transient enhancement. With the exception of this transient effect our observations are consistent with those from intracellular studies of postsynaptic potentials in NC for other thalamic inputs (Buchwald et al., Exp. Neurol. 38: 311-323, 1973). These preliminary findings suggest a heterogeneous organization of the CN in contrast to the usual view of a homogeneous organization.

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126 THE GLOBUS PALLIDUS OF THE MONKEY: NEURONAL ACTIVITY IN RELATION TO MOVEMENT. Apostolos P. Georgopoulos and Mahlon R. DeLong. Dept. Physiol., The Johns Hopkins Sch. Med., Baltimore, MD 21205.

Single neurons in the external (GPe) and the internal (GPi) segments of the globus pallidus of the monkey were studied in a step and a pursuit tracking task. The manipulandum was a light-weight handle which the monkey could grasp and move along a horizontal path with minimal friction. The display consisted of 2 rows of 128 lamps each (10/inch): the upper indicated the target position and the lower the position of the handle. After holding in a starting position for a variable period of time, step movements were elicited by suddenly jumping the target lamp to a new position, while pursuit movements of different but constant velocities were obtained by activating sequentially adjacent lamps. The monkey was rewarded with liquid for successful acquisition and maintenance of the target. Data for spike and behavioral events, and movement parameters (position, velocity and acceleration of the manipulandum) were collected for each movement. EMG activity from 16 arm, neck and paraspinal muscles was also collected durthe performance of the task.

236 units were isolated in 27 penetrations through a lateral approach: 151 from GPe and 85 from GPi. Nearly all of the task-related units were modulated in both the step and pursuit tasks; a detailed analysis of the relations of unit activity to the amplitude, velocity and acceleration of the movement and to EMG activity is currently being done. Further observations were made by careful examination of the animal to assess the functional properties of units in response to "passive" manipulations with the animal relaxed, and to active movements that the animal made outside the context of the task. The patterns of spontaneous activity in the two pallidal segments conformed to those described previously by DeLong. A majority of the cells were clearly modulated by active movements of individual contralateral limbs or by chewing, licking and facial movements. Passive driving, when present, was generally weak, and was related to deep structures in and around the region involved in the active movement. A few units did respond very sensitively to tapping of muscle tendons. Cutaneous driving was seen only rarely. Most of the movement-related cells were located in the ventroposterior 2/3 of the globus pallidus; units isolated from the dorsorostral 1/3 of the nucleus were not clearly related to the movements tested. Within the GPe, leg-related units were located most dorsally, those related to orofacial movements most ventrally, and arm-related units in between. Within the GPi, leg-related neurons were situated dorsal to arm-related neurons, whereas units related to chewing, licking and facial movements occupied the ventromedial portions.

127 PECKING AND CIRCLING IN PIGEONS FOLLOWING DESTRUCTION OF A TEGMENTOPALEOSTRIATAL PATHWAY, A POSSIBLE NIGROSTRIATAL HOMOLOGUE. Irving J. Goodman and Albert J. Azzaro*. Depts. of Psychology and Neurology, West Virginia University, Morgantown, WV 26506.

Apomorphine induced compulsive pecking may be significantly or totally blocked temporarily (3-14 days) with bilateral destruction of the pigeon's dopamine-rich paleostriatum augmentatum (PA), a homologue of the mammalian caudate-putamen. The present study explored the mechanisms of apomorphine induced stereotyped behavior by focusing on dopaminergic PA afferents, which originate in and around the nucleus tegmenti pedunculo-pontinus, pars compacta (TPc) and form the tegmentopaleostriatal (TPc - PA) pathway, a possible nigrostriatal homologue. Stereotaxic placement of unilateral electrolytic (DC, 2 mA/15 sec) or 6 hydroxydopamine (6-OHDA, 8 ug/2 ul saline) lesions were made in TPc or just rostral to it, in area ventralis (Tsai) (AVT). Apomorphine (2.0 - 3.5 mg/kg, i.p.)-produced wall, body, air or floor directed compulsive pecking, which tended not be biased toward the right or left side in presurgical baseline tests, deviated markedly toward the lesioned side after either type of lesion or placement. In most cases this was accompanied by circling with the same directional bias. Both effects were observed consistently from day 1 after surgery until 6 mo. later when testing was terminated. Histological and biochemical analyses confirmed lesion placements and dopamine depletion levels. An earlier study in our laboratory (Goodman & Stitzel, 1977) had noted similar directional biases in pecking following unilateral PA lesions, with circling a less frequent event. The anticipated outcome of the present study was that TPc/AVT lesions would favor pecking and circling away from the lesioned side, based upon findings in unilateral lesioned nigrostriatal rats and the accompanying explanation of supersensitivity of denervated caudate cells. Considering sensitivity changes as a means of explaining the above findings, we bilaterally lesioned TPc in other pigeons with the idea that if supersensitivity resulted from denervation in the pigeon, apomorphine induced pecking should increase in the bilateral preparation. In fact, animals showed a marked absence or reduction of apomorphine pecking for up to 30 days post-lesion, and recovery rarely reached baseline levels. If post-lesion sensitivity changes in PA cells is a correct explanation of the above experimental results, then, just the opposite, reduced sensitivity to apomorphine stimulation is suggested. No sooner have we begun to make a case for an avian homologue of the nigrostriatal pathway when we turn up, perhaps, an important behavioral exception.

128 STRIOSOMAL ORGANIZATION OF THE CAUDATE NUCLEUS: I. ACETYLCHOLINESTERASE HISTOCHEMISTRY OF THE STRIATUM IN THE CAT, RHESUS MONKEY AND HUMAN BEING. Ann M. Graybiel and Clifton W. Ragsdale, Jr.*, Dept. of Psychology, Mass. Institute of Technology, Cambridge, MA 02139.

A defining characteristic of the mammalian striatum is its high content of the enzyme, acetylcholinesterase. We have examined the distribution of acetylcholinesterase activity in the striatum of the adult cat, monkey and human using the histochemical staining methods of Geneser-Jensen & Blackstadt and Karnovsky & Roots.

In cross sections through the head of the caudate nucleus, marked local discontinuities in enzyme activity appear in all three species. These take the form of a variable number of circumscribed pale zones that lie embedded in a background of much denser staining. Even in single transverse sections, the individual zones of low enzyme activity are usually highly variable in shape, some being long and thin, others being rounded, still others being branched. The average transverse diameter of the zones is about 0.5mm in the cat, 0.5-0.8mm in the human. In the brains so far examined, these histochemical discontinuities were most prominent in the head of the caudate nucleus, especially its central sector, and faded in its dorsolateral quadrant. Comparable pale zones have been difficult to detect in the putamen. An observation that could have developmental implications is that, within the caudate nucleus, the pale zones often appeared to stretch away from the ventricular face.

Detailed study was made of uninterrupted sets of serial sections through part of the head of the caudate nucleus in the cat (80 50μm sections) and human (75 75μm sections). Important observations are first, that despite sometimes abrupt changes in their shapes from section to section, many of the profiles could be traced in continuity over several millimeters. Second, at least over the short distances so far analyzed in serial sections, many of the apparently unconnected pale zones in individual sections are also actually continuous with one another, forming elements of a highly branched three dimensional labyrinth.

The heterogeneity in staining described here provides support for the concept of a fundamental subdivision of the striatum in the human being, cat and monkey into at least partially segregated, histochemically distinct units. These units are here given the general name, striosomes. It is an unresolved problem how the cholinesterase-poor compartments described here relate to other inhomogeneities observed in the striatum, a particularly interesting instance being the "dopamine islands" observed by Tennyson et al.(1972) and Olson et al.(1972). Study of such relationships may be essential to understanding the significance of the compartmentalization here described.

Supported by NSF grant BNS 75-18758.

129 QUANTIFICATION OF BEHAVIORAL AND NEUROCHEMICAL CHANGES FOLLOWING INTRASTRIATAL KAINIC ACID INJECTIONS. Robert D. Grubbs, Mary L. Michaelis, and Elias K. Michaelis. Dept. Human Development, U. of Kansas, Lawrence, KS 66045

The use of microinjections of kainic acid (KA) to induce neuronal degeneration has become an important tool for determining the neurochemical nature and interrelationships of cells within discrete brain areas. Within the striatum, there appears to be a strong correlation between the neurochemical and morphological changes induced by KA microinjections and the changes observed in postmortem striatal samples of Huntington's Disease patients. Furthermore, behavioral changes have been noted anecdotally following intrastriatal injections of KA. In the present study, an attempt was made to quantify these abnormal involuntary movements (AIMs) by means of a three class code in which the AIMs were operationally defined. The animals were intrastriatally injected with 2μg/μl of a KA solution while under ether anesthesia and videotaped beginning 2 hours after the injection for a period of 60 minutes. Two 15 minute segments of the videotape were then scored using the code. Glutamic acid decarboxylase (GAD) activity was measured 2 hours after injection to determine if a correlation existed between the AIMs and changes in GAD specific activity. GAD activity was also measured at 2 days post-injection. At 2 hours, no difference was observed in GAD activity between uninjected control caudate and KA injected caudate, while at 2 days, the KA injected caudate showed an average 27% decrease from control values. It was determined experimentally that the efficacy of the KA injection was largely dependent on the parameters of the injection procedure. Subsequently, a total volume of 1 μl of a 2μg/ μl solution of KA was administered intrastriatally by thereotaxic injection over a 5 minute period and the needle kept in place an additional 5 minutes. Using this injection procedure, a 58% decrease in GAD activity was observed 72 hours post-injection. In light of reported decreases in adenylate cyclase activity following KA administration, we looked for an effect on striatal cAMP-phosphodiesterase (PDE) activity following treatment. A 61% decrease in V_{max} for cAMP-PDE specific activity was observed, while essentially no change in K_m was seen. This decrease is comparable in mangitude with changes reported by others for ACh and GABA systems following KA injections.

Supported by Univ. of Kansas General Research Support Grant 3646, by DHEW research service award HD-07066 from NICHHD to Kansas Center for Mental Retardation & Human Development, by AA01911 from NIAAA and by GM 22357 from NIGMS.

130 DENDROAXONIC NEUROTRANSMISSION I: POSSIBLE SITES OF SYNTHESIS, STORAGE AND RELEASE OF DENDRITIC DOPAMINE AND ACETYLCHOLINE. T. Hattori, P.L. McGeer and E.G. McGeer. Kinsmen Lab. Neurol. Sci., Dept. Psych., University of B.C., Vancouver, B.C., Canada, V6T 1W5.

It has been known for some time that neurotransmitters such as dopamine exist in dendrites. In a companion paper (McGeer, McGeer and Innanen, Dendroaxonic Neurotransmission II), it has been shown by specific binding assays that receptors may exist on nerve endings for dendritically released dopamine in SN and acetylcholine in the neostriatum. This paper presents an ultrastructural evidence indicating possible sites of synthesis, storage and release of dopamine in SN dendrites and acetylcholine in neostriatal dendrites. Postsynaptic structures were carefully examined in the rat SN and striatum following post fixation with 4% unbuffered O_sO_4 and preembedding staining with 2% uranyl acetate. In the SN, round vesicle-like structures of 20 to 60 nm diameter were often seen attached to the inner surface of postsynaptic membranes. When the nigra was immunohistochemically stained for tyrosine hydroxylase (TH)[1], heavy staining was seen around vesicle-like structures. Microtubules were stained unevenly as if globules containing the enzyme were embedded in the tubules. These could be the precursors of the dendritic vesicles. When fixation was carried out following the administration of the false transmitter for dopamine, 5-hydroxydopamine (5-OHDA), it was found to be localized to smooth endoplasmic reticulum, where large vesicular structures approached the dendritic surface. In the striatum, round, vesicle-like structures of 20 to 60 nm diameter were also seen attached to or embedded in the postsynaptic thickenings of spines which received dopaminergic nerve endings as evidenced by 6-OHDA irduced degeneration. Immunohistochemically, these vesicles, as well as globules within the microtubules were heavily stained for choline acetyltransferase (CAT). In both nigral and striatal dendrites, the vesicle-like structures seemed to accumulate at postsynaptic sites and were rarely seen along other regions of the dendritic membrane. The origin of the vesicle-like dendritic structures is unclear. However, following the administration of horseradish peroxidase in vivo, the enzyme appears to be localized to similar structures following both anterograde and retrograde transport. Since the enzyme is also concentrated in the smooth endoplasmic reticulum, the possibility exists that these two intracellular structures are related. These morphological findings support biochemical data suggesting dendroaxonic transmission of dopamine and acetylcholine.

[1]We thank Dr. Menek Goldstein of the New York Medical Centre for supplying antibodies to TH.

This research was supported by a grant from the MRC of Canada.

131 NONDOPAMINERGIC AND DOPAMINERGIC NIGROSTRIATAL PATHWAYS IN RATS. John Hedreen. Dept. of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, Baltimore, Md. 21205.

Rat brains stained by the Fink-Heimer method after electrolytic nigral lesions show no degenerating pathway in the ventral tegmental area, where the dopaminergic nigrostriatal axons travel. But a group of degenerating axons is seen which separates from the nigrothalamic path and proceeds rostrally in the zona incerta along the internal capsule. The axons pass through the entopeduncular nucleus in the network of cellular areas and transversely oriented fiber bundles, then pass principally through the ventral half of the globus pallidus and enter the neostriatum. Degenerating axons in the neostriatum are especially concentrated in the region lateral to the ventral half of the globus pallidus. The identification of this pathway as nondopaminergic, and of all or most of the dense terminal degeneration in the neostriatum in these cases as dopaminergic, is supported by the following experiments. Cases with nigral lesions and Fink-Heimer staining following long-term pretreatment with 6-hydroxydopamine show no change in this pathway or in the abundance and distribution of degenerating fibers in the neostriatum, while striatal terminal degeneration is much decreased. If the Fink-Heimer method is applied acutely after 6-hydroxydopamine the pathway described above is not seen; only a group of axons of entirely different staining characteristics is seen in the ventral tegmental area and zona incerta, and rarely also in the neostriatum. These are not seen in nigral lesion cases. Thus a limitation of the Fink-Heimer technique - its inability to stain degenerating dopaminergic axons after a nigral lesion - may be turned to advantage, allowing the demonstration of the nondopaminergic nigrostriatal pathway without interference from dopaminergic axons.

The dopaminergic nigrostriatal pathway, as described by Ungerstedt ('71), is revealed in autoradiography cases after nigral injections of tritiated amino acids. The axons in this path traverse the ventral tegmental area before entering the zona incerta. More rostrally, where they remain identifiable by their very strong labeling, they follow a partly different course from the nondopaminergic path, for example traversing the entopeduncular nucleus without favoring the cellular areas, and fanning out through the whole extent of the globus pallidus.

Clearly the two pathways provide a substrate for two kinds of nigral influences upon neurons of the neostriatum. The possibility that the nondopaminergic pathway consists of collaterals of nigrothalamic axons invites investigation.

Supported by NSF (GB-41755), and NIH (NS-10920, & RR-05378).

132 LESIONS OF THE STRIATONIGRAL PATHWAY DO NOT REVERSE METHAMPHETAMINE-INDUCED TYROSINE HYDROXYLASE DEPRESSION. Adair Hotchkiss* and James W. Gibb*(SPON: L.W. Jarcho). Depts. Pharmacology, Univ. of Utah, Salt Lake City, UT 84132.

The apparent Vmax of neostriatal tyrosine hydroxylase (TH) is decreased by subacute administration of methamphetamine (METH) (Koda and Gibb, Pharmacologist 13:253, 1971). It was postulated that this depression is mediated through feedback inhibition of the nigral dopaminergic neurons via the striatonigral pathway (Buening and Gibb, Eur. J. Pharmacol. 26:39, 1974). This hypothesis was tested by evaluating the effect of subacute METH on striatal TH activity in rats with unilateral lesions of the striatonigral pathway produced by two different methods.

One type of lesion was produced by intrastriatal injection of kainic acid (2 µg in 1 µl/rat) in rats weighing 230 to 250 g. Ten days later METH was administered (10 mg/kg, s.c.) every 6 hrs for 5 injections. Thirty-six hrs after the first METH injection TH was depressed in both neostriata with no significant difference found between ipsilateral and contralateral neostriata or between sham- and kainic acid-injected animals. Concurrent administration of haloperidol (3 mg/kg, i.p.) prevented TH depression in both sides (Buening and Gibb, ibid); γ-acetylenic GABA (γAG, 20 mg/kg, i.p.) similarly prevented TH depression in both sides (Hotchkiss and Gibb, Fed. Proc. 37:510, 1978). A second group of animals was lesioned electrothermically in the crus cerebri just anterior to the substantia nigra as described by Gale et al. (Sci. 195:503, 1977). Preliminary data indicate equal TH depression in the paired neostriata after the METH regimen, 14 days following electrothermic lesion. With both lesion types, animals exhibited ipsilateral rotation following METH. In the kainic acid lesion model, rats receiving haloperidol and METH rotated in the contralateral direction, whereas rats receiving γAG and METH rotated in the ipsilateral direction.

These data suggest that inhibition of nigral dopaminergic cells by the striatonigral projection may not be responsible for the METH-induced TH depression. Likewise, reversal of TH depression by haloperidol and γAG does not require this pathway to be intact. The mechanism for the METH-induced depression of neostriatal TH is still uncertain and other possibilities such as presynaptic inhibition must be considered. Alternative hypotheses are being explored in an effort to explain the METH-induced depression of striatal TH activity. (Supported by USPHS grants GM 00153 and DA 00869.)

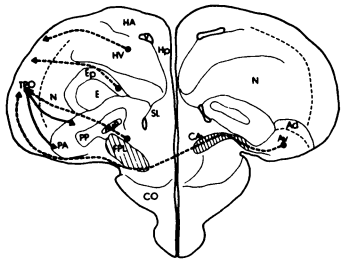
133 NEURAL CONNECTIONS OF THE AVIAN LATERAL CORTICOID AREAS. Cheryl A. Kitt & S. E. Brauth, Dept. Psychology, Univ. of Md. College Park.

In pigeons, HRP was injected into the tempero-parieto-occipital area (TPO) and area corticoidea dorsolateralis (CDL) of the telencephalon. Retrograde transport of HRP was observed to three ipsilateral cell fields: the hyperstriatum ventrale (Hv), periectostriatal belt (Ep) and to a region of neurons ventral to the paleostriatum primitivum (PP). TPO and CDL neurons also receive projections from cells in the contralateral ventral archistriatum (Av) via the anterior commissure. Other injections of HRP into the lateral neostriatum (N) and Av labelled cells in two different discrete fields of the frontal and caudal neostriatum and not cells within Ep, Hv or Av. Injections of HRP into the parahippocampal area (APH) labelled neurons ipsilaterally in the hippocampus (Hp) lateral septal area (SL) and in the diagonal band of Broca (FDB) In addition, cells in the nucleus superficialis parvocellularis (SPC)were labelled bilaterally in this case.

HRP injections were placed within the dorsal, central and lateral portions of TPO and CDL. No topography of the above described afferent projections was apparent, however virtually all retrogradely labelled cells observed in Ep were situated in the medial portions of this field.

Injections of 3H-leucine and 3H-proline into the TPO region revealed anterograde projections from these regions into the paleostriatum in aggreement with prior work (Brauth, Ferguson & Kitt, 1978). Fibers from TPO could be traced into PA, into the region ventral to PP, and as far medially as the ventromedial portion of the lobus parolfactorius. This latter projection was confirmed by means of HRP injections into LPO. The vast majority of TPO efferents terminate, however, in PA.

These results indicate that neurons in TPO and CDL receive unique projections from Ep, Hv, the region ventral to PP, and from the contralateral Av and project extensively upon the paleostriatum



This drawing represents a schematic of the major projections of the lateral corticoid areas in the pigeon telencephalon.

134 PROJECTIONS OF CENTRUM MEDIANUM TO CAUDATE NUCLEUS IN THE CAT AS DEMONSTRATED BY RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE. David C. Kuo*, Ernest S. Pile* and George M. Krauthamer. Dept. Anat., CMDNJ Rutgers Med. Schl. Piscataway, NJ 08854.

In order to determine more precisely the anatomical relationship between the medial thalamus and the head of the caudate nucleus (Cd), small, focal deposits of horseradish peroxidase were placed in Cd by electrophoresis. The brain sections were treated by a variation of the DeOlmos, Hardy & Heimer method, using tetramethyl benzidine.

Results showed a discrete input from centrum medianum (CM) which was clearly outlined by many labeled neurons in close proximity to each other. No other thalamic nuclei were labeled. Cell bodies, proximal dendrites and, presumably portions of axons were densely filled with granular reaction product. There were many apparent contacts between the processes of adjacent labeled neurons suggestive of a synaptic interrelation. In agreement with the present findings, a monosynaptic input from CM to Cd has been demonstrated (Kocsis et al.) and antidromic responses have been recorded in CM (Krauthamer et al.).

Other labeled neurons were seen in substantia nigra, pars compacta and lamina V of the corresponding cortical projection field. In contrast to the massive, densely labeled CM projection, these consisted of scattered and lightly labeled cells.

The results demonstrate that at least some regions of Cd receive their thalamic input from CM rather than n. centralis lateralis as commonly believed. Since CM, in turn, receives projections from the deep layers of superior colliculus and vestibular nuclei (McGuinness et al.), fairly direct access to Cd is provided for the polysensory activities related to orienting responses.

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Krauthamer, Feltz P. & Albe-Fessard, D. J. Neurophysiol., 30 (1967) 81-97.

McGuinness, C., Dalsass, M., Proshansky, E. & Krauthamer, G. Neurosci. Absts. 2 (1976) 67.

(Supported by NIH grant NS10922)

135 FUNCTIONAL ORGANIZATION OF NEURONS RELATED TO ARM MOVEMENT IN THE PUTAMEN. Samuel L. Liles. Dept. of Physiol., LSU Med. Ctr., 1100 Florida Ave., New Orleans, LA 70119.

Recent anatomical evidence suggests that motor cortex projects in a somatotopically organized manner to the putamen, such that axons arising in "leg", "arm" and "face" areas terminate in dorsolateral, intermediate and ventromedial regions, respectively, of the putamen (H. Künzle, Brain Res., 1975). The observations that arm movement-related (MR) units are found almost exclusively in intermediate regions of putamen (S. Liles, Fed. Proc., 1978) and units related to orofacial movements occur in ventromedial regions (Liles, unpublished observations) suggest, at least at the gross anatomical level, a possible relationship between afferent input and functional organization of neurons in the putamen. The present study concerns the fine anatomical distribution of arm MR units within intermediate regions of putamen.

Monkeys trained to push or pull a manipulandum to obtain a fruit juice reward (S. Liles, Fed. Proc., 1978) were prepared for single unit recording after the technique of Evarts (1968). Electrode penetrations were made at an angle of 36° from vertical. Unit response magnitudes were estimated from digitally integrated peri-response time histograms composed from 25 movement trials. About 80% of MR units showed activity during both push and pull movements, although most of these units were preferentially related to either the push or pull movement. Most MR units showed a tendency to occur in groups or clusters of 500 μm or less along recording tracks. During some electrode penetrations, seven or eight MR neurons were all encountered over an axial distance of only 400-500 μm; during other penetrations three or four such clusters of MR neurons were found. MR neurons within each cluster invariably showed similar activity patterns (viz., all units within a cluster were related preferentially to either push or pull movements). In seven instances it was possible to record the activity of two units simultaneously from the same site. Each of these pairs of units showed similar MR activity patterns.

These data suggest that arm MR neurons are not scattered randomly throughout the intermediate region of the putamen, but frequently occur in small neuronal colonies or clusters. The fact that neurons within these clusters show similar MR activities suggests that these colonies may represent functional groupings of neurons. Future studies utilizing more discrete conditioned movements (e.g., movements of wrist or digits) combined with microstimulation of motor cortex are needed to determine possible specific relations between afferent input from motor cortex and MR properties of neurons in putamen. (Supported by USPHS Grant NS-08907 and the E.G. Schleider Foundation).

136 NIGRO-NEOSTRIATO-NIGRAL NEUROANATOMY: TOPOGRAPHY, TRAJECTORIES, AND TARGET ELEMENTS. Raymond Marchand* and Larry L. Butcher. Dept. Psychol. and Brain Res. Inst., UCLA, Los Angeles, CA 90024.

Nigro-neostriato-nigral relationships were assessed by protein-incorporation autoradiography, horseradish peroxidase (HRP) histochemistry, and colchicine-induced build-up of dopamine (DA) and acetylcholinesterase (AChE) in the nigro-striatal pathway. To assess interconnections of (1) nigro-striatal neurons with AChE-containing neostriatal interneurons and (2) striato-nigral fibers with pars compacta dendrites projecting into pars reticulata, we used protein-incorporation autoradiography in combination with AChE histochemistry (see Butcher, 1978; in: Cholinergic Mechanisms and Psychopharmacology; Jenden, Ed.; Plenum Press, New York).

Nigro-striatal fibers—containing both AChE and DA and traveling in the ventromedial mesencephalic tegmentum, field H_2 of Forel and adjacent regions, and the internal capsule and globus pallidus—appeared to make contact with a small but definite proportion of neostriatal AChE neurons, primarily on the proximal portions of dendrites and/or somata of those target cells. Striato-nigral fibers, coursing prominently through the internal capsule, were observed to contact numerous AChE (DA)-containing dendrites of pars compacta neurons projecting into pars reticulata.

HRP injected dorsolaterally or dorsomedially in the rostral neostriatum (i.e., rostral to anterior commissure) resulted in accumulation of the enzyme in numerous cell bodies of the medial third of pars compacta throughout its entire rostro-caudal extent. Neurons of the more lateral segments of pars compacta showed little or no enzyme accumulation. HRP infusion into the caudate-putamen nucleus at the level of the decussation of the anterior commissure resulted in enzyme accumulation in pars compacta neurons in the lateral and intermediate segments of that structure; again, this accumulation was observed throughout the rostro-caudal extent of the cellular mass.

Since HRP is transported in anterograde as well as retrograde directions the neostriatal injections also disclosed a topographic, reciprocal relationship between the striato-nigral terminating fibers in the substantia nigra, pars reticulata and the HRP-containing somata of pars compacta apparently giving rise to the nigro-striatal AChE (DA)-containing pathway (see Butcher, 1977; Life Sci. 21, 1207-1226). [This research supported by USPHS grant NS 10928 to L.L.B. R.M. is a recipient of a fellowship from the Medical Research Council of Québec, Canada.]

137 SENSORY INATTENTION PRODUCED BY 6-HYDROXYDOPAMINE INJECTIONS ALONG THE ASCENDING DOPAMINERGIC FIBERS: SPONTANEOUS RECOVERY AND PHARMACOLOGICAL CONTROL. John F. Marshall. Department of Psychobiology, University of California at Irvine, Irvine, CA 92717

Rats that received injections of 6-hydroxydopamine (6-OH-DA) into the area ventralis tegmenti displayed a syndrome of impaired orientation (inattention) to tactile, olfactory, and visual stimuli that was evident upon neurological examination. When the 6-OH-DA was injected unilaterally, these impairments were apparent only on the contralateral body side. Rats that received bilateral 6-OH-DA injections failed to attend to stimuli impinging on either side. When rats with bilateral 6-OH-DA injections were given small doses (0.05-0.20 mg/kg i.p.) of apomorphine 2-8 days postoperatively they showed a dramatic but short-lived restoration of orienting to touch, visual stimuli, and odors that was maximal in animals given 0.10 mg/kg apomorphine 3 days postoperatively. Lower doses of apomorphine had little obvious behavioral effect, while higher doses resulted in intense stereotyped sniffing, particularly on the 5th and 8th day postoperatively. The apomorphine-induced reversal of the sensory inattention was completely abolished by pretreatment with the dopamine receptor blocking agent haloperidol (0.05 mg/kg, i.p.), but not by its vehicle.

Further examination of the contralateral inattention to somatosensory stimuli in rats with unilateral 6-OH-DA injections revealed that many rats spontaneously recovered the ability to orient to touch of the affected body surface during the first postoperative month. This recovery occurred first to touch of rostral points of the body surface and only later to touch of progressively more caudal regions. When given i.p. alpha-methyl-para-tyrosine (70-100 mg/kg) or spiroperidol (0.05 mg/kg) 2-3 mo. postoperatively, these recovered rats showed a restitution of somatosensory inattention that was restricted to the contralateral body surface. After drug administration the somatosensory loss spread in a caudal to rostral direction. As the drug effects dissipated, orientation was restored rostrocaudally, recapitulating in brief the process of spontaneous recovery.

These findings suggest that the inattention syndrome seen after 6-OH-DA injection is related to a loss of dopamine receptor activity and that the spontaneous recovery of orientation depends critically upon dopaminergic mechanisms.

138 LOCALIZATION OF GLUTAMINASE IN THE RAT NEOSTRIATUM. E.G. McGeer and P.L. McGeer. Kinsmen Lab. Neurol. Sci., Dept. Psych., UBC, Vancouver, B.C., Canada V6T 1W5.

The subcellular concentration of glutaminase in the synaptosomal fraction of brain (Bradford and Ward, Brain Res. 110, 115 (1976)) suggests that it may play an important role in the maintenance of the transmitter pools of glutamate, GABA, or both. The rat striatum provides a system to test this hypothesis since the extensive glutamergic innervation from the cortical-striatal tract can be destroyed by cortical lesions without effect on the striatal GABAergic systems, while the latter can be extensively lesioned by local injections of kainic acid (KA) without adverse effect on the afferent glutamergic nerve endings. Thus, injections of 5 nmoles of KA cause a decrease in GAD activity to about 30% of control without effect on high affinity glutamate uptake, while cortical lesions cause a decrease in glutamate uptake to about 60% of control with no effect on GAD activity.

Using a radioactive assay for glutaminase, based on a coupled reaction with bacterial GAD, the activity was found to decrease significantly in the striatum following KA injections but not following cortical lesions.

The decrement in glutaminase in KA-lesioned striata correlated significantly with the decrement in GAD. The intercept on the x axis suggested that 60% of the glutaminase activity in the striatum is located in structures (presumably GABAergic neurons) destroyed by the KA-injections while 40% is in some unaffected compartment(s). Subcellular fractionation indicated some 60% of the glutaminase in control striata was in the P_2 fraction. These data suggest that about 60% of the activity in the striatum is probably in GABAergic structures with about 40% in glia. They do not support the hypothesis that the neurotransmitter pool of glutamate may be derived directly from glutamine in glutamergic nerve endings.

139 AN ALLELE OF DYSTONIA MUSCULORUM EXHIBITING LESIONS IN SOME AREAS OF THE EXTRAPYRAMIDAL SYSTEM. Anne Messer, Norman L. Strominger[+] and Lorraine Flaherty*. Div. of Labs and Research, NYS Dept. of Health and [+]Department of Anatomy, Albany Medical College, Albany, NY 12201 (12208).

An autosomal recessive mutation, characterized by severe motor deficits, arose spontaneously in the BALB/cByJ mouse stock maintained by Dr. Flaherty at the NYS Dept. of Health Labs. At about two weeks of age mutant animals show slight movement disorders and abnormal limb placements. The syndrome is steadily progressive to extreme ataxia with writhing side-to-side movements, and some shaking of the hind limb on first one side and then the other; there is almost no normal use of hind limbs. Front limbs exhibit stiff, flipper-like motions, and the entire body flexes into bizarre postures. The syndrome is fully developed by four weeks and coordination actually improves slightly after a few months. Although sometimes smaller than littermates, mutants can survive many months.

Because of a similarity in symptoms with the mutant dystonia musculorum, an allelism test was performed. Crosses between $+/dt^{J}$ and a known heterozygote from our colony revealed that the two mutations are allelic. Therefore the mutation found here will be called dt^{Alb}. The pathology of dt^{Alb} is now being compared to that of dt^{J}.

Duchen, Strich and Falconer (Brain 87, 367-378, 1964) reported that the major pathology seen in dystonics was "degeneration of nerve fibres in the peripheral nerves, in the sensory roots and ganglia of spinal and cranial nerves, and in the spinal cord and brain-stem." They found no obvious abnormalities in the basal ganglia or cerebellum but hypothesized that these may be functionally abnormal since a severe sensory ataxia may not account for the entire clinical syndrome. The dt^{Alb} shows clear abnormalities in the red nucleus and in part of the striatum, in addition to some of the pathological features described above. Formalin-fixed, paraffin-embedded serial sections (15 μ) stained with thionin show pathological changes in large coarse neurons with an overall reduction in number in the red nucleus. The striatum contains an abnormal number of small darkly staining cells when compared to controls. Notably, this pathology is asymmetric, although the clinical symptoms appear to be bilateral and equal within the limits of observation. Neurochemical correlates of these phenomena are being examined.

(Supported by a grant from the H. D. Foundation, Los Angeles, CA [AM] and an NIH Grant No. N5-12208 [NLS]).

140 ELECTRICAL ACTIVITY IN THE IN VITRO CAUDATE PREPARATION. J.J. Miller, D.P. Rutherford* (SPON: T. Calvert), Dept. Physiology, University of British Columbia, Vancouver, B.C. V6T 1W5.

Recent reports have demonstrated the advantages of the in vitro slice preparation, particularly in the hippocampus, as a suitable model for studying synaptic transmission in the brain. In order to determine whether this technique may be applied to components of the basal ganglia, the present investigation was undertaken to examine the electrophysiological properties of the in vitro caudate slice preparation. Transverse sections of the rat caudate nucleus (500 μm in thickness) were maintained in a modified Ringer's solution at 34-36°C and superfused with 95% O_2-5% CO_2. Bipolar semi-micro stimulating electrodes were positioned at the confluence of the internal capsule or in the cellular zones between the radially oriented axon fascicles. Extracellular unit activity, recorded from the cellular regions consisted of spontaneous and orthodromically evoked single spikes at latencies of 2.5-4.0 msec. These responses followed stimulus frequencies of 10-20 Hz and were decreased or eliminated under conditions of anoxia. When the stimulus intensity was increased (2xT) a negative going population spike, on which single spikes were often superimposed, was evoked at similar latencies. When a Ca^{2+}-deficient medium was perfused, the evoked population response and unit activity were eliminated and both showed recovery upon replacement with the normal medium. Antidromically evoked cells and fiber responses were elicited at latencies of 1-2 msec. These responses followed high frequency stimulation (>100 Hz) and were resistant to the removal of Ca^{2+}. The activation of spontaneously discharging neurones was frequently followed by periods of inhibition lasting up to 50 msec. Paired pulse stimulation resulted in inhibition of the evoked response elicited by the test stimulus at C-T intervals up to 150 msec. In Cl^- deficient medium this inhibition was eliminated and a dramatic increase in the amplitude of the evoked population spike and background discharge rate was observed. Paired-pulse stimulation also resulted in an enhanced test response at intervals of 10-100 msec. Recovery of the control response was obtained when the Cl^- content in the media was restored. Although the cellular origins of the evoked responses have not been determined, these results provide preliminary evidence of the viability of the in vitro caudate slice and its suitability for studies of the pharmacological and ionic mechanism underlying synaptic transmission in this region.

Supported by the Medical Research Council.

141 STRIOSOMAL ORGANIZATION OF THE CAUDATE NUCLEUS: II. EVIDENCE THAT NEURONS IN THE STRIATUM ARE GROUPED IN HIGHLY BRANCHED MOSAICS. Sandra Moon Edley*, Ann M. Graybiel and Clifton W. Ragsdale, Jr.*, Dept. of Psychology, Mass. Institute of Technology, Cambridge, MA 02139. (SPON: W.J.H. Nauta).

The fiber projection from the caudate-putamen to the pallidum forms one of the two great efferent systems of the mammalian striatum. In order to study the distribution within the striatum of the cells giving rise to the strio-pallidal connection, we have injected the retrograde tracer, horseradish peroxidase (HRP), into the pallidum in the cat. Two points of technique were dictated by our attempts to obtain as nearly complete cell-labelling of the striatum as possible: first, we injected very large amounts of HRP; and second, we used the BDH and TMB histochemical techniques of Mesulam to detect labelled neurons. We report here on findings in 3 cats in which large pallidal deposits produced extremely dense labelling of striatal neurons.

The most striking finding in these cases is that, despite massive labelling of both small and large neurons in the caudate nucleus, the distribution of HRP-positive neurons was by no means uniform. Instead, in each case, a pattern of cell-labelling appeared in which large fields of labelled neurons were suddenly and repeatedly interrupted by variably shaped, roughly 0.5mm-wide zones containing relatively few and often scarcely any labelled neurons. These sparsely labelled zones were easily visible with the naked eye in cross-sections through the head of the caudate nucleus. Some of the zones were vermiform, long enough to cross nearly the full width of the caput (2-3mm), but others were rounded or of complex shape. By tracing individual zones from section to section it became clear that in many instances the profiles in serially adjoining sections were continuous with one another.

The enzyme deposits were in all cases large, involving not only both segments of the globus pallidus (GP) but also adjoining parts of the fundus striati and (2 cases) deep putamen rostrally, and the subthalamus caudally. In one case the substantia nigra (SN) itself was also included in the injection site, and in no case could the possibility be excluded of infiltration of perforant nigrostriatal fibers by the HRP. It is not yet clear, therefore, whether separate mosaics of striatal neurons give rise to the caudate's projection to GPe, GPi and SN, or whether the sparsely labelled zones here described represent neural populations with intrinsic striatal connections. Experiments are underway to study this problem and also to examine the relation between these neuronal compartments and the AchE-poor labyrinths described in the accompanying abstract. From the few intercalcated sections processed in the present experiments for AchE, it seems evident that the two striosomal patterns at least in part do overlap.

Supported by NSF grant BNS 75-18758 and an NFF stipend.

142 A DESCENDING PATHWAY INVOLVING NIGRAL-INDUCED HEAD TURNING MOVEMENTS. Fereshteh Motamedi and Donald H. York, Dept. of Physiology, University of Missouri, Columbia, Missouri 65212

Previous studies have shown that stimulation of various structures in the basal ganglia of the awake conscious rat, cat, or monkey will produce head turning movements to the contralateral side. The question of which precise neuronal output system from the basal ganglia results in these head turning movements is not resolved. The present study was undertaken to evaluate nigral-induced head turning movements in rats with ipsilateral brain transections just anterior to SN in order to eliminate the effects of striato-nigral or corticofugal fibers which may affect SN.

Experiments were carried out in Sprague-Dawley rats (350-450 gms) which were divided into two groups. Group I (control) were anesthetized and had a concentric bipolar electrode stereotaxically inserted into SN (pars reticulata, pr.) (A2.75, L2.3, $D^{-1.5}$) (Skinner, 1971). Group II rats were anesthetized and a transection of the mesencephalon (A4.25, L0.9 - 4.35, D-2.2) just anterior to SN was performed. A bipolar stimulating electrode was also inserted into SNpr ipsilateral to the lesion. A period of one week elapsed before behavior studies were undertaken.

Stimulation of SNpr (65-200μA, 0.1-0.2 msec, 50 Hz for 6 sec) produced consistent head turning to the contralateral side in both groups of animals. There was no significant difference in the threshold current required to produce head turning in these two groups. Haloperidol (0.5 mg/Kg) consistently elevated the stimulus threshold current required to produce head turning in the lesioned group (Group II). Higher doses of haloperidol (1 mg/Kg) abolished head turning. In the non-lesioned group, haloperidol also initially raised the threshold to induce turning in some animals, but was found to lower threshold in others. The lowering of stimulus threshold was never observed in the lesioned group.

Recent neuroanatomical findings have defined a pathway from SNpr to the superior colliculus and midbrain tegmentum. In order to discover if this pathway was responsible for head turning movements, animals of Group II were given a further lesion involving the ipsilateral superior colliculus (A0.85, L1.5, D1.5). A week was allowed before further testing commenced. Stimulation of SNpr did not consistently produce a contralateral head turning. There was either no effect or an equal tendency to turn left or right in these animals.

These results suggest that there is a dopamine link in the output pathway from SNpr to cervical spinal cord motoneurons. The superior colliculus may also play a role in the nigral output pathway involved with head turning movements.

(Supported by Dalton Research Center)

143 ELECTROPHYSIOLOGICAL DEVELOPMENT OF THE GLOBUS PALLIDUS IN KITTENS. G.D. Novack, M.S. Levine, E. Cherubini*, C.D. Hull and N.A. Buchwald. Ment. Retard. Res. Ctr., Sch. Med., UCLA, Los Angeles CA 90024

These experiments are part of a series of studies assessing the maturation of connections of the caudate nucleus (CD) and globus pallidus (GP) in the kitten. We showed previously that inputs to the CD from cortex (CX), thalamus and nigra are functional in the first few days of life. Stimulation of any of these structures evoked action potentials from extracellularly recorded CD neurons in 1 or 2 day old kittens. Extracellular records were adequate to demonstrate the excitatory connections to the CD. To demonstrate the development of inhibitory potentials we were forced to record intracellularly from neonatal CD neurons. In adult animals, the typical response of CD cells to stimulation of their afferents is an excitation followed by an inhibition (EPSP-IPSP sequence). In kittens we found that the IPSP developed very slowly and did not reach adult levels in terms of frequency of occurrence, duration, and amplitude until over 40 days of age. The present experiments concern development of caudate outputs to the globus pallidus. Extracellular spike responses were evoked in GP neurons by stimulation of CD or CX. CD stimulation was used to assess development of striopallidal connections directly. Responses to CX stimulation assessed the ability of the CD to relay information to the GP. There is little evidence for a direct CX-GP pathway. Results of this study showed that CD-GP connections exist as early as 2 days of age. Latency of the CD evoked spike decreased with increasing age from a mean of 40 ms in the 1-10 day range to 20 ms at 60 days. For CX stimulation, the latency to response decreased from 50 to 40 ms at the same ages. Concomitantly, the percent of units responding to CD increased from 70% to 90%, while the percent of units responding to CX stimulation remained constant (60%). The majority of responses to both CD and CX stimulation in younger kittens were excitatory. In older kittens, the majority of responses were either purely inhibitory or involved excitatory-inhibitory sequences. About 40%-50% of the units responded to stimulation of both sites regardless of age. Of these, the percent responding with the same type of response decreased with age from about 73% to 45%. These experiments show that the majority of excitatory input connections to both the CD nucleus and GP are well developed at birth. In contrast, inhibitory connections in both these nuclei develop more slowly. The results also suggest that the CD, early in postnatal development, probably serves as a passive relay rather than as a complex processor of incoming information. Supported by USPHS grants HD-05958, MH-7097 and NS-12324.

144 PAW USAGE IN ACAUDATE AND AFRONTAL CATS. Ch. E. Olmstead and J.R. Villablanca. Depts. Psychiat., Anat., and MRRC, UCLA, CA 90024.

A modified Wisconsin General Test Apparatus was used to evaluate and compare the paw usage of 50 adult cats distributed as follows: 26 intact[14 colony-reared(CI)and 12 pound-derived(PI); 15 with bilateral aspiration lesions of the caudate nuclei [7 operated as kittens(KBAc)and 8 as adults(BAc)];6 with unilateral caudate removals(UAc-all adult);12 with bilateral frontal cortical removal [9 as kittens(KBFr)and 3 as adults(BFr)];and 6 sham[all as kittens(ShO)]. Many of the adult operated were evaluated both pre- and post-operatively.The ability and accuracy in retrieving chunks of meat through a narrow opening were evaluated for 10 trials on each of 5 days. Paw attitude was scored on a 5-point scale and the number of swipes per trial was counted.In all intact and ShO animals, the dominant movement was a sweeping forward, lateral and medial thrust of the paw to envelop or hook the food.More of the PIs showed a definite handedness(left)than did the CIs or ShOs.All of the lesion groups showed changes in both paw attitude and the number of swipes to obtain meat.Most severely affected were the BAc and KBAc groups where only the adult animal with the smallest lesion showed any normal performance.The dominant attitude for both groups was a straight clubfooted movement that was inaccurate.Successful retrievals were usually followed by perseverative responding at that site despite a new meat position.Differences between the BAc and the KBAc were that the BAc tended to use their claws more often and that none of the KBAc ever showed the normal swipe.Although the KBAc generally took more swipes per trial than the BAc, the difference was not statistically significant.Neither group showed a significant change in either attitude or accuracy with continued training.In the BAc group, there were no changes in handedness from that seen preoperatively.The UAc group showed bilaterally many of the changes seen in the BAc group but all improved with training.There were no changes in handedness despite the fact that the paw contralateral to the lesion was significantly($p<.02$)less accurate than the one ipsilateral.The most striking difference between the adult and the kitten lesioned animals appeared in the BFr and KBFr groups where a)the BFrs were extremely inconsistent in their behavior and when they retrieved showed only a random mixture of normal swipes and dismetric jabs while the KBFr showed fairly consistent, albeit dismetric, approximations to the normal paw movements;b)what dismetria there was in the KBFr improved with training while the BFr showed none.These data suggest that a)the caudate nuclei have a role in postural adjustments for paw usage;b)these defects probably contribute to the deficits in bar pressing previously reported for both BAc and KBAc;c)the slowness in the KBFr and BFr cats was probably due to inconsistent behavior and dismetria (USPHS Grants HD-05958, MH-07097, and HD-94612).

145 IS THERE A PALLIDOHABENULAR PATHWAY IN MONKEYS? A. Parent and R. Boucher, Lab. Neurobiol., Fac. Méd., Univ. Laval, Québec, Canada.

In the rat, the entopeduncular nucleus (EN)(the equivalent of the internal pallidum of primates) has been shown to be the major source of forebrain afferents to the lateral habenula (LH) with the horseradish peroxidase (HRP) method (Herkenham and Nauta, J. Comp. Neur., 173: 123, 1977). In the cat, however, we have shown by means of the same procedure that the largest collection of forebrain neurons projecting to the LH occurs along the ventromedial aspect of the EN but not in the EN itself (Gravel et al., Neurosc. Abstr., 3: 198, 1977).

In order to see if the latter condition is peculiar to cats or if it may also be found in other non-rodent mammals, single injections of HRP (30% sol'n, 0,15-0,25 µl) were made in the habenula of 6 squirrel monkeys (Saimiri sciureus). Retrogradelly labelled cells were visualized after a survival period of 24 to 48 hrs by means of the original LaVail method. In 3 monkeys, the injection sites were mainly confined to the LH and only slightly involved the medial habenula or the dorsomedial thalamic nucleus. In such cases, the largest number of labelled neurons are found along the ventromedial border of the rostral third of the internal pallidum (IP). Many of these numerous and strongly positive cells are intermingled with the fibers of the inferior thalamic peduncle and of the ansa lenticularis. A small number of labelled neurons also occurs along the lateral border of the IP, i.e. within the internal medullary laminae, and along the dorsal aspect of the IP, beneath the internal capsule. No HRP-positive cells are found in the viscinity of the caudal two-third of the IP. Although the rostral IP appears nearly completely surrounded by labelled cells, only a few HRP-filled neurons are present within the IP itself. In another monkey, the HRP injection site involved mainly the dorsomedial nucleus and diffused only slightly into the LH. In such a case, the number of HRP-positive cells was greater in the internal medullary laminae but much lesser along the ventromedial border of the IP than after LH injection.

Therefore, in monkeys as in cats, the main source of forebrain afferents to the LH appears to be the peripallidal cells, especially those lying along the ventromedial edge of the IP. These peripallidal neurons may well be part of certain limbic structures intimately surrounding the pallidum, such as the substantia innominata and the lateral hypothalamus.

(Supported by grant MT-5781 of the Medical Research Council of Canada).

146 LEARNING IMPAIRMENTS IN RATS WITH EXPERIMENTAL DEGENERATION OF THE NEOSTRIATAL NEUROPIL. Michele Pisa, Paul R. Sanberg and Hans C. Fibiger. Div. Neurol. Sci., Dept. Psychiatry, Univ. of B.C., Vancouver, B.C., Canada, V6T 1W5.

In contrast to conventional methods of lesions, injections of kainic acid (KA) in the caudate-putamen result in a relatively selective degeneration of neostriatal neurons with apparently little damage to both afferents terminals and axons in transit. The neurotoxic lesion therefore appears to afford a more specific attribution of the resulting behavioral disorders to neostriatal dysfunction. Three nmoles of KA in 0.5 µl of phosphate buffered saline were injected in the rostral neostriatum of male Wistar rats. The control rats received injections of the vehicle solution only. In the first experiment KA treated rats showed retarded acquisition, normal retention, increased resistance to extinction, normal reacquisition of a 1-way active avoidance response and impaired acquisition on subsequent training of a passive avoidance response using the shock-shock conflict procedure. In a second experiment all KA treated rats, in contrast to the controls, failed to learn a food-reinforced spatial alternation task in a T maze. Similar effects have been documented in earlier studies in which both the neostriatal neuropil and neocortical connections in transit were damaged. The present findings indicate that impairments of associative processes can also result from lesions mostly limited to neostriatal tissue. The results suggest that the neostriatal degeneration of patients with Huntington's disease may account, at least in part, for the associated cognitive impairments.

147 ANTIDROMIC AND SYNAPTIC ACTIVATION OF HRP IDENTIFIED SUBSTANTIA NIGRA AND RETRORUBRAL NEURONS IN THE CAT: ANATOMICAL AND ELECTROPHYSIOLOGICAL STUDY. R.J. Preston*, R. A. McCrea, H. Chang* and S. T. Kitai (SPON: M.H. Cooper). Dept. of Anatomy, Mich. State Univ., E. Lansing, MI 48824.

The nigro-striatal system was investigated by recording the intracellular responses of horseradish peroxidase (HRP) identified substantia nigra (SN) and retrorubral (RR) neurons. These neurons were activated by stimulation of the caudate (Cd) nucleus or the internal capsule (IC) and medial forebrain bundle (MFB). Cats were anesthetized with surital (35 mg/kg) and α-chloralose (80 mg/kg). Stimulating electrodes were positioned stereotaxically in the head of the Cd nucleus and in the IC-MFB (A. 10, Reinoso-Suàrez). Recording microelectrodes were filled with 2M KCl (30-50 megohms) or with 4% HRP in 0.2 M KCl-tris buffer (pH 7.6). Cd nucleus stimulation evoked IPSPs with latencies of 3-15 msec ($\bar{x}$=7.9). IC stimulation produced mostly IPSPs (1.0-9.0 msec; $\bar{x}$=3.5) but occasionally these IPSPs were preceded by depolarizing potentials with latencies of 1.2 - 6.0 msec ($\bar{x}$=2.9 msec). Both MFB and Cd stimuli evoked antidromic potentials with mean latencies of 4.0 and 9.3 msec, respectively. Conduction velocity for SN and RR axons ranged from 1 - 3 m/sec. Neurons identified by intracellular injections of HRP were located in pars compacta of SN or in the RR nucleus. Their spine free pyramidal or fusiform somata ranged in size from 15 - 29 µm. Three to six spine free primary dendrites branched sparsely into spine laden secondary and tertiary dendrites which coursed without branching until terminating in complex thickets at radial distances of 240-860 µm. Axons arose mainly from primary dendrites. For some neurons, the axon coursed rostrally without collateralizing while for others, the axon coursed caudally and gave off 2-5 collaterals which appeared to terminate locally. These data indicate that neurons of both SN and RR send axons to the Cd nucleus and in turn receive mainly inhibitory input from the caudato-nigral pathway. (This work was supported by NIH Grant NS00405.)

148 EVIDENCE FOR A MIDBRAIN RETICULAR - ZONA INCERTA LOOP. N.Ropert*, A. Kitsikis*, A. Parent and M. Steriade. Lab. Neurophysiol. and Lab. Neurobiol., Faculty of Medicine, Laval University, Quebec, Canada.

Ascending influences of the brain-stem reticular formation are thought to distribute mainly through medial-intralaminar thalamic nuclei and subthalamic areas. This view stemmed from experimental approaches which could not circumvent difficulties resulting from the presence of a great number of *en passage* fibers in these regions, especially in the subthalamus. To test the hypothesis that the upper reticular formation and related subsystems play a role in forebrain activation processes, we first decided to identify the target structures of rostrally projecting cells in the mesencephalic central tegmental field (FTC) and to disclose some control circuits involving the FTC. Both electrophysiologic and morphologic methods were used and their results were concordant. Within the frame of the physiologic investigation, extracellular unit recordings of FTC, zona incerta (ZI) and medial-intralaminar thalamic neurons were performed in chronically implanted, behaving cats while stimulating various brain-stem, thalamic, anterior hypothalamic and neocortical areas. Fiber recordings were rejected. Here only the FTC-ZI interrelationships will be reported. Antidromic invasion of FTC neurons following stimulation in the ipsilateral ZI region occurred within a latency range of 0.5 ms to 5.0 ms. The question whether the testing stimulus affected FTC terminals ending in ZI was positively answered in parallel experiments when unit discharges belonging to ZI cell bodies were monosynaptically elicited (1-3 ms) following focal stimulation in FTC. This midbrain reticular→ZI projection is supported by autoradiographic studies of Edwards and de Olmos (J. Comp. Neurol., 165: 417, 1976). On the other hand, backfiring of ZI cells was elicited at 0.5-1.5 ms latencies, following stimulation of one or several foci in the ipsilateral FTC. That fibers arising in ZI terminate in the FTC was corroborated by recording monosynaptic discharges of FTC cells following ZI stimulation. In order to substantiate the electrophysiologic findings, the retrograde transport of horseradish peroxidase was studied with the diamino-benzidine method and the improved, tetrametyl-benzidine procedure of Mesulam (J. Histochem. Cytochem., 26: 106, 1978). Labelled neurons were found in large number in both medial and lateral parts of ZI after injections in the midbrain tegmentum, whereas only a few positive cells occurred in the adjacent hypothalamic areas. The significance of these reciprocal pathways between FTC and ZI for activation-deactivation processes is now being investigated by recording spontaneous firing and evoked activities of physiologically identified cells at various levels of alertness. (Supported by MRC grants MT-3689 and MT-5781).

149 LOCOMOTOR ACTIVITY, EXPLORATION AND NEOPHOBIA IN RATS WITH KAINIC ACID-INDUCED DEGENERATION OF THE NEOSTRIATUM. Paul R. Sanberg, Michele Pisa and Hans C. Fibiger. Div. Neurol. Sci., Dept. Psychiatry, Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Locomotor, exploratory and neophobic behaviors were assessed in male Wistar rats with neurotoxic degeneration of the neostriatal neuropil. Kainic acid (KA; 3 nmoles in 0.5 µl of phosphate-buffered saline) was bilaterally injected into each caudate-putamen of the experimental rats. The controls received injections of the vehicle only. No differences between groups were found in daytime locomotor activity in photocell activity cages. Exploration of the arms of a T-maze was assessed in 5 daily sessions, each 5 min long. The experimental rats explored less than the controls in the first and the last two sessions, as reflected by the significant interaction of lesion treatment with sessions. The experimental rats also tended to ambulate less then the controls at the onset of each session. This was especially evident in the subsequent spontaneous alternation test in the T-maze, in which the experimental rats failed to leave the start box or to complete the trials significantly more frequently than the control rats. Although the KA treated rats alternated less than the controls on the average, the difference was not significant, owing to great variability in their behavior. There were no differences between groups on locomotion and rearing in an open field. The rats with lesions, however, showed significantly less thigmotaxic, i.e. wall hugging, behavior. Activity was finally measured in a maze with 7 parallel alleys joining a start box and a goal box. The KA treated rats showed significantly longer latencies both to leave the start box and to eat the first food pellet in the goal box. Their locomotor activity in the alleys did not differ from that of the controls, however.

These results suggest that neostriatal lesions may not affect gross daytime locomotor activity, expecially if it is measured in homogenous environments. On the other hand, in conditions involving locomotion from one place to another, neostriatal lesions appear to result in decreased activity, presumably on account of an increased fear of novelty. These finding indicate a role of the neostriatum in the control of emotional reactions. It is of interest that patients with Huntington's disease, which involves gross neostriatal degeneration, also suffer intense emotional disorders.

Supported by the Medical Research Council of Canada.

150 EFFECTS OF INTRASTRIATAL KAINIC ACID ON MOTOR BEHAVIOR IN RATS. E.K.Silbergeld, R.E.Hruska, J.R.Walters, S.Kennedy*, N.Eng* & S. deSantis* (SPON: T.N. Chase). Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20014.

Kainic acid (KA) injected intrastriatally has been reported to produce relatively specific neurotoxic sequelae involving cholinergic and GABAergic neurons in the striatum. KA-treated rats have been proposed as models for Huntington's disease by Coyle & Schwarcz (Nature 263:244, 1976). However, this analogy has not been supported by studies of the effects of KA on motor behavior.

Rats (250-300 gm) were injected stereotactically into the striatum with 1 µg KA dissolved in 0.5 µl 0.1 M NaH_2PO_4 buffer, pH 7.4. For behavioral studies, injections were bilateral, at these coordinates (Koenig & Klippel): 7.9 mm anterior, 2.6 mm lateral, and 5.7 mm below skull surface. Biochemical changes were measured in striata and nigras of rats, injected unilaterally with the same dose, 7 and 14 days after injection. In KA-injected striata, high affinity choline uptake was 30% (7 days) and 27% (14 days) of control striata (non-injected side); glutamic acid decarboxylase (GAD) activity was 18% (7 days) and 16% (14 days) of controls; striatal dopamine levels were not significantly changed, nor was the accumulation of DOPA after inhibition of DOPA decarboxylase. In nigras ipsilateral to KA injected striata, GAD activity was also lower: 30% (7 days) and 35% (14 days) of contralateral nigras.

Motor behavior was assessed 7 and 14 days after bilateral injections. Rats were intubated for 4 days after injections; but survivors lost 25-40% body weight. Gait was studied quantitatively by recording footprints of rats freely walking on an enclosed, inclined runway. Length of stride, placement of hind and forefeet, and angles between contralateral footfalls were found to be consistent among control rats. Motor behavior was also studied by videotaping freely moving rats and analyzing dynamic records as to duration of stride, swing time for each limb, and footfall sequence. KA rats were found to be significantly different from controls in: variability of stride length, lack of superimposition of hind over forefootfall, distance covered per stride, duration of stride, and swing time. In addition, observation of nonquantifiable aspects of motor behavior indicated abnormal posture and problems in controlling nonlocomotive forefoot movements in KA rats. These changes were not seen in rats having lost comparable body weight through food restriction, or in rats injected bilaterally with buffer.

151 STRIATAL CYSTEINESULFINIC ACID DECARBOXYLASE. Wm. A. Staines*, A.M. Benjamin* and E. G. McGeer (SPON: A. Jakubovic). Div. Neurological Sciences, University of British Columbia, Vancouver, B.C. V6T 1W5, Canada.

Cysteinesulfinic acid decarboxylase (CSAD), the enzyme catalyzing the penultimate step in the major route of taurine synthesis, is reported to be predominantly synaptosomal and may thus serve as a marker for taurine-containing neurons if such exist. The acute and long term effects of kainic acid (KA)-induced lesions on CSAD activity in rat striatum and substantia nigra were studied using a modification of the assay of Pasantes-Morales et al (Brain Res., 1976, 107, 575-589). In some cases taurine levels were measured on an amino acid analyzer. Activities of CSAD in control tissue were 85 µmoles/hr/mg protein in striatum and 162 µmoles/hr/mg protein in substantia nigra (SN). The Km determined for striatal tissue was 5.56 mM. Control taurine level in striatum was 8.63 µmoles/gm initial weight.

Previous work indicates that injections of KA under the conditions used here destroy striatal neurons but leave intact nerve endings and fibres of passage. One week after unilateral striatal injections of 3 nmoles of KA the CSAD activity (like those of choline acetyltransferase and glutamic acid decarboxylase) were reduced to approximately 50% of control. Similar injections of vehicle, 5 nmoles KA and 10 nmoles KA reduced CSAD activity in the striatum to 94%, 38% and 27% respectively. Taurine levels in the striatum showed a significant reduction to 64% of control levels after an injection of 5 nmoles of KA. A smaller but dose-dependant reduction in CSAD activity was also seen in the ipsilateral SN after intrastriatal KA injections.

Six to eight weeks after striatal injections both CSAD activities and taurine levels seemed to be reduced to a lesser extent than in the more acute experiments.

In as much as KA is believed to be a neuron-specific toxin, the loss following its injection give some further support to a neuronal localization for CSAD activity. The long term recovery in both taurine levels and CSAD activity may be due to a compensatory increase in glia. Intrastriatal injections of KA have been suggested as an animal model of Huntington's chorea. Reports of amino acid levels in this disease indicate no abnormalities with respect to taurine so that our data on animals acutely lesioned indicate a difference from the diseased state. The long term effects of small doses may still, however, cause insignificant changes in the taurine system as seen in chorea.

This work was supported by the Huntington Chorea and the Garfield Western Foundations.

152 ELECTROPHYSIOLOGICAL ANALYSIS OF EXTRINSIC INPUTS TO RAT CAUDOPUTAMEN. Cam P. VanderMaelen*, Anthony C. Bonduki*, and S.T. Kitai (SPON: J. A. Rafols.) Dept. Psychol., Wayne State Univ. Detroit, MI, and Dept. Anat., Michigan State University, E. Lansing, MI 48824.

Intracellular recordings were obtained from neurons in the caudoputamen of hooded rats. Animals were anesthetized with urethane, placed in a stereotaxic holder, and a craniotomy performed to allow for the placement of stimulating and recording electrodes. Stimulating electrodes were placed in the cerebral neocortex (CX), centromedian-parafascicular area (CMP) of the thalamus, and the substantia nigra (SN). Recording electrodes consisted of glass micropipettes filled with 2M KCl or K-citrate with DC resistances of 30-70 Megohm. Electrical stimulation of CX, CMP, or SN elicited monosynaptic EPSPs in caudoputamen neurons. Response latencies ranged from about 1.7-4.5 msec (Median=3.0) for CX stimulation; 2.6-3.6 msec (Median=3.2) for CMP; and 2.3-5.5 msec (Median=3.5) for SN. Convergence of monosynaptic inputs from stimulation of all three areas was demonstrated. Such monosynaptic convergence was also recently reported in the cat caudate nucleus (Kocsis et al., Brain Res., 124, 1977, 403-413). Most neurons exhibited a hyperpolarizing potential following the initial EPSP, which was sometimes followed by rebound excitation with action potentials. EPSP amplitude was increased by injecting hyperpolarizing current through the recording electrode, and was decreased by the passage of depolarizing current.

Double stimulation of SN or CMP with an appropriate interstimulus interval (e.g. 40 msec) resulted in 50-100% reduction in the test EPSP amplitude. But double stimulation of CX at various interstimulus intervals resulted in little or no reduction in test EPSP amplitude. Similarly, test EPSPs from CX stimulation were not substantially reduced by conditioning stimulation to CMP or SN. However, test EPSPs from SN or CMP were reduced by conditioning CX stimulation. This finding in the rat of "prepotency" of the cortical input to the striatum is in agreement with results reported for the cat (Hull et al., Exp. Neurol., 38, 1973, 324-336). (This research was supported by NIH grants NS00405 and NS05576.)

153 KAINIC ACID AND $[^3H]$-GLUTAMATE BINDING IN RAT STRIATUM. Steven R. Vincent and Edith G. McGeer. Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

Kainic acid is a neurotoxic amino acid which has been suggested to act via glutamate receptors in the central nervous system to exert both its neurotoxic and neuroexcitatory actions. In the present study we have examined the effects of kainic acid on the specific binding of $[^3H]$-glutamate to rat striatal membranes. The specific binding of $[^3H]$-glutamate (defined as that displaceable with 5 mM unlabelled glutamate) was found to be sodium-dependent and saturable. Kinetic studies revealed a dissociation constant (Kd) of 2.1 µM and the maximum number of binding sites (Bmax) was 1.1 nmoles per mg. protein. Kainic acid inhibited the Na^+-dependent $[^3H]$-glutamate binding, however, the slope of the displacement curve was less than that found for glutamate, suggesting that kainic acid was acting at a different site than glutamate. This was confirmed in kinetic studies, where kainate was found to inhibit non-competitively the $[^3H]$-glutamate binding with a Ki of 0.54 mM.

It has been suggested that Na-dependent glutamate binding represents binding to a glutamate uptake site. However, drugs such as glutamic acid diethyl ester and ibotenic acid which have no effect on high affinity uptake were found to inhibit Na-dependent $[^3H]$-glutamate binding, while oubain was without effect. Also, lesions of the striatum with kainic acid did not reduce Na-dependent, high affinity glutamate uptake, but did result in a 50% reduction in the density of Na-dependent $[^3H]$-glutamate binding sites, without affecting their affinity. Thus it appears that kainic acid can inhibit the sodium-dependent binding of glutamate to neuronal membranes by acting at a site distinct from the glutamate receptor.

(Supported by the Medical Research Council)

154 NEUROLEPTICS AND NIGROSTRIATAL DOPAMINERGIC NEURONS. Suzanne M. Wuerthele and Kenneth E. Moore. Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

Increased activity of nigrostriatal dopaminergic neurons is associated with increased concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) in the striatum (Roth et al., Eur. J. Pharm. (1976) 36: 163-172). In the present study a radioenzymatic procedure was employed to measure the concentrations of dopamine and DOPAC in the striatum and substantia nigra of rats treated with drugs reported to increase or decrease nigrostriatal nerve activity. Systemic administration of haloperidol (0.1 mg/kg, i.p.) increased the concentrations of DOPAC in the striatum and substantia nigra to 270% and 140% of control, respectively, but failed to alter dopamine concentrations in either region. Several days after a unilateral injection of kainic acid (2.5 µg in 2 µl), which destroys cholinergic and GABAergic neurons in the striatonigral feedback loop, the concentration of dopamine was unaltered while the concentration of DOPAC was increased in the ipsilateral striatum. Systemic administration of haloperidol caused a further increase in the DOPAC concentration in the kainic-acid lesioned striatum, suggesting that the neuronal feedback loop is not essential for this action. To determine if blockade of putative autoreceptors located on nigrostriatal cell bodies are involved in the DOPAC-elevating actions of neuroleptics, haloperidol (0.1, 0.5, 1 µg in 2 µl) was injected unilaterally into the substantia nigra of conscious rats through chronically implanted cannulae. Thirty minutes after intranigral injections of haloperidol the concentration of dopamine and DOPAC in the striatum and substantia nigra were unaltered. On the other hand 30 minutes after intranigral injections of baclofen (2.5 µg in 2 µl) the concentration of dopamine was increased and the concentration of DOPAC was decreased in the striatum. Baclofen also decreased DOPAC concentrations in substantia nigra. A lower dose of baclofen (0.1 µg) which was without effect on striatal DOPAC concentrations per se prevented the haloperidol-induced increase of striatal DOPAC. These data suggest that although nigrostriatal activity can be influenced by intranigral baclofen, haloperidol-induced increases in nigrostriatal activity may not be mediated through an action on dendritic receptors located in the substantia nigra. (Supported by USPHS Grant MH13174)

155 ALTERED CAUDATE NUCLEUS FIELD POTENTIALS FOLLOWING SUSTAINED STIMULATION TO DIFFERENT SUBSTANTIA NIGRA REGIONS. R.R. Yeoman*, B.M. Rigor*, N. Dafny, (Spon: Richard Wiggins). Department of Neurobiology and Anatomy, The University of Texas Medical School at Houston, Houston, Texas 77025.

It has been demonstrated neuropharmacologically that electrical stimulation of central pathways causes changes in terminal area transmitter concentrations. The present experiments were initiated to confirm electrophysiologically if field potentials from the head of the caudate nucleus are also changed subsequent to sustained stimulation of the substantia nigra pars compacta and pars reticularis. Urethane anesthetized male rats were stereotaxically implanted with bipolar concentric recording electrodes localized in the caudate nucleus. In experiment one, the pars compacta-medial lemniscus area was stimulated through bipolar concentric electrodes with square wave 0.1 msec pulses at 1/3 sec for a control recording. After the responses from eight test stimuli were recorded and averaged, a single five minute train of 0.2 msec biphasic square pulses at 50 cycles per second were given. Immediately after the train, 8 test stimuli were again given and the responses recorded and averaged for comparison to pre-burst activity. Responses stimulated from pars compacta were characterized by two fast spikes followed by a wave, P_3-N_3. This wave was markedly increased in amplitude immediately after the stimulation train, yet returned to control level within five minutes. In experiment two, the pars reticularis was similarly stimulated and tested. The characteristic waveform from this area consisted of a biphasic spike followed by a wave, P_2-N_2-P_3. The P_2 peak was consistently decreased after a burst in this area yet it also returned to control within five minutes. The burst affected peak had similar latencies of 10-15 msec in both experiments. Histological verification of the stimulus site was done in all animals. The apparent substantia nigra stimulus localization effect on caudate nucleus field potentials suggests different transmitter pathways may be involved and modified by the electrical stimulation train.

BRAIN METABOLISM AND NUTRITION

156 EFFECTS OF CARBIDOPA OR PRECURSOR (TYROSINE) LOADING ON URINARY CATECHOLAMINE LEVELS IN RATS. R. Alonso*, J. Agharanya* and R. J. Wurtman. Dept. Nutrition & Food Sci., MIT, Cambridge, MA 02139.

We have used urinary norepinephrine (NE) and epinephrine (E) levels to examine the effects of carbidopa or tyrosine (Tyr) administration on NE and E release from peripheral sympathetic nerves and adrenal medulla of rats. We previously showed that carbidopa, a peripheral inhibitor of aromatic amino acid decarboxylase, suppresses the decarboxylation of endogenous ^{3}H-dopa formed from ^{3}H-tyrosine in cardiac sympathetic nerves (Nature 265: 79, 1977). Tyr is the circulating precursor for NE, and its administration enhances NE synthesis in (Science 185: 183, 1974) and release from (Gibson & Wurtman, Life Sci., in press) CNS noradrenergic neurons. The present results show that carbidopa (100 mg/kg i.p.) decreased urinary NE of male rats and completely blocked the rise in NE excretion caused by cold exposure (Table). The carbidopa-induced decrease in urinary NE was associated with a reduction in cardiac NE content (from 333.3 ± 21.7 ng/g to 136.0 ± 18.9 ng/g), but no change in brain NE. Hence, carbidopa reduces the NE content of rat urine by inhibiting its synthesis in peripheral sympathetic nerves, without affecting brain NE neurons. These data provide an additional basis for clinical trials of high doses of carbidopa when it is desired to decrease peripheral sympathetic activity without affecting brain function. In studies on Tyr, male rats fasted overnight and placed in a cold (4°C) environment were injected with Tyr (100, 200, or 400 mg/kg i.p.) or its diluent and given water (5 ml) by stomach tube. The excretion of both catecholamines during the following 3 hr increased in rats treated with 200 or 400 mg/kg Tyr, and the effect was linearly dose-related in the case of E. These observations suggest that catecholamine synthesis in, and release from, peripheral tissues, as in rat brain, can be precursor-dependent. It remains to be determined whether the amounts of Tyr available for peripheral NE and E synthesis depend solely on plasma Tyr levels, or also on plasma concentrations of other neutral amino acids. Perhaps induced changes in plasma amino acid levels may be useful in treating disorders of sympathetic nervous function.

	Control	Cold	Carbidopa	Carbidopa + Cold
NE (ng/12h)	427.3±49.7	691.6±112.1*	231.3±52.9†	222.0±37.7†
	Control	Tyr(100mg/kg)	Tyr(200mg/kg)	Tyr(400mg/kg)
NE (ng/3h/	153.3±17.4	150.5±7.9	195.9±11.7*	194.2±11.5*
E 100g)	19.3± 3.6	25.3±3.6	43.9± 4.8‡	68.7± 7.2§

*P<0.05 vs control; †P<0.0025 vs control; ‡P<0.01 vs control; §P<0.005 vs 200 mg/kg.

157 CORTICAL OXIDATIVE METABOLISM IN NORMAL AND MILDLY ISCHEMIC BRAIN. George Austin, Britton Chance, Clyde Barlow, and Ronald Jutzy*. Sect. of Neuros., Loma Linda Univ., Loma Linda, CA., 92350 and Johnson Research Foundation, Univ. of Penns., Phila., PA. 19174

In an area of normal cortex or an area of relative brain ischemia, where considerable function still exists in the cortical network, it is possible to increase relative brain O_2 tension, (bPO_2) by: 1) increasing the mean B.P., 2) increasing the FiO_2 at least up to 60%, and 3) increasing the CBF by additional CO_2 inhalation. These studies were carried out in a series of cats under N_2O/O_2 anesthesia, in a 2/1 ratio. They were based on the use of 25 μ teflon coated platinum electrode inserted 1 - 2 mm in the prefrontal cortex to record bPO_2. In all cats it was necessary to exceed autoregulation of flow or of bPO_2 by increasing the mean B.P. or FiO_2 by a degree beyond that which allows the autoregulatory mechanisms to effectively perform. In a follow-up group of normal and mildly ischemic cats the results of a significant rise in bPO_2 on cortical oxidative metabolism were investigated by the use of non-invasive optical techniques to measure the relative redox level of some members of the mitochondrial electron transport system. These included Cyt. a,a_3 (by dual beam, dual wavelength spectrophotometer), flavoprotein (by flying-spot fluorometer), and NADH (by fluorometer). The results of these studies show that cortical oxidative metabolism can be increased, at least in some areas of the brain, by driving up the bPO_2. The results of these ongoing animal studies form the basis for intraoperative monitoring of cortical oxidative metabolism in patients undergoing microanastomosis. They support the conclusion that the increase in bPO_2 and increase in oxidized state of members of the electron transport chain, offer a reasonable explanation for the acute improvement following microanastomosis.

158 NEW APPROACH TO EARLY TREATMENT OF PHENYLKETONURIA (PKU). Helen K. Berry, Mary K. Bofinger*, and Richard E. Butcher*. Dept. Pediatrics, Univ. Cincinnati. Sch. Med. Cincinnati, OH 45229.

We have shown that phenylalanine levels in brains of PKU rats treated with valine, isoleucine, and leucine (VIL) were lower than in untreated PKU rats. We also demonstrated reduction in CSF phenylalanine (Phe) in an adult woman with untreated PKU after administration of VIL, although serum Phe levels were unchanged. It seemed logical to test the effect of VIL on treatment of infants with PKU.

An infant recognized to have PKU as a result of newborn screening was treated with VIL to determine if transport of Phe into CSF could be inhibited. Newborn screening for Phe was positive at 4 days of age. Serum Phe measured at 18 days was 55 mg% (normal<1). Tyrosine was 1.2 mg% (normal<2). Phenylpyruvic acid excretion by Phenistix was 15 mg%. Following confirmation of the diagnosis the infant was hospitalized for treatment. Prior to beginning the low-phenylalanine diet baseline studies were carried out to determine the effect of treatment with VIL on cerebral Phe transport. Blood and cerebrospinal fluid were collected while the infant was fed with Similac (24 oz/day). The infant was then fed with Similac supplemented with valine, 135 mg/kg; isoleucine, 129 mg/kg; and leucine, 241 mg/kg. At the end of 48 hours blood and CSF were collected; VIL treatment was discontinued and a low Phe formula was begun. During the 48 hour period on VIL serum Phe remained unchanged at 3174 uM, while CSF Phe decreased from 1049 to 882 uM.

Similar studies were carried out in two additional infants with comparable results. Trials of VIL combined with low Phe diet are now being undertaken to determine if CSF Phe levels can be reduced to a greater degree than by diet alone.

159 EXTRACELLULAR POTASSIUM CHANGES IN NORMAL AND GLIOTIC CEREBRAL CORTEX DURING ISCHEMIA. William F. Blank. Dept. Pathology, Washington U. Medical School, St. Louis, Mo 63110.

Extracellular potassium concentration (K_o) was measured continuously in gerbil cortex during ischemic insults caused by occluding one or both carotid arteries. The gerbils were anesthesized with 30 mg/kg pentobarbital and body temperature kept constant at 37°C. Portions of the cortex were frozen for 15 seconds with a dry ice/acetone slurry in order to produce astrocytosis.

The control K_o before the insult averaged 2.7 mM in normal cortex and 4.8 mM in gliotic cortex. After the carotid arteries were clamped the K_o increased. In normal cortex the K_o rise was a complex exponential and resembled spreading depression. The maximum rate of rise averaged 4.75 mM/sec and the K_o reached a mean of 99 mM/L. The K_o rise in gliotic cortex was linear and averaged 3.1 mM/min. The maximum K_o reached 26 mM before reversal of the insult.

The insults were reversed by unclamping the carotids. The K_o fall was described by a single exponential function in both normal and gliotic cortex. The rate constant averaged -.025/sec in normal cortex and -.004/sec in gliotic cortex. Undershoots of K_o below control levels of up to 1.2 mM (mean .65 mM) occurred in normal cortex but were not seen in gliotic cortex.

Since neurons and axons are absent in the gliotic cortex, it is postulated that the presence of neurons and axons are necessary for the occurrence of the exponential, spreading depression-like rise and the undershoot of K_o following reversal of the insult. The exponential rise probably occurs during ischemia (energy depletion) because of increased neuronal permeability due to massive transmitter release from synaptic endings. The linear rise in gliotic cortex is probably due to inhibition of $Na^+ - K^+$ ATPase of the astrocytes and diffusion of K^+ from the normal (though ischemic) surround. (See Blank, W.F. and Kirshner, H.S.: Brain Research 123:113-124, 1977).

160 ROLE OF ASPARTIC AND GLUTAMIC ACIDS IN THE ATAXIA PRODUCED BY THIAMINE DEFICIENCY. Roger F. Butterworth, Edith Hamel* and André Barbeau. Dept. Neurobiology, Clinical Research Institute of Montreal, Montreal, Quebec, Canada.

Thiamine deficiency is considered to produce its effect on the central nervous system by one (or more) of the following mechanisms: (i) inhibition of brain transketolase (ii) inhibition of brain pyruvate and α-ketoglutarate dehydrogenases (iii) by a direct action on the neuronal membrane.

Pyrithiamine, a central thiamine antagonist, when administered to rats in doses of 0.5 mg per kg per day, produces within 18 days, neurological symptoms of thiamine deficiency including ataxia. Brain levels of the amino acids glycine, GABA, glutamine, aspartic acid, glutamic acid and taurine were measured by a double-labelling dansyl microassay (Anal. Biochem., 64, 389 (1975)) in whole brain, retina, dorsal root ganglion and in the following discrete brain regions of affected animals: cerebellum, medulla oblongata, cortex, hypothalamus, hippocampus, midbrain, caudate nucleus and olfactory bulbs. In cerebellum, medulla oblongata and midbrain, regions of the brain most affected pathologically in thiamine deficiency, aspartic and glutamic acids were decreased by 47% and 26% respectively ($p < 0.01$).

In a separate series of experiments, rats were maintained on a thiamine-deficient diet for up to 7 weeks, at which time signs of deficiency (loss of righting reflex, ataxia) were present. Significant decreases in glutamic and aspartic acids were again observed in medulla oblongata, cerebellum and midbrain of affected animals when compared to pair-fed controls, suggesting that the amino acid changes were not secondary to the anorexia associated with thiamine deficiency. These results suggest that the putative excitatory amino acids, glutamic and aspartic acids may play a key role in the production of the neurological symptoms of thiamine deficiency. Mechanisms implicating either inhibition of pyruvate (and α-ketoglutarate) dehydrogenase(s) or a direct role for thiamine in membrane excitability will be discussed.

Supported by grants from Université de Montréal CAFIR and The Association Canadienne de l'Ataxie de Friedreich.

161 PROPAGATION OF FOCAL MOTOR SEIZURES IN THE MONKEY William F. Caveness, Motohiro Kato*, Shinichi Hosokawa*, Barbara L. Malamut*, Shinichiro Wakisaka*, and Raymond R. O'Neill*, NINCDS, National Institutes of Health, Bethesda, Maryland 20014

Focal seizures were induced in 3.5 Kg. Macaca mulatta by injecting 25,000 units of Penicillin in 0.25 m. water into the face-hand area of the right cerebral motor cortex. The paroxysmal activity was monitored by electroencephalography and electromyography. After thirty minutes each head was removed, frozen and serially sectioned. The cortical and subcortical metabolic activity was then determined by the Sokoloff Method: 2-deoxy-D[^{14}C]glucose, injected by vein at the beginning of the seizure, competes with glucose for transport across the blood brain barrier and for the enzyme hexokinase. While glucose completes its metabolic cycle, the deoxyglucose is trapped in brain tissue at the phosphorylated phase where its [^{14}C] label may be quantitatively measured by autoradiography. Using these measurements with those of the isotope in arterial blood, the actual rate of local glucose utilization may then be calculated in mg per 100 grams of brain per minute.

The pattern of glucose utilization in four control monkeys was bilaterally symmetrical with a range in individual brain components from 8-9 mg/100/gm/min to 2-3 mg/100 gm/min. The right-left difference, % (R-L)/L, was negligible. With contralateral face seizures, in four monkeys, there was a distinct though uneven increase in activity with a right-left difference of 90-100% in the lateral globus pallidus, VPM of the thalamus, and motor cortex, with somewhat less difference in VPL, VL and medial globus pallidus. With extension to contralateral face and hand seizures, in four monkeys, there was a dramatic change in pattern, predominantly unilateral, with values as high as 22-28 mg/100 gm/min. The right-left difference was 400% in medial, and 340% in lateral globus pallidus, twice that of any other structure; 120-160% in the motor and sensory cerebral cortex; 90-120% in VL, VPL and VPM; and a left-right difference of 50-60% in the cerebellar cortex.

These data provide fresh insight into the location and extent of increased neuronal activity in focal motor seizures, that must be accommodated when considering basic mechanisms. Further, they should be considered in the search for new modes of therapy.

162 SYNTHESIS OF MYELIN-ASSOCIATED GLYCOPROTEINS IN PROTEIN-DEFICIENT RATS. Mary J. Druse-Manteuffel and Nancy L. Krett*. Department of Biochemistry and Biophysics, Loyola University Medical Center, Maywood, Il. 60153.

The synthesis of fucosylated glycoproteins was studied in the 15-, 20- and 27-day-old offspring (control and protein-deficient pups) of female Sprague-Dawley rats that were maintained on normal laboratory chow or a protein-deficient diet (8% protein) during the first twenty days after parturition. A double-label isotope technique was used whereby a control rat was given an intraventricular injection of ^{3}H- or ^{14}C-fucose 18 hours prior to sacrifice and a protein-deficient rat was given fucose labeled with the other isotope. Brain homogenates from a control and protein-deficient pup were combined. Purified myelin (JNC 21: 749, 1973) was subfractionated (BBA 329: 305, 1973) into light, medium and heavy myelin. ^{3}H and ^{14}C radioactivity was determined in separated myelin subfraction proteins.

These experiments provide evidence that the major myelin-associated glycoprotein in young protein-deficient pups has a higher apparent molecular weight than that from the myelin subfractions from age-matched control pups. These results may be explained by the presence of a new, abnormal glycoprotein in the protein-deficient rats. Alternatively, the developmental shift to a lower apparent molecular weight glycoprotein which has been seen in normal rats (Brain Res. 58: 506, 1973) may have been delayed in the protein-deficient rats.

This research was supported by a Basil O'Connor Starter Research Grant from The National Foundation-March of Dimes. Dr. Mary Druse Manteuffel is the recipient of a Schweppe Foundation Career Development Award.

163 EFFFCT OF CHRONIC PROTEIN MALNUTRITION ON PENTYLENETETRAZOL-INDUCED AND KINDLED MOTOR SEIZURES IN THE RAT. W.B. Forbes, C.A. Tracy*, W.C. Stern, P.J. Morgane, and O. Resnick. Worcester Foundation for Exptl. Biology, Shrewsbury, MA 01545.

In a previous study (Brain Res., 79:375-384, 1974) we found that rats malnourished during lactation exhibit, at adulthood, greater susceptibility to electroconvulsive shock applied via ear-clip electrodes. In order to evaluate the generality of that finding, we tested the susceptibility of malnourished and normal rats to pentylenetetrazol (PTZ) induced and kindled motor seizures.

Female rats were fed one of two isocaloric diets, differing only in percent casein content (8% or 25%), for five weeks prior to mating, throughout gestation and lactation. Following weaning, pups were fed the same diet as their mothers, ad lib., either the high (group HH) or low (group LL) casein diet. For the study of kindled motor seizures, two additional groups of animals were switched to the opposite diet at weaning (groups HL and LH). Testing of the pups was performed at adulthood, i.e., 60 - 120 days.

Susceptibility to PTZ-induced seizures was evaluated by administering repeated doses of the drug (15 mg/kg, i.p.) at 15 min. intervals and measuring the latency from the first injection to the occurrence of a convulsion. No difference between dietary treatment groups in latency to convulse was seen. Kindled motor seizures were induced by daily elicitation of amygdaloid after-discharges. Electrical stimulation at threshold levels was applied daily until full motor convulsions were observed. In comparison with group HH, group LL exhibited after-discharges of shorter duration early in the experiment (HH=18±7 sec, LL=10±7 sec) but when full motor convulsions developed, did not differ significantly from group HH in this respect. Group LL required significantly more stimulations for the elicitation of a full motor seizure than group HH (LL=15±3; HH=10±2). Only the HL dietary treatment group differed significantly from group HH in threshold for after-discharge elicitation (HL=333±78μA; HH=184±65μA).

These data do not support the generality of the previous observation that chronic malnutrition enhances seizure susceptibility. It appears that the effect of malnutrition on this aspect of brain functioning is situation dependent. The present findings of decreased amygdaloid after-discharge duration and slower kindling of motor seizures in group LL are in keeping with our previous observation of higher brain levels of serotonin and norepinephrine in chronically malnourished rats (Exp. Neurol., 49:314-326, 1975). However no general conclusion regarding the effect of chronic malnutrition on seizure susceptibility is possible at this time. (Supported by NICHHD Grant HD06364.)

164 PIAL ARTERY PRESSURE IN THE DOG: EVIDENCE THAT AUTONOMIC INNERVATION DOES NOT AFFECT SURFACE BRAIN ARTERIES.
Patricia A. Grady, Otis R. Blaumanis* and Erland Nelson. Univ. of Md. Sch. Med., Dept. Neurol. Balt., Md. 21201

Among the most controversial aspects of the physiology of the cerebrovascular system is the functional role, if any, of the innervation of cerebral blood vessels. Numerous investigators have argued on the basis of morphological and physiological observations that the autonomic nerves which accompany the pial arteries exert a significant influence on cerebral blood flow (CBF). In recent years attention has been focused on the possible role of the central adrenergic system which appears to innervate the cerebral microcirculation and originates within the central nervous system itself. It has been suggested that either or both of these systems is involved in the neurogenic component of the autoregulation of CBF. Whether or not the nervous system influences CBF by some direct action remains unknown. Several investigators have found that the larger pial arteries are the sites of a significant proportion of the total cerebrovascular resistance. Others have found that this segment of the cerebrovascular system is richly endowed with autonomic nerves. Numerous technical problems have impeded a resolution of the question of whether perivascular innervation has a significant effect on the caliber of the pial arteries.

The present study was carried out using mongrel dogs anesthetized with Pentobarbital. Aortic and middle cerebral artery pressures were measured. We hypothesized that if the pial arteries, known to contribute substantial resistance to overall CBF, were involved in autoregulation, downstream pressures in small arteries should remain relatively constant as systemic arterial pressure varied. Direct cannulation of vessels as small as 200μ showed that this was not the case. When mean aortic pressure was decreased from 200 to 20 mmHg by reducing blood volume, pial artery pressures followed in a linear relationship. Pial artery pressures were always less than those in the aorta, but the difference between the two was constant. The mean arterial pressure in these experiments was varied over the entire range of the cerebral autoregulatory curve. The pial artery pressures varied accordingly. These relationships were not altered after blockade of the sympathetic nervous system by phenoxybenzamine (5mg/kg) administered 30-60 minutes before decreasing the blood pressure.

These data suggest that the well innervated cerebral arteries do not participate to any significant degree in the overall autoregulatory mechanism of the cerebral circulation. These findings suggest that the microcirculation of the brain is the major site of regulation of cerebral blood flow.

165 ULTRASTRUCTURAL CHANGES IN PURKINJE CELLS DUE TO PERINATAL UNDERNUTRITION.
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Protein deficiency during perinatal development results in decreased body and brain weight. Reduced number of some neuronal and glial cell types as well as cell size, are believed to be responsible for the decreased nervous tissue mass. Though quantitative alterations have been described, qualitative changes which are indicative of a deprived nutritional status during development have not been reported. In studies using a rat model to determine the impact of 8% protein diets, with normal calories, on the development of the nervous system, characteristic ultrastructural changes in dendritic cytoplasmic constituents of Purkinje cells were observed. Endoplasmic reticulum (ER) formed stacked arrays in about 30% of the offspring which were reared from mothers deprived of normal amounts of protein both pre- and postnatally. These arrays consisted of 2-6 cisternae which were adjoined to each other by dense particles between the cytoplasmic surfaces of these adjacent membranes. The ER of the soma appeared normal, while in dendrites, the arrays lined the plasma membrane of large processes and were found throughout smaller dendrites. Quantitative measurement showed that stacking was accompanied by a 30% increases in the perimeter of the ER profiles per unit area of dendritic section.

In a small percentage of the animals with the ER changes, giant spines were found on the spiny branchlets of Purkinje cells. These dendritic profiles had even greater amounts of ER. The diameter of the giant spines was 2-3 times larger than normal and displayed equally elongated synaptic contacts. About 30% of the spines in these animals were giant spines.

This alteration in the ultrastructure of Purkinje cells may be due to factors extrinsic to the Purkinje cell such as fewer granule cells and a reduction of presynaptic sites. A second possibility is that the deficiency may act intrinsically by interfering with the Purkinje cells' ability to produce a cytoskeleton of tubules and filaments which are necessary to obtain a full sized neuron. In either case, the excess ER may result since the dendritic trees are small. (Supported by the USPHS Grant HD-10934).

166 **A TEST OF AUTO-OXIDATION OF PARTIALLY DISRUPTED BRAIN TISSUE IN VITRO.**
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Mincing of the brain starts the process of autolysis because of: 1) mechanical damage of cell membranes and 2) ischemic disorder of metabolic activity. A hypothesis tested here was that after the electron transport chain is injured by ischemic insult, restoration of oxygen may facilitate lipid peroxide production and catabolic subcellular components. Three groups of minced brains were incubated at 37°C with bubbling of 1) room air, 2) 100% nitrogen and 3) 100% oxygen, respectively; and frozen by liquid nitrogen 5, 15, 30 and 60 minutes after the beginning of incubation. The frozen samples were then assayed for energy metabolites, and the free radical reaction indices, malonyl dialdehyde (MDA), oxidized form of glutathione (GSSG), and the reduced form (GSH). Brain tissue incubated with 100% oxygen had the highest amount of ATP and PCr, but also demonstrated the highest values of MDA and GSSG/GSH ratio. The anoxic incubation resulted in the lowest energy reserve, and the lowest values in free radical indices. The earliest liquefaction of the incubated media was seen in the oxygenated group. These results strongly suggest that: 1) the level of energy metabolites does not necessarily represent the ability of the tissue to maintain structural integrity, and that 2) excess oxygen to the injured brain cell initiates free radical reactions.

167 ROLE OF ERYTHROCYTE CARBONIC ANHYDRASE IN OXYGEN DELIVERY TO BRAIN.
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The brain is critically dependent for its moment to moment function and survival on an adequate supply of oxygen. This supply of oxygen is in turn dependent on a precise spatial temporal relationship between the unloading of oxygen from hemoglobin in the capillaries and the acidification of blood by carbon dioxide from the tissue. Since an erythrocyte spends only about 0.6 seconds in the capillary, the reaction time required for the hydration of CO_2 becomes crucial. The enzyme carbonic anhydrase (CA) (EC 4.2.1.1) may play an important role in oxygen delivery to brain tissue by facilitating the hydration of metabolically produced CO_2 in erythrocytes in brain capillaries, thus permitting the Bohr effect to occur while the erythrocyte is still in the capillary. We have tested this hypothesis by examining the effect of IV acetazolamide (30 mg/kg), a potent inhibitor of carbonic anhydrase, upon cerebral oxygen consumption ($CMRO_2$) in lightly anesthetized, passively ventilated rhesus monkeys. Cerebral blood flow (CBF) and $CMRO_2$ were measured with oxygen-15 labeled water and oxygen-15 labeled oxyhemoglobin, respectively, injected into the internal carotid artery and monitored externally. Acetazolamide produced an immediate and significant increase in CBF (64.7 to 83.8 ml/min/100g), an increase in arterial carbon dioxide tension (40.7 to 47.5 torr), and, most importantly, a 32% decrease in $CMRO_2$ (from 4.16 to 2.82 ml/min/100g). Because the change in $CMRO_2$ occurred within minutes of the administration of acetazolamide, this effect probably was due to an interference with oxygen unloading in the brain, and not a direct effect on brain cells containing CA (e.g., glia). The critical nature of the spatial temporal relationships critical to oxygen delivery to the brain is further emphasized by companion studies involving the addition of CA to plasma. CA normally resides only in red cells, thus potentially delaying CO_2 hydration by the time necessary to diffuse through plasma. We tested this hypothesis by the addition of bovine CA to plasma. This caused a significant increase in $CMRO_2$.

These results demonstrate the delicate balance between oxygen delivery and consumption by the brain and point out, for the first time, the important role played by erythrocyte CA in this process.

Supported in part by USPHS Grants NS-11059; NSO-6833 & HL13851.

168 PRENATAL INFLUENCE OF PROTEIN MALNUTRITION IN RATS. Maravene Miller*, Oscar Resnick*, J. Patrick Leahy* and Peter J. Morgane. Worcester Foundation for Experimental Biology, Shrewsbury, Ma. 01545.

Our group has reported marked increases in brain serotonin (5-HT) levels from birth through adulthood in rats whose dams received a low protein diet (8% casein) starting 5 weeks prior to conception as compared to rats whose dams received a normal diet (25% casein). These alterations were correlated with increases in tryptophan (Trp) availability to the brain due to changes in plasma albumin (Alb) and fatty acid (NEFA) levels (Exp. Neur. 57: 142, 1977). To determine which of these changes were the result of prenatal deficiencies and which could be caused by lactational deficiencies, pups born to dams fed 25% or 8% protein diets were cross-fostered at birth to dams of the opposite diet, i.e., pups from 8% dams were fostered on 25% dams; 25% pups were fostered on 8% dams. At 21 days of age these rats were compared to unswitched rats of each diet. These results (table below) show that while some parameters can be induced as a result of lactational deficiencies, most parameters could not be completely rehabilitated by an adequate diet during lactation. As a result, protein inadequacies during gestation appear to cause long lasting metabolic changes in offspring of rats. While the mechanism(s) responsible for these changes are unknown, these results demonstrate for the first time that prenatal protein malnutrition alone can importantly influence brain indole metabolism in the postnatal period.

Effects of Pre- and Postnatal Diets at 21 Days of Age
% Casein (n=16 per group)

Prenatal	8%	8%	25%	25%
Postnatal	8%	25%	25%	8%
Brain				
5-HT ng/g	558 ± 13	565 ± 12	389 ± 10	530 ± 12
Trp ng/g	4903 ± 135	4970 ± 67	3191 ± 46	4761 ± 41
Weight mg	1210 ± 23	1444 ± 23	1335 ± 27	1275 ± 13
Plasma				
T* Trp ng/ml	9390 ± 410	15399 ± 708	20019 ± 881	8445 ± 598
F† Trp ng/ml	3914 ± 213	2970 ± 100	2339 ± 75	2208 ± 119
Alb µg/ml	2314 ± 143	3502 ± 252	4321 ± 125	2339 ± 91
Pro** µg/ml	4421 ± 429	7084 ± 207	7453 ± 145	6221 ± 420
NEFA µeq/ml	.633 ± .05	.616 ± .07	.357 ± .03	.340 ± .04
Body Wgt. g	26.4 ± 0.5	63.4 ± 1.5	65.3 ± 1.3	24.0 ± 0.3

*T = Total; †F = Free; **Pro = Total Protein

Supported by grant HD 06364

169 EFFECT OF TIMING ON MANNITOL ADMINISTRATION ON BRAIN DENSITY. Michael E. Miner, Steve H. Graham*, and Gerald Wantz*. Department of Surgery (Neurosurgery), University of Texas Medical School at Houston, 77030.

Osmotic agents such as mannitol are used to treat human cerebral edema of a wide variety of etiologies. However, a paradoxical increase in white matter edema has been noted by some investigators when these agents are used. We investigated the timing of mannitol administration after producing a brain lesion known to result in cerebral edema.

Utilizing a density gradient column of bromobenzine and kerosine white matter density was measured in rats. In 20 control animals the specific gravity was 1.0425 ± .0003. Intravenous mannitol (2 gm/kg) was given to a series of animals. After two hours the specific gravity rose to 1.437 ± .001 but at 6 and 24 hours was within the control range. A liquid nitrogen cold lesion resulted in an ipsilateral decrease in specific gravity most pronounced at six hours (1.0402 ± .0005). The contralateral white matter density decreased slightly less. Intravenous mannitol (2 gm/kg) was then given to a series of rats one hour after making the cold lesion. The white matter density was increased at two hours (1.0432 ± .0003) similar to the series given only mannitol with no lesion, and was only slightly less than control values at 6 and 24 hours. However, a fourth series of animals was given intravenous mannitol (2 gm/kg) 10 minutes after making the same cold lesion and the ipsilateral white matter density was indistinguishable at 6 hours (1.0398 ± .0004) and 24 hours from rats with the cold lesion alone. The contralateral white matter increased in density at 2 and 6 hours, but was of less density at 24 hours than any other group.

This data demonstrates that mannitol given very soon after a cold lesion does not significantly dehydrate the brain and at six and 24 hours the density is no different than animals who only have the cold lesion and no mannitol. However, when mannitol was given one hour after the cold lesion a significant increase in density was observed and at six hours there was a marked reduction of the decrease in brain density compared to creating only the lesion. This data implies that mannitol may at least partially cross the blood brain barrier early after a cold lesion. This may be dependent on lesion size and when the mannitol is presented. This "rebound" effect did not occur when the drug was given an hour post-lesion. This data has implications in treating human cerebral edema and may help to explain the absence of the rebound phenomenon seen in the patients treated with mannitol.

170 TRANSPORT OF THYROID HORMONES ACROSS THE BLOOD-BRAIN BARRIER. William M. Pardridge. UCLA School of Medicine, Los Angeles, CA 90024.

The transport of ^{125}I-triiodothyronine (T_3) across the brain capillary wall, i.e., the blood-brain barrier (BBB), was investigated with a tissue sampling-single injection technique. Approximately 0.15 ml of buffered Ringers solution containing 1.25 µCi/ml of ^{125}I-T_3 and 25 µCi/ml of ^{3}H-water, a highly diffusable internal reference, was rapidly injected via the common carotid artery in barbiturate-anesthetized rats with decapitation 15 sec later. High affinity binding of labeled T_3 to glass vials and plastic syringes was prevented by the addition of 0.1% albumin to all injection solutions. The clearance of T_3 by brain was estimated by calculating the brain uptake index (BUI), i.e. the ratio of ^{125}I/^{3}H DPM in brain divided by the same ratio in the injection solution (x100). The ratio of ^{125}I/^{3}H was determined by double isotope liquid scintillation counting. The BUI for a tracer concentration (2 nM) of T_3 was 32.1 ±1.5% for whole brain analyses. Since albumin is known to avidly bind thyroid hormones, the effect of varying doses of this compound on the BUI of T_3 was studied; increasing the albumin concentration from 0.001%, an albumin level that should bind only 5% of T_3 (based on the affinity constant of albumin for T_3), to 0.1%, a dose that should bind 80% of T_3, resulted in no change in the BUI for T_3, suggesting both free and bound hormones are transported into brain. The addition of increasing concentrations of unlabeled T_3 or thyroxine (T_4) decreased the BUI of labeled T_3; double reciprocal plots of the saturation data were linear (r=0.92) and provided estimates of the kinetic constants of thyroid hormone transport: K_m=2.7 µM and V_{max}= 0.2 nmol/min/g for T_3 and K_i=2.5 µM for T_4. Transport showed little stereospecificity as D-T_3 competed actively for transport with labeled L-T_3. Tyrosine, leucine, and potassium iodide at levels of 1000 µM had negligible effects on T_3 transport. Conclusions: (1) The major route of entry of thyroid hormones into the CNS is transport through the BBB via a carrier system specific for thyroid hormones. (2) The affinity of the BBB transport system for T_3 exceeds the affinity of albumin for T_3 (K_d= 4 µM); therefore, albumin-bound T_3, which comprises 20% of the circulating pool of this hormone, may be stripped off the plasma protein by the BBB thyroid hormone transport system.

171 METABOLIC MAPPING OF FUNCTIONAL NEURAL PATHWAYS IN MAN WITH ^{18}F-FLUORODEOXYGLUCOSE. M. Reivich, A. Alavi*, J. Greenberg*, J. Fowler*, D. Christman*, A. Wolf*, A. Rosenquist*, P. Hand and R. Tusa*. Cerebrovascular Res. Center of Dept. of Neurology and Depts. of Anatomy and Animal Biology, Schls. of Med. and Vet. Med., Univ. of Penna., Phila., PA 19104

The ^{18}F-fluorodeoxyglucose method for measuring local cerebral glucose consumption (Reivich, et al Acta Neurol. Scand. 56: suppl 64, 190, 1977) has been used to map functional neural pathways in man. Alterations in functional activity change the metabolic rate of the involved regions of the brain which can be detected with a positron emission tomographic scanning system. The effect of visual and somatosensory stimuli have been investigated. Four visual system studies have been carried out in 3 normal male volunteers. One subject was tested twice, first with both eyes open and second with both eyes blindfolded. The occipital cortex was symmetrically labeled bilaterally in both tests, but in the blindfolded condition there was a 23% decrease in glucose utilization in both left and right occipital cortices from a value of 11.2 to 8.6 mg/100 gm/min. Two other subjects were required to look into a clear plastic hemisphere and fixate upon a small light located at its center. This light randomly dimmed so slightly as to be detectable only by foveal vision. Reports of dimming events indicated a greater than 95% accuracy in both subject's fixation. The right visual field was blackened and received little or no patterned visual stimulation during the test. The left visual field was stimulated with high contrast black and white line and dot stimuli during the test. In both subjects a clear asymmetry was seen in the occipital pole with the right pole showing greater glucose utilization than the left, presumably due to visual stimulation of the left visual field. In the somatosensory study, rapid brush stroking of the fingers and hand of the right arm of two right-handed subjects produced a dramatic increase in the metabolic rate for glucose in the left postcentral gyrus at tomographic levels OM+8 cm and OM+9 cm. This cortical region corresponds to the finger and hand area as described by Penfield and Rasmussen (1950). This asymmetry was not seen in control subjects without somatosensory stimulation. These studies demonstrate that ^{18}F-fluorodeoxyglucose can be used to map the regions of the brain with altered metabolic activity in response to alterations in local functional activity in man. (Supported by USPHS Grant NS 10939-06)

172 MITOCHONDRIAL VULNERABILITY TO ISCHEMIC INJURY: STIMULUS-EVOKED RESPONSES OF METABOLISM DURING AND FOLLOWING ISCHEMIA IN CAT CEREBRAL CORTEX IN SITU.

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Examination was made of reduction/oxidation ratio changes of nicotinamide adenine dinucleotide (NADH/NAD^+), the initial co-enzyme of the mitochondrial respiratory chain, during and following incomplete and complete ischemic episodes in cat cerebral cortex. These measurements were made noninvasively by monitoring the fluorescence at 460 nm when the tissue was presented with excitation light at 366 nm (NADH fluoresces, NAD^+ does not). Ischemia was produced by various combinations of ligation and/or clamping left subclavian, left and right common carotids and left innominate arteries after previous ligation of other possible ascending pathways in cerveau isolé cat preparations. Incomplete ischemia was accompanied by increased levels of reduced NAD which returned toward baseline during arterial clamping. Complete ischemia was accompanied by complete reduction of NAD. The rates of return of NADH to baseline after successive 1 min periods of complete ischemia were faster in each successive case. Blood volume, however, returned at a constant rate and hemoglobin oxygenation, measured by reflection spectrophotometry, returned more slowly in successive insults, indicating an uncoupled mitochondrial system. When stimulus pulses were presented to the cortical surface at intensities sufficient to evoke small shifts of the steady potential, incomplete ischemia resulted in a decrease in the amplitude of the transient NADH oxidation. Complete ischemia produced an amplitude decrease but also initially increased and then decreased the rate of NAD^+ re-reduction. Stimulation sufficient to provoke spreading cortical depression (SD) resulted in accentuation of these ischemia-related metabolic changes. There appears to be a critical level of perfusion at which no change in SD kinetics occurs when perfusion is adequate for normal NADH/NAD^+ regulation. Decreased perfusion results in marked changes in excitability and a slowing of the NAD re-reduction rate. These findings confirm that short ischemic periods can produce alterations in oxidative metabolic capabilities indicative of uncoupling, resulting in decreased excitability and decreased capacity to respond to increased metabolic demand. (Supported by PHS grants NS 14319 & NS 14325).

CEREBELLUM

173 TOPOGRAPHY OF AFFERENT BRAIN STEM PROJECTIONS TO POSTERIOR VERMAL CEREBELLUM OF THE RAT. S. Ausim Azizi*, Richard A. Burne and Donald J. Woodward (SPON: J. Kirkpatrick). Dept. of Physiology, Univ. TX Health Sci. Ctr., Dallas, Texas 75235.

This study was undertaken to determine the origin of the afferent projections to the auditory and visual zones of the posterior vermis of the rat cerebellum. We describe here the topographical organization of the projections from the pontine gray (PG), inferior olive (IO), and the brain stem reticular nuclei to the vermal regions of lobules V-IX and their relation to the descending cortical and tectal projections to the brain stem. The midline cerebellar zones of thirty five rats (200-300 g.) were hydraulically injected with 0.06-0.12 µl of horseradish peroxidase in H_2O solution to localize the origins of afferent projections.

With injections localized to the midline, all resulting HRP labeled cells were observed bilaterally within the PG, IO and reticular nuclei. The basic trend of projections noted was: Lobules V and VIa-b receive a sparse and non-focal projection from the PG, but a substantial input from the medial accessory olive (MAO), lateral reticular nuclei (LRN) and caudal principal olive (PO). Lobules VIc and VIIa receive projections from rostral PG, whereas lobules VIIb, VIII and IX receive primarily from paramedial and lateral zones in the caudal PG. With respect to olivocerebellar projections, the posterior vermis (lobules VIc-IX) receive primarily from the medial aspect of MAO, whereas lobules V, VIa and b receive from the lateral MAO. All the midvermal lobules studied receive a projection from the caudal PO, with the exception of lobule VIIb, which receives solely from the MAO. In addition the studied lobules receive from paramedian reticular nuclei and/or LRN.

We conclude that pontine projections to the lobules VIc and VIIa originate from areas receiving descending input from the visual (VC) and auditory cortices (AC). Pontine areas projecting to lobules VIII and IX receive input from the somatosensory cortex (SC), as revealed by other studies in this laboratory. With respect to lobules V and VIa-b, the sparse pontine innervation noted does not appear to correspond with the known descending projections from the VC, AC, SC or tectal regions. In addition, the MAO regions which receive visual projections from the tectum, project to lobules VI-IX supporting a visual climbing fiber input to the midvermal region. Such results support the electrophysiological evidence for visual and auditory input to posterior vermis (Lobules VIc-IX) in the rat and cat cerebellum. A general result is that each sublobule in posterior vermis receives a highly discrete set of afferents. These can be expected to mediate diverse functions in visual and auditory aspects of sensori-motor integration. (Supported by NSF BNS 77-01140 to D.J. Woodward)

174 UPTAKE AND TRANSPORT OF ^{3}H-GABA INJECTED EXTRACELLULARLY IN THE CAT DENTATE NUCLEUS. H. Bantli and D.L. Tolbert. Dept. Neurosurg., Minn. Med. Sch., Minneapolis, Minn. 55455

Recently small neurons in the dentate nuclei of the rat have been shown to accumulate extracellularly injected tritiated gamma amino butyric acid ^{3}H-GABA, (Chan-Palay, Cerebellar Dentate Nucleus, Springer-Verlag, N.Y., 1977). On the basis of these observations, it was proposed that some of the small neurons in the dentate nucleus are inhibitory local circuit neurons. However, results obtained following multiple injections of horseradish peroxidase in the cat brainstem (McCrea, et al, 1978, J. Comp. Neurol.) indicated that probably all neurons in the cerebellar nuclei project to extracerebellar structures. Based on these findings, it may be inferred that neurons which accumulate ^{3}H-GABA also project to extracerebellar nuclei. To test this hypothesis, ^{3}H-GABA was injected into the dentate nuclei in four cats. As a control, ^{3}H-GABA was also injected in the cerebellar cortex, since it is well known that all cortical neurons except granule cells accumulate ^{3}H-GABA to varying degrees. The results obtained from these four animals suggested that if neurons in the cerebellar cortext were labelled with ^{3}H-GABA, thereby indicating a successful experiment, then labelled fibers could also be observed to course from the dentate nucleus towards the lateral folia of Crus I and dorsomedial folia of Crus II and from the ventrolateral aspect of the nucleus to the white matter of the dorsal parafloccu-lus. Because of the short survival period, these fibers could only be followed for approximately 4 mm from the injection site. In the same animals, other labelled fibers were observed leaving the dentate nucleus from its ventromedial aspect and projecting into the brachium conjunctivum. When these labelled fibers were present, dense accumulations of silver grains could also be observed overlying nissl-stained cells in the dentate nucleus, indicating that these cells had taken up the ^{3}H-GABA. These findings suggest that neurons in the cerebellar dentate nucleus, which accumulate ^{3}H-GABA, also appear to orthogradely transport labelled GABA to extracerebellar sites. The labelled fibers directed to the cerebellar cortex could have resulted from the uptake of ^{3}H-GABA by Purkinje cell axon terminals and the subsequent retrograde transport of this compound. Alternatively, the uptake of ^{3}H-GABA by dentate neurons which resulted in the labelling of axons in the brachium conjunctivum may have also orthogradely labelled fibers in the nucleocortical pathway since at least some of these fibers are collaterals of cerebellar efferent projections. The significance of these observations as to the possible use of GABA by extracerebellar projecting neurons can only be speculative on the basis of our data. NIH contract # N54-2332 and Fellowship # NS-05577.

175 CEREBRO-CEREBELLAR MICROCIRCUITS: MICROMAPPING THE FINE-GRAINED PROJECTIONS BETWEEN TACTILE AREAS IN CEREBRAL (SI) AND CEREBELLAR (GRANULE CELL) TACTILE AREAS OF RATS. Donald H. Beermann, John M. Gibson, James M. Bower, Georgia M. Shambes and Wally I. Welker, Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706

We found highly specific patterns of connections between the somatotopically organized tactile areas in the hemispheres of cerebral and cerebellar cortex. These cerebro-cerebellar projections originate in SI and terminate in granule cell (GC) layer of cerebellar cortex.

Two independent, monopolar, ball-tip, tungsten microelectrodes were used to record multiple unit responses from homologous tactile areas in SI and GC cortex. The receptive fields (RF's) in both areas were defined by threshold mechanical stimulation of cutaneous tissues. In-depth microstimulation (single, biphasic pulses; 5-10µA, 5/sec.) through the SI electrode evoked responses in the GC cortex. Two sampling strategies were employed for micromapping the cerebro-cerebellar projections. (1) The SI electrode remained stationary after the approximate best activating depth was located; then the cerebellar electrode was used to map the areal extent of the GC cortex which was activated from the stimulating SI locus. In each successive cerebellar puncture the peripheral RF was also determined. These strategies yielded recording maps. (2) In other experiments the SI stimulating electrode was used as the mapping electrode while the cerebellar recording electrode remained stationary. These strategies yielded stimulating maps. In this case RF's were defined for each puncture in SI cortex before it was stimulated electrically. Sampling densities of up to 75 punctures/mm^2 were required to fully delineate the fine-grained spatial pattern of these projections. The borders and areal extents of the interconnected focal areas were defined by these two mapping strategies.

Major results are: (1) the SI sources and the GC destinations of the cerebro-cerebellar circuit are small, less than 1mm^2; (2) the interconnected SI and GC foci were located within portions of the two tactile areas which have the same peripheral RF's (e.g., SI upper lip focus projected to GC upper lip patch); (3) each SI source projects only within the confines of a homotypical GC columnar patch in the mosaic of the cerebellar tactile area defined by Shambes et al. (Neuroscience Abstr., 2: 117, 1976); (4) the foci of optimal responsiveness in GC columns to SI and peripheral stimulation were congruent; (5) however, the SI source is located just below layer IV (cells in layer IV respond best to peripheral RF stimulation); (6) minimal latencies of SI-GC evoked activity were 3-4 msec. (Supported by NSF grants BNS #'s 75-08124 and 77-16230 and by USPHS grants NS06225 and NS07026).

176 THE ORGANIZATION OF DESCENDING PROJECTIONS FROM THE BRAIN STEM ACTIVATED BY THE OUTPUT OF THE DENTATE NUCLEUS. J.R. Bloedel, E. G. Hames*, H. Bantli, and J.F. Rowlands*. Depts. Neurosurg. & Physiol., Minn. Sch. Med., Minneapolis, Minn., 55455.

Experiments were performed in unanesthetized decerebrate cats and cats anesthetized with alpha choloralse to determine: 1) the organization of descending systems from the brain stem activated by the output of the dentate nucleus and 2) the effects of these systems on segmental interactions occurring in the spinal cord. In addition to our previous demonstration of the dentato-reticulospinal system, evidence was also obtained for the existance of a dentatorubrospinal system projection. The latter was demonstrated in a series of electrophysiological studies in which the effects of various lesions on the responses evoked in the cervical spinal cord following dentate stimulation were assessed. It was shown that the response presumably evoked via the dentatorubrospinal projection could be eliminated by lesions in the lateral brachium conjunctivum as well as in the contralateral red nucleus. In addition, this response was present in decerebrate animals. The possibility that this response was evoked via spread of the stimulus current to the interposed nucleus was ruled out by showing that the response was unaffected by electrolytic lesions in this structure adjacent to the position of the stimulating electrode in the dentate nucleus. The segmental effect of these dentatoreticulospinal and dentatorubrospinal projections was found to produce marked changes on interneurons as well as on reflexes activated by cutaneous afferent fibers. In addition to demonstrating that dentate stimuli produced marked changes in the excitability of certain spinal interneurons impinged upon by low threshold cutaneous afferents, the results indicated that the reflexes evoked in motoneurons by the same afferents were also affected. These data indicate that the output of the dentate nucleus activates at least two descending projections from the brain stem to the lumbar region of the spinal cord and that the activation of these projections affects the excitability of neurons and reflexes activated by low threshold cutaneous afferent fibers. This was supported by Grant# NS 09447.

177 VISUAL INPUT TO THE PARAFLOCCULUS: AN ELECTROPHYSIOLOGICAL STUDY. Richard A. Burne and Donald J. Woodward. Dept. Cell Bio., Univ. Tx. Health Sci. Ctr., Dallas, Tx 75235

Previously we presented anatomical evidence suggesting that the paraflocculus is a cerebellar target zone for visual cortical and tectal input. The present study was undertaken with the general aim of determining the electrophysiological properties of the visual sensitive Purkinje cells within the paraflocculus of the rat cerebellum as determined by 1) electrical stimulation of regions within the visual cortex and superior colliculus, or 2) presentation of controlled optical images in the visual field of the rat.

Single unit recordings of Purkinje cell activity in halothane anesthetized rats and post-stimulus-time-histogram (PSTH) analysis were employed to determine the response characteristics following cortical and tectal stimulation with monopolar and bipolar concentric electrodes, respectively (0.1 ms, 0.1-0.6 ma, 1-10 Hz). Of 58 identified Purkinje cells in the paraflocculus, PSTHs of 49 cells (84%) showed evidence of a mixed excitatory-inhibitory mossy fiber input (42 cells, 86%), or a pure inhibitory (5, 10%) or excitatory (2, 4%) input following cortical stimulation. Following tectal stimulation, 33 cells (80%) responded to either a mixed excitatory-inhibitory mossy fiber input (24 cells, 59%), or a pure inhibitory (7, 17%) or excitatory (2, 4%) input. The mean latencies to onset of excitation were 10.3 ± 0.5 msec and 7.7 ± 0.53 msec, and to onset of inhibition, 13.7 ± 2 msec and 11.8 ± 1 msec for cortex and tectum, respectively. Sixty-eight percent of the Purkinje cells tested responded to both cortical and tectal stimulation. In addition, complex spike responses (via climbing fibers) were elicited at a latency of 22 ± 1.7 msec and 17 ± 2 msec following cortical and tectal stimulation, respectively.

Single unit recordings of an additional 7 paraflocular Purkinje cells in unanesthetized, immobilized rats showed evidence, through PSTH analysis, for mixed excitatory-inhibitory mossy and climbing fiber input following visual field stimulation. Single and complex spike responses were primarily elicited from light spots or bars projected against a dark background and moving in the nasal or temporal direction at velocities of 0.93-18 m/sec. In addition, on-off complex spike responses were elicited by 400 msec pulsed (at 1 Hz) stationary spots of light (10° dia.) when projected upon different quadrants of the visual field.

These results confirm our previous anatomical work suggesting a strong visual input to the paraflocculus, and also indicate that a significant input from visual cortex and superior colliculus converges upon paraflocular Purkinje cells. Our hypothesis is that the paraflocculus may serve as a strong link in visual sensori-motor integration. (Support by NSF BNS77-01174 to DJW).

178 ISOLATION OF OLIGODENDROCYTES FROM MOUSE CEREBELLUM USING MAGNETIC MICROSPHERES. Graham LeM. Campbell*, Oded Abramsky,* and Donald H. Silberberg, Franklin Inst. Res. Labs.and Dept. of Physiology, Temple University Med. School, Phila., Pa. and Dept. of Neurology University of Pennsylvania School of Medicine, Phila., Pa. 19104

Isolation and separation of highly enriched populations of oligodendrocytes from brain regions not enriched in white matter using density gradient techniques is inherently difficult. Techniques for cell separation based on differential binding of ligands to cell surface moieties provides an alternative methodology. The development of magnetic microspheres to which ligands may be conjugated provides a facile and effective technique for the separation of oligodendrocytes from the cerebellum of 10 day old mice.

Cerebellar cells were isolated and glial cell enriched population prepared as described previously (Campbell et al, Brain Res. 127: 68-86, 1977). Glial cell enriched population was incubated with rabbit anti-bovine oligodendrocyte anti-serum, washed and then incubated with magnetic microspheres conjugated with goat anti-rabbit IgG. The resultant mixture was washed and introduced into a magnetic field. Cells to which the microspheres adhered were retained, whereas the remaining cells passed through the magnetic field. After the magnetic field is removed, the cells with adherent microspheres were released and collected. This retained population yielded 1-2% of the total isolated cerebellar cell bodies and represents approximately $0.5x1.0x10^5$ cells/cerebellum.

Observations using SEM and TEM indicate that over 90% of the cells examined were morphologically similar to cells isolated by bulk preparative techniques. The size range is 5-8 u with a high nuclear to cytoplasmic ratio. Occasional cells (less than 5%) were seen to have myelin fragments. In addition, these cells extend processes in vitro on polylysine coated coverslips within 1 hr. incubation at 37°C. Further characterization of these cells by measuring the enzyme 2'3' cyclic nucleotide phosphohydrolase and the uptake of S^{35} into sulfatide will also be presented.

Supported by: Benner & Clainer Funds, NIH NS11037, TW02359 and National Multiple Sclerosis Society 894-B-2.

179 INCREASED SPINOVESTIBULAR PROJECTIONS AFTER NEONATAL HEMICEREBELLECTOMY IN RATS. Anthony J. Castro and Diane E. Smith, Depts. Anatomy, Loyola Univ. of Chgo., Stritch Sch. Med., Maywood, Ill., 60153 and Louisiana State Univ., Sch. Med., New Orleans, La., 70112.

In light of recent reports of aberrant cerebellar efferent projections that develop after neonatal hemicerebellectomy, this study was undertaken to examine possible changes in afferent spinal-cerebellar pathways after similar neonatal lesions. Under hypothermic anesthesia, hemicerebellectomy lesions were made by aspiration with small pipettes in 2-3 day old rat pups. Two to 12 months later these same animals under sodium pentobarbital anesthesia sustained spinal cord lesions at mid-thoracic levels on the side ipsilateral to the neonatal hemicerebellectomy. Control animals received only spinal cord lesions. Animals were sacrificed 4-10 days after spinal cord lesions, and their brains were processed using the Fink-Heimer method. Lesion sites and brainstem cytoarchitecture were examined with Nissl stains.

In control animals, spinocerebellar fibers were observed to enter the cerebellum ipsilaterally and cross within the cerebellum to project bilaterally to the fastigial nuclei as well as specific cortical areas of the anterior and posterior lobes. Control spinobulbar fibers projected to several brainstem areas. Of particular interest to this study are the ipsilateral projections to the vestibular nuclear complex.

After hemicerebellectomy, few spinal afferents were observed within the spared side of the cerebellum indicating a lack of neuroanatomical rerouting of spinocerebellar projections to the contralateral side. However, analysis of ipsilateral projections revealed an increase of spinovestibular projections to the lateral vestibular nucleus in comparison to the relatively small input found in control animals. The increase of ipsilateral projections to the lateral vestibular nucleus would appear to be a plausible alternative locus for remodelling considering the functional interrelationship between the vestibular and fastigial nuclei.
(Supported by NIH Grant NS 13230.)

180 TWO MODES OF INTEGRATION OCCURRING IN THE CEREBELLAR CORTEX. T.J. Ebner* and J.R. Bloedel. Depts. of Neurosurg. & Physiol., Univ. of Minn. Sch. of Med., Minneapolis, Minn. 55455.

Experiments were performed in decerebrate, unanesthetized cats to determine the effects of natural stimuli on the temporal correlations between the stimultaneously-recorded discharge of two to three Purkinje cells. Alterations in temporal correlation were compared to the excitability changes evoked in Purkinje cells by a natural stimulus. The pairs and triplets of Purkinje cells were located on surface folia either parallel or perpendicular to the orientation of parallel fibers. Purkinje cells were identified by the presence of spontaneous climbing fiber responses The natural stimuli consisted of stretch of the ipsilateral gastrocnemius-soleus muscle, with or without a plate in contact with the planter aspect of the hindfoot. The cross correlation between the discharge of pairs of Purkinje cells was computed during spontaneous firing and during the application of the natural stimulus. The excitability changes evoked by the stimulus were determined by constructing PST histograms. The results suggested that there are two types of integrative processes occurring in the cerebellar cortex which may be relatively independent. Stimuli, in addition to evoking the well documented modulation in Purkinje cell excitability, also resulted in changes in the cross correlation between pairs of cells. On some occasions an increase in excitability was associated with an increase in the temporal correlation. In other cases, although each cell in the pair responded to the stimulus, there was no alteration in the temporal correlation. Also, in many pairs the duration of the responses in the PSTH were different than the time course of the changes observed in the cross correlation. Furthermore, in certain pairs of cells the addition of the cutaneous stimulus during muscle stretch produced marked alteration in the structure of the cross correlation, but only minor alterations in the PSTH. These findings imply that peripheral inputs may result not only in excitability changes, but also in changes in the temporal correlation between Purkinje cell discharge, and that these two processes may be independent. This research was supported by Grant # NS 09447. Timothy Ebner is a Life Insurance Medical Scientist Scholar.

181 THE PONTO-CEREBELLAR PROJECTION IN THE RAT: DIFFERENTIAL PROJECTION TO SUBDIVISIONS OF THE UVULA. Leonard M. Eisenman. Dept. Anat., Thomas Jefferson Univ., Philadelphia, PA 19107.

Microinjections of 5 to 10% solutions of horseradish peroxidase (HRP) were made in different subdivisions of the uvula cerebellar vermal cortex (lobule IX of Larsell, '52). These injections resulted in retrograde labeling of cells located in the pontine nuclei, in addition to cells in other brainstem nuclei e.g. the vestibular nuclei and inferior olive. Those experiments in which the injected HRP labeled only sublobules IX, a, b or c of the uvula, revealed that two columns of cells in the candal half of the pontine nuclei project to this part of the cerebellar cortex. The most ventral lobule of the uvula (IX c) receives a projection from both pontine columns, one medial and one ventrolateral. The intermediate uvular lobule, IX b receives a projection from similarly placed columns but there is evidence that the columns are more extensive in dorsoventral extent. Finally the most dorsal of the uvular sublobules, IX a, appears to receive a different input from only one pontine columns of cells. This column is in the lateral portion of the pontine nuclei but more dorsal to the column projecting to sublobules IX b and c.

These differential projections from the pontine to subdivisions of the uvula, especially sublobule IX a as compared to sublobules b and c suggest that there may be functional differences between these parts of the uvular portion of the vermis. Although injections were placed in medial and lateral parts of the lobules no clear distinction in labeled pontine cells is evident which may suggest a sagittal organization in this projection system.

182 LENGTH OF PARALLEL FIBERS IN CAT CEREBELLAR CORTEX: STEREOLOGICAL DETERMINATION. V.L.Friedrich,Jr. Department of Biobehavioral Sci., Univ. Connecticut, Storrs, CT. 06268.

Parallel fiber degeneration extends for lengths of 5 to 7 mm from knife cut lesions of cerebellar cortex (Brand et al., Exp. Brain Res. 26:39). This finding is in conflict with previous estimates of parallel fiber length based on other methods. I here report a preliminary estimate of the length of parallel fibers, based on a stereological analysis of the molecular layer. The result is consistent with the degeneration data.

In sections transverse to folia (and thus to the parallel fibers), the expected areal density of parallel fiber profiles in the molecular layer (N_A) is related to the average length of parallel fibers (L) and the number of parallel fibers per unit volume of molecular layer (N_V^{pf}) by $N_A=LN_V^{pf}$. Since each parallel fiber is associated with one granule cell, $N_V^{pf}=N_V^{gc}V^{gl}/V^{ml}$, N_V^{gc} the density of granule cells in the granular layer and V^{gl} and V^{ml} the volumes of granular and molecular layers, respectively. Thus, $L=(N_A/N_V^{gc})\ (V^{ml}/V^{gl})$.

Parasagittal slices of vermis used previously to determine N_V^{gc} and V^{ml}/V^{gl} (Anat. Rec. 190:398) were trimmed to well fixed regions and sectioned for electron microscopy. The fields photographed were selected by a calculator programmed to generate random stage coordinates positioned within the molecular layer; this procedure eliminated operator bias in the selection of fields.

The fractional area of parallel fibers in the micrographs (A_A) was determined by point hit counting, the average area per profile (a) by planimetry and the areal density by $N_A=A_A/a$; the results were $A_A=0.44$, $a=0.07\ um^2$, $N_A=6.3\ um^{-2}$ and L=5.4 mm.

My preliminary estimate of 5.4 mm , which represents the average length of parallel fibers, is in good agreement with the maximal length of 5 to 7 mm as estimated by the degeneration method. This agreement is compelling evidence for the validity of both values. Evidently, previous estimates in the range of 2 mm are in error. The lateral spread of parallel fibers and their maximal conduction time are thus greater than thought previously.

(Supported by NIH Grants 1F32 NS05533 and NS09904.)

183 PURKINJE CELL ACTIVITIES DURING THALAMOCORTICAL EPILEPTOGENESIS (PENICILLIN FOCUS;CAT). T.L.Frigyesi, W.B.Jarzembski* and J.B. Lombardini*. Depts. Physiology, Biomedical Engineering, and Pharmacology, Texas Tech U. Sch. Med., Lubbock, Texas 79409.

Motor cortical penicillin epilepsy is, for the most part, the function of thalamic multisynaptic machinery that is also governed by cerebellar activities. Hence, the behavior of Purkinje cells is germaine to thalamocortical epileptogenesis. An acute penicillin focus was established in the motor cortex of 20 encèphalé isolé cats; simultaneous recordings were obtained from the motor cortical surface, and unit and focal potentials from the thalamic ventrolateral nucleus (VL) ipsilaterally, and from cerebellar Purkinje cells, contralaterally. In all animals, interictal spike relations at these three recording sites were 1:1:1 (save for the initial 10-15 minutes when Purkinje cells failed to fire). In 12/20 cats, the VL potentials preceeded and the Purkinje potentials followed the cortical interictal spikes. This sequential order of interictal spikes could be modified by 1) i.v. taurine: an additional focal negativity developed in the Purkinje layer, the onset of which was either coincident with or preceeding the onset of the VL interictal spikes; i.v. DPH abolished the early Purkinje firing and restored the sequential firing order of the control; i.v. barbiturate abolished the focal potentials in the Purkinje layer but failed to alter the control time relation of VL-motor cortical firing; 2) following severance of the climbing fibers, Purkinje focal negativities occurred later; 3) following severance of the ipsilateral brachium conjunctivum, the Purkinje focal negativities occurred prior to the cortical interictal spikes. Purkinje unit discharges were as frequently observed before or concomitant to as following the thalamocortical interictal complexes. High doses of DPH i.v. induced prolonged Purkinje unit discharges with coincident abolition of all thalamocortical epileptic activities. However, topical DPH onto the neocerebellum induced sustained Purkinje discharges with exacerbation of thalamocortical interictal and ictal activities. Topical DPH onto the motor cortex attenuated the cortical and abolished the Purkinje interictal spikes. During fully developed icti, Purkinje cell activities were enhanced; however, in 6 animals, Purkinje activities were suppressed during the tonic phases. Purkinje burst activities were only occasionally encountered at the termination of thalamocortical bursts. Topical DPH onto the neocerebellum increased Purkinje cell activities with resultant increases in thalamocortical seizures. These and other data indicate that Purkinje cells are momentously involved in thalamocortical penicillin epileptogenesis. However, the cerebellar suppression of thalamocortical paroxysms involves mechanisms other than the Purkinje cell induced disfacilitation of thalamocortical projection activities.

184 EVIDENCE FOR A NEW SPINOCEREBELLAR PROJECTION FROM THE NUCLEUS DORSALIS (CLARKE'S COLUMN) IN THE CAT. E.G. Hames*, D.L. Tolbert, J.R. Bloedel. Depts. of Neurosurg. & Physiology, Univ. of Minn. Med. Sch., Minneapolis, Minn. 55455.

The origin of spinocerebellar pathways from the nucleus dorsalis in spinal segments T-1 through L-4 was examined following injections of horseradish peroxidase (HRP) into the anterior lobe. A series of experiments were performed to determine if a spinocerebellar projection in addition to the dorsal spinocerebellar tract (DSCT) originates from this nucleus. Unilateral injections of HRP into the anterior lobe in a cat with bilateral lesions of the superior cerebellar peduncles confirmed the projection of DSCT as ipsilateral to the cerebellum and as principally originating from the nucleus dorsalis, at least at these spinal segments. These experiments also defined the region of the nucleus dorsalis at these spinal segments as an area whose cell bodies are primarily located immediately dorsal and lateral to the central canal. In another set of experiments in which unilateral anterior lobe injections were made in animals with a lesion of the ipsilateral inferior cerebellar peduncle, the labelled neurons were distributed bilaterally in the nucleus dorsalis. The number of neurons on each side of the cord appeared qualitatively similar. This finding suggests that a projection other than the DSCT originates in the nucleus dorsalis. To examine the spinal course of this spinocerebellar projection from the nucleus dorsalis through the superior cerebellar peduncle, a unilateral anterior lobe injection in an animal with an ipsilateral lesion including the dorsal column and the entire lateral funiculus was performed at T-9. This lesion should eliminate the labelling of DSCT neurons ipsilaterally. Again, a qualitatively similar distribution of labelled cells was found bilaterally in the nucleus dorsalis. After a bilateral injection of the anterior cerebellar lobe following a lesion of the dorsolateral fasciculus at T-9, labelled neurons in the contralteral nucleus dorsalis resulted from uptake both by ipsilateral DSCT terminals as well as by the terminals of bilateral projections into the cerebellum via the superior peduncles. The nucleus dorsalis located ipsilaterally below the lesion contained only HRP positive neurons retrogradely labelled through the bilateral projection coursing through the superior cerebellar peduncles. This evidence suggests that spinocerebellar projections other than the DSCT originate in the nucleus dorsalis and project bilaterally to the cerebellar anterior lobe through the superior cerebellar penduncles. This research was supported by NIH Grant # NS 09447. Dr. Tolbert was supported by NIH Fellowship # NS 05577.

185 THE TOPOGRAPHICAL ORGANIZATION OF THE CEREBELLO-OLIVARY PROJECTION IN THE RAT. Alan J. Haroian, Leo C. Massopust, Paul A. Young, and Michael G. Murphy. Depts. of Anat. and Surg., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Topographical projections of the deep cerebellar nuclei to the inferior olivary nucleus have been well documented in the monkey, cat, and opossum. Faull and Carman (1978) observed cerebello-olivary fibers in the descending limb of the brachium conjunctivum after ablating the superior cerebellar peduncle in the rat. Chan-Palay (1977) has shown autoradiographically that the rat dentate nucleus projected primarily to the contralateral principal nucleus. At the level of the olive, some fibers recrossed the midline to terminate ipsilaterally. The purpose of the present investigation was to determine the cerebello-olivary projection from each of the deep nuclei in the rat.

Following injections of 3H-leucine in the deep cerebellar nuclei, the rostral two-thirds of the contralateral inferior olivary complex was heavily labelled and the ipsilateral olive lightly labelled. The nucleus interpositus anterior (NIA) projected primarily to the rostral three-quarters of the dorsal accessory olive (DAO), the nucleus interpositus posterior (NIP) to the rostral two-thirds of the medial accessory olive (MAO), and the dentate nucleus (DN) to both the dorsal and ventral lamellae of the principal olive (PO). No fastigial projections to the inferior olive were observed. Preliminary results from micro-injections of 3H-leucine into parts of the individual nuclei revealed that a much more complex topographical organization exists. The central part of the middle third of the NIA projected to a similar zone in the DAO, the medial part of the caudal NIP to the lateral part of the middle third of the MAO, and the dorsal part of the middle third of the DN to the lateral half of the dorsal lamella of the PO. The basic mammalian pattern reported for the monkey, cat, and opossum was also observed in the rat; however, the topographical organization from parts of the individual cerebellar nuclei to their respective counterparts in the olive differs from species to species.

(Supported in part by USPHS grant FR 05388.)

186 FETAL ALCOHOL INTOXICATION AND CEREBELLAR DEVELOPMENT. S.E. Kornguth, E. Sunderland*, U. Juhl*, F. Siegel, and I. Carlson*. Wis. Univ. Med. Ctr., Madison, WI 53706.

Albino rats were placed on a liquid diet (Ensure) containing either 9% or 5% ethanol during the second to twentieth day of pregnancy. Each animal was matched with a pair fed control. The control diet contained an amount of sucrose equivalent calorically to the ethanol ingested by the experimental animal of the pair. The offspring of these animals were sacrificed on days 7, 11, 14, and 21 after birth and the following parameters analyzed: body weight; brain weight; cerebellar weight; cerebral and cerebellar activities of ornithine decarboxylase (ODC), aspartyl amino transferase (AAT), and malic dehydrogenase (MDH); sera levels of thyroxine (T_4); histological development of the cerebellum revealed by the Golgi silver and Nissl stains.

The 5% rats drank the maximal amount of food presented (100 ml per day of a 70% Ensure diet). In some cases the amount of alcohol ingested by rats on the 5% diet was equivalent to that ingested by animals on the 9% diet since the latter group had decreased total food intake (40-50 ml per day). The pups of mothers that ingested more alcohol during pregnancy (greater than 6 ml absolute ethanol per day) were significantly smaller by day 14 after birth than pair fed controls or pups of mothers that drank less than 4 ml alcohol per day. The cerebellar ornithine decarboxylase activities of pups from mothers on the 5% ethanol diet were elevated even at day 14 (1.3 picomoles CO_2/min/mg protein) compared with pair fed controls (0.3 pM/min/mg protein). Pups from the 9% experiment and control groups had slightly elevated ODC activities at day 14 which may reflect the reduced total food intake in this group. The T_4 levels at 11 days postnatum of pups exposed to the 9% ethanol were reduced (2 µg/dl) compared with their pair fed controls (4 µg/dl) or with the 5% group (3-5 µg/dl). The cerebellar AAT and MDH activities of pups exposed to ethanol were similar to their pair fed controls at each stage of development studied. Histologically, the development of cerebellums was impeded at day 7 postnatum in pups exposed to either 5 or 9% alcohol. The Purkinje cells of pair fed controls were in a single lamina at this time whereas those exposed to alcohol were still clustered. The experimental cerebellums also had a decreased molecular layer width compared with controls. These data indicate that the exposure of rats during embryonic and fetal life to alcohol, affects both cerebellar development and serumthyroxine levels. Alcohol may impede cerebellar development either directly or indirectly; in the latter case hypothyroidism may be one cause.

187 SODIUM AND CHLORIDE CHANGES DURING SPREADING DEPRESSION AND ANOXIA IN RAT CEREBELLUM. R.P. Kraig, C. Nicholson & J.M. Phillips*. Dept. Physiol. & Biophys., N.Y. Univ. Med. Ctr., 550 First Avenue, New York, NY 10016

Spreading depression (SD) and anoxia are two related phenomena. In the teleost fish cerebellum large decreases in $[Na^+]_0$ and $[Cl^-]_0$ are observed during SD (Kraig and Nicholson, Neuroscience, in press). We have now examined changes in these ions in a mammalian preparation, the rat cerebellum, and also compared them with anoxic changes.

Rats were anesthetized with urethane and the exposed cerebellum was superfused with Ringer solution. By using hypotonic Ringer solution (100m Osm.; $[Na^+]$: 46mM, $[Cl^-]$: 30mM, other constituents as Feldberg and Fleischhauer, J. Physiol. 150: 451, 1960) SD could be readily induced with 20 Hz local surface stimulation. Anoxia was induced with N_2 breathing under normal Ringer superfusion. $[Na^+]_0$ was measured with theta-micropipettes containing monensin-based ion-exchanger (Kraig and Nicholson, Science 194: 725, 1976) or neutral carrier ligand (Güggi et al, Helv. Chim. Acta 59: 2417, 1976). $[Cl^-]_0$ was measured with micropipettes containing Corning exchanger 477315. $[K^+]_0$ and $[Ca^{2+}]_0$ were also monitored for comparison.

The reduced NaCl Ringer lowered ambient $[NaCl]_0$ cerebellar molecular layer to between 50 and 70 mM below normal baseline values. When SD occurred, a further, transient, fall in both $[Na^+]_0$ and $[Cl^-]_0$ took place to levels approximately 1/3 of those in the cerebellum before superfusion with hypotonic Ringer. During anoxia, with normal Ringer superfusion, both $[Na^+]_0$ and $[Cl^-]_0$ fell to levels similar to those seen at the peak of SD, but no recovery took place during the anoxic period. $[K^+]_0$ and $[Ca^{2+}]_0$ changes during both SD and anoxia resembled those reported previously (Nicholson et al, Proc. Natl. Acad. Sci. USA, 74: 1287, 1977).

The falls in $[Na^+]_0$ and $[Cl^-]_0$ during SD and anoxia in the rat cerebellum are similar, in both magnitude and proportion of normal baseline values, to those seen during SD in the fish (Kraig and Nicholson, Neuroscience, in press). These results show that $[Na^+]_0$ and $[Cl^-]_0$ fall to relatively invariant levels during SD and anoxia in diverse species and under different initial NaCl concentrations. This suggests that these ionic changes are a manifestation of a fundamental ionic mechanism of the brain. (Supported by Public Health Service, Grant NS-13742).

188 DENDRITIC CALCIUM SPIKING IN MAMMALIAN PURKINJE CELLS: IN VITRO STUDY OF ITS FUNCTION AND DEVELOPMENT. R. Llinás and M. Sugimori*, Dept. Physiology & Biophysics, New York Univ. Med. Ctr., 550 First Ave., New York 10016.

Intracellular recordings from soma and dendrites of rat Purkinje cells were obtained from cerebellar slices kept in vitro by perfusing with an oxygenated Krebs solution. Sodium conductance blockage by TTX (10^{-5}) demonstrated calcium action potentials followed by prolonged potassium-dependent after-hyperpolarizations. It was shown that this calcium-potassium system is capable of changing the effective time constant of the cell and of producing long-lasting modulation of membrane potential. Intradendritic recording, under direct vision, further indicated that this voltage-dependent conductance change is especially prominent at the mid-dendritic level. Under normal conditions, in vitro Purkinje cells fire repetitively in a spontaneous manner, quite similarly to the burster neurons in invertebrates. This bursting property is underlayed by slow modulation of the membrane potential which remains unchanged following sodium conductance blockage. In the absence of calcium current (after blockage by 1 mM cadmium), Purkinje cells ceased to show rhythmic bursting. If cadmium is applied without blockage of the sodium current, high frequency sodium-dependent spikes (up to 1500/sec) may be observed during direct depolarization, indicating that in this cell sodium conductance behaves mainly as a booster, membrane excitability being apparently governed by the calcium-potassium system. A second set of in vitro experiments in newborn rats has shown that prior to the development of the main dendritic tree (5th to 6th days after birth) only sodium-dependent spikes can be obtained. At the 5th or 6th day after birth, development of the main dendritic tree begins, accompanied by climbing fiber activation and by the presence of vigorous calcium spikes. It is proposed that calcium is necessary for normal integration and for the growth of Purkinje cells. (Supported by USPHS grant NS-13742 from NINCDS)

189 BIOCHEMICAL AND MORPHOLOGICAL CHANGES IN THE MOUSE CEREBELLUM FOLLOWING NEONATAL ADMINISTRATION OF METHYLAZOXYMETHANOL ACETATE. K. L. Lovell and M. Z. Jones, Dept. Pathology, Mich. State Univ., E. Lansing, MI 48824.

Methylazoxymethanol acetate (MAM), when injected subcutaneously into neonatal mice, destroys dividing neurons of the external differentiating cell layer resulting in a deficit of cerebellar interneurons and aberrant development of the remaining cells. To assess the effects of MAM-induced cell depletion and disruption of cytoarchitecture on the norepinephrine (NE) and gamma-aminobutyric acid (GABA) transmitter systems, biochemical assays of cerebellar NE concentration and glutamic acid decarboxylase (GAD) activity were conducted at several ages. In these experiments 0.045 µl MAM/gm body weight, or an equivalent amount of saline solution, was injected into mice within 24 hr of birth. As in previous studies, animals that received MAM showed decreases in body and cerebellar weights, delayed development of the external differentiating cell layer, depletion of interneurons (granule, basket and stellate cells), and misalignment of Purkinje cells. NE was measured fluorometrically and GAD activity was assayed by the $^{14}CO_2$ trapping method (Kanazawa, I., et al., J. Neurochem., 27: 1267, 1976). The amount of NE per gram of tissue was increased in MAM-injected animals, while the amount per cerebellum was not significantly different from control animals. This suggests that the total number of NE terminals (present in the cerebellum before birth) is unchanged by MAM treatment. The GAD activity per gram of tissue was higher in MAM-injected animals compared to saline-injected controls, while the total cerebellar GAD activity was decreased. A loss of some GABA containing neurons (mainly basket and stellate cells) could explain the decrease in activity per cerebellum while an increased density of remaining GABA-containing cells (e.g. Purkinje cells or neuroglia) in a smaller cerebellum might account for the increase in GAD activity per gram of tissue. Thus these studies provide correlations between aberrant cerebellar development and neurotransmitter biochemistry.

This research was supported by a NIH postdoctoral fellowship to K.L.L.

190 DEVELOPMENT OF THE INFERIOR OLIVARY NUCLEUS IN THE AMERICAN OPOSSUM: A LIGHT AND ELECTRON MICROSCOPIC STUDY. Bruce E. Maley* and James S. King, Department of Anatomy, The Ohio State University, Columbus, Ohio, 43210.

In order to study the development of the inferior olivary nucleus (ION) a series of young opossums at different snout-rump (SR) lengths were removed from their mothers' pouches and processed for light and electron microscopic analysis. The ION is not present as a discernable nuclear group at birth (12-13 days post conception), but it is apparent by the 4th-5th day (23mm) in the pouch. At this SR length the ION is a homogenous mass of cells with no apparent nuclear subdivisions. Separate principal and accessory nuclei cannot be identified until the animals attain a SR length of approximately 50mm. During this period of time (SR 23mm to SR 50mm) the ION increases in length from 650µm to 920µm. Analysis of Nissl and Golgi preparations reveals that olivary neurons are 5-10µm in diameter and exhibit relatively short dendrites with infrequent sessile spines. By the 50-55mm stage comparable neurons have increased in size to 10-15µm with varicose dendrites which have spines and spiny appendages. Often the tips of these dendrites end in a moniliform appearance. It is not until animals attain a length of over 90mm that the neurons resemble those seen in the adult (Bowman and King, 1973, J.C.N., 148: 491).

Examination with the light as well as the electron microscope discloses, at the 23mm stage, that the neurons within the developing ION are densely packed with scant neuropil. At this SR length all neurites are unmyelinated with only an occasional synaptic contact observed. Synaptic vesicles encountered were either of the clear, spherical type or the large granular variety. By the 50-55mm SR length the olivary neurons are surrounded by more neuropil which also includes profiles described as growth cones (Skoff and Hamburger, 1974, J.C.N., 153: 107), as well as processes that could be the ultrastructural correlates of varicose dendrites. There is an apparent increase in synaptic vesicles within the individual neurites as well as the total number of synaptic contacts. Most synaptic contacts in both the 23mm and 50mm stages are on neurites, however on a rare occasion a somatic synapse has been observed. In the 50mm stage, the synaptic vesicles are either clear, spherical or large, granular. Neuroglia are present in both the 23mm and 50mm stage, but no glial enclosed synaptic clusters, as seen in the adult ION, are present (King, 1976, J.C.N., 165: 387).

(Supported by N.I.H. Grants NS-08798 and NS-07410.)

191 PURKINJE CELL ACTIVITY IN THE INTERMEDIATE CEREBELLAR CORTEX DURING A VARIABLE BALLISTIC MOTOR TASK. James McElligott, Eric Hansen*, and Robert Kester*, Dept. of Pharmacology, Temple University School of Medicine, Philadelphia, Pa. 19140.

The intermediate area of the cerebellar cortex has been considered to regulate the details of on-going movements in contrast with the lateral area which is involved with preprogramming a movement (Allan & Tsukhara, 1974). Previous work (Thach,1968,1970) showed that intermediate zone Purkinje cells modulate their firing patterns during discrete phases of a stereotyped wrist movement. The object of this study was to investigate Purkinje cell activity in the intermediate cerebellar cortex during a variable ballistic motor task and to determine if alterations in the motor task are reflected by changes in Purkinje cell firing patterns. We were also interested in observing the types of sensory input that influence these cells.

Four female cats were prepared for chronic extracellular single unit recording from the cerebellar cortex. They were habituated to a head restraining apparatus and trained to make a ballistic forelimb response. This response consisted of lifting the forelimb from a predetermined position and rapidly extending it to cover a spot of light presented at various positions on a large screen oscilloscope mounted in front of the animal.

Eighty percent of the recorded Purkinje cells (n=60) modulated their activity before as well as after the initiation of the forelimb response. Increases (54%), decreases (26%) and biphasic (20%) responses were observed. The modulation of the firing pattern for an individual cell was qualitatively similar for each trajectory. However, a variation in trajectory was generally accompanied by a change in latency, duration or firing rate modulation. For adjacent locations of the target light, the changes in the neuronal response appeared to be on a continuum. In no instance was there an abrupt change, e.g., from strong excitation to no excitation or to inhibition.

A number of the Purkinje cells (30%) related to the forelimb response also responded to a tone which initiated each trial. This response was usually a short latency (<30 msec) excitation of brief duration (<50 msec) that occurred independently of the forelimb response. No cells were found that responded to the presentation of the target light.

These results provide evidence that Purkinje cells in the intermediate cerebellar cortex can regulate and reflect details of specific motor responses. The short latency response to auditory input may prepare the motor system for action and may be similar to that observed by Mortimer (1975) during an acoustically elicited startle reflex.

(Support by PHS #NS 10488 and McLaughlin Fdn. Fellowship to E.H.)

192 CEREBELLAR STIMULATION IN MAN; CHANGES IN JOINT COMPLIANCE AND MUSCLE COACTIVATION. Barbara Miller*, Richard D. Penn, Gerald Gottlieb, Gyan C. Agarwal* (SPON: R. D. Penn). Dept. Neurosurg., Rush Medical College, Chicago, IL 60612.

Chronic electrical stimulation of the superior surface of the cerebellum is now being used to alleviate the motor problems of cerebral palsy patients. This operation provides, for the first time, an opportunity to study the physiological changes that occur due to cerebellar stimulation in man. To quantitate the motor effects, tests of ankle joint compliance and stretch reflexes of the leg were performed on 10 cerebral palsy patients with and without stimulation. The seated subjects have one foot strapped to a footplate which rotates about a horizontal axis through the medial malleolus. Surface EMG electrodes are placed over the anterior tibial (AT) and gastrocnemius-soleus (GS) muscles. The footplate is rotated by a d.c. torque motor. A computer generates the motor-drive voltage and digitizes the data. The applied torque, angular rotation, and GS and AT EMGs are stored on digital magnetic tape. Two separate series of experiments are conducted. First, the computer directs a series of sinusoidal oscillations to the foot. Recordings are made for 10 seconds with frequencies of oscillation from 3 to 30 Hz. The joint compliance is determined from the angular rotation and the constant torque level at each drive frequency. Second, the myotatic (stretch) reflex is evoked by sudden step-like dorsiflexions and plantarflexions of the ankle given in a random series directed by the computer. In both studies the magnitude and latency of EMG activity is measured with respect to the onset of stretch. Simultaneous increases in AT and GS activity represent coactivation of agonist and antagonist muscles.

In those cerebral palsy patients who are clinically diagnosed as spastic, sinusoidal oscillation of the ankle reveals a low ankle compliance (which is characteristic of a "stiff" joint) and coactivation of GS and AT muscles during the dorsiflexion phase of the cycle. Quick stretch of the ankle results in simultaneous responses of AT and GS at the latency of the stretch reflex, regardless of the direction of stretch; this activity is never seen in normal subjects. Some patients who received regular cerebellar stimulation showed increases in joint compliance and reduction in muscle coactivation, but the degree and time course of change varied enormously. At one extreme a 50% increase in compliance was found within 5 minutes of stimulation, at the other extreme no significant change was found after 6 months. The prestimulation tests suggest that these cerebral palsy patients have abnormally active stretch reflexes as well as deranged spinal connections which result in coactivation. Stimulation decreases these abnormalities in some cases.

193 PROXIMAL LIMB MOVEMENTS ELICITED BY MICROSTIMULATION OF CEREBELLAR NUCLEI IN MONKEY. E.B. Montgomery*, W. Schultz*, R. Marini*, and G.I. Allen. Lab. Neurobiology, Dept. Physiol., State Univ. of New York, Buffalo, New York 14214.

The dentate and interpositus nuclei of the primate cerebellum have been assumed to play an important role in the initiation and control of skilled movements. Recently, it has been observed that the lateral cerebellum exerts a direct descending influence upon the spinal cord and this may mediate a postural mechanism. In order to clarify this problem, movements elicited by microstimulation of dentate and interpositus nuclei were studied in nine adult monkeys (Cebus apella) under light thiopental anesthesia. Microstimulation of dentate and interpositus nuclei and the areas of efferent fibers anterior to them elicited a response consisting of a combination of the following five movements regardless of the site of stimulation: flexion of the elbow, and elevation of the shoulder in the forelimb. and hip flexion, knee flexion and dorsiflexion of the ankle in the hindlimb. Thresholds were as low as 5 μA. No movement was accepted where threshold exceeded 100 μA. A response consisting of at least one forelimb and one hindlimb flexion with current up to 100 μA was elicited from 42% of all effective stimulus sites. Four out of these five movements could be elicited by stimulus strengths of 100 μA at 22% of all effective stimulus sites. Movements of the fingers or toes were seen only at 7 of 281 sites. Ipsilateral facial movements were seen at 25% of effective stimulus sites. These stereotyped proximal flexion movements were not dependent on the motor cortex or red nucleus as demonstrated by continued response to stimuli following ablation of the contralateral motor cortex or intercollicular brain stem transection. These responses were eliminated only with transection of the brachium conjunctivum caudal to its decussation. The stereotyped forelimb and hindlimb proximal flexion without apparent somatotopy suggests activation of descending postural mechanisms independent of structures rostral to and including the red nucleus. This is not inconsistent with a role of the lateral cerebellum in skilled distal movements via motor cortex. The dentate and interpositus nuclei may affect the posture of the proximal musculature in preparation for skilled distal movement which is then superimposed.

194 A FORMAL THEORY FOR CEREBELLAR FUNCTION: THE PREDICTIVE DISTRIBUTED PROPERTY OF THE CORTICO-NUCLEAR CEREBELLAR SYSTEM AS DESCRIBED BY TENSOR NETWORK THEORY AND COMPUTER SIMULATION. A. Pellionisz and R. Llinás, Dept. Physiology & Biophysics, New York Univ. Med. Ctr., 550 First Ave., New York 10016.

It is difficult to describe in abstract terms the properties of a neuronal network in which inputs are distributed over a large cortical area, carrying information in a parallel manner. Generally these networks are treated as "loops" of a few individual neurons, or the system is simply deemed redundant. This spatially distributed parallel organization, being characteristic for the brain in general and for the cerebellum in particular, has compelled us to devise a new set of premises for the analysis of such systems. Using computer simulation methods, two features of a new theory of cerebellar function (Pellionisz and Llinás, *Neuroscience*: in press) will be demonstrated. At the single cell level the theory identifies the function of individual Purkinje cells as taking different order time-derivatives of the parallel fiber input. *The first tenet of the theory holds that the activity of individual cells over a cortical area may be represented by a spatially distributed, finite, series expansion of a time-function, which is then reconstructed on a set of nuclear neurons.* For the cerebellum, given a proper ratio of Purkinje cells taking 0,1,2,3.... order derivatives, these cellular activities can be treated as a Taylor expansion such that the reconstruction of the time function on the nuclear neurons can provide a running tally which predicts (by extrapolation from the past neuronal activities) a future value at a delta look-ahead time. While it is intuitively obvious that prediction is essential for coordination of time functions, our theory embeds the above assumption into a second tenet in order to formulate a general theory for the cerebellar coordination. *Neuronal networks are considered as tensors (in full generality), each identifying a scalar valued function of a number of vector variables which is linear in each variable.* Rather than conceptually oversimplifying neuronal networks into "loops" or "reflexes", we regard these entities as tensors; thus the function of the cerebellum may be explained by the available tensor theory. For example, the Purkinje cell network becomes a tensor of rank two, which assigns to a given mossy fiber vector (curve-point in m-space at t) a Purkinje cell vector (curve-point in n-space at time t + delta) by virtue of the connectivity matrix. The application of these principles will be demonstrated by computer simulation. In addition to providing a theory for cerebellar coordination, the model demonstrates the principle of distributed organization of a system which is, by its nature, quite impervious to diffuse lesions. (Supported by USPHS grant NS-13742 from NINCDS)

195 LOW THRESHOLD CLIMBING FIBER PROJECTIONS FROM FORELIMB TO CEREBELLAR CORTEX IN THE CAT. D. S. Rushmer, L. T. Robertson, K. D. Laxer*, M. H. Woollacott. Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, Oregon.

The olivocerebellar projection has been shown by a number of electrophysiological and anatomical investigations to have a parasagittal orientation. Previous work reported by this group described a parasagittally oriented strip of Purkinje cells receiving climbing fiber projections in lobules Vb and Vc of the cerebellar intermediate cortex that were highly responsive to small displacements of the forepaw. The present work elaborates on these findings and describes a second more laterally located climbing fiber strip that is responsive to natural stimulation of receptors in the dorsal forepaw and forearm. Extracellular single unit responses of Purkinje cells to natural stimulation of the forelimb were recorded in cats anesthetized with pentobarbital. Peristimulus histogram techniques were utilized to compute characteristics of climbing fiber responses (CFRs) and simple spikes. The stereotaxic location of all units was recorded and the final electrode penetration was verified histologically.

The center of the medial strip was located 4 mm from the midline, was 700-900 μm wide and extended rostrocaudally 13 mm. The CFRs in this strip were highly responsive to small displacements of the forepaw or low threshold stimulation of the paw pads. Lateral to the medial strip was a 2 mm wide unresponsive zone in which the spontaneous CFRs were identified, but were not correlated with any form of natural stimulation of the ipsilateral or contralateral fore- or hindlimbs. Beyond the unresponsive zone was a 1.5 mm wide lateral strip that was sensitive to natural stimulation of the dorsal surface of the forepaw and forearm. The rostrocaudal extent of this strip appeared to be approximately the same as the medial strip.

Many of the CFRs within the lateral strip could be elicited by pulsatile forces as small as 10 gm, had an average latency of 18 msec, and followed stimulus frequencies of 10/sec. The lateral strip was topographically organized. The more medial portion was responsive to forces applied the dorsal surface of the forepaw between the wrist joint and the distal phalanx. Cells in the lateral aspect of the strip were responsive to stimulation of the dorsum of the forearm between the elbow and wrist joints.

196 TOPOGRAPHY OF DESCENDING PROJECTIONS TO THE INFERIOR OLIVE FROM THE PARAFASCICULAR REGION, MESENCEPHALON AND SUPERIOR COLLICULUS IN THE CAT. J.A. Saint-Cyr and J. Courville. Centre de Recherche en Sciences Neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec.

Experiments on 20 cats utilizing the retrograde transport of horseradish peroxidase (HRP) revealed the sources of descending projections to the inferior olive from the upper brainstem. The cells of origin lie ventrolateral to the central gray and are continuously distributed from the vicinity of the fasciculus retroflexus to the rostral pole of the red nucleus. These cells are located in regions which include the following structures: nuclei parafascicularis and subparafascicularis, nucleus of Darkschewitsch (ND), interstitial nucleus of Cajal (INC) and parvocellular red nucleus. HRP-labeled neurons were also found in the deep layers of the superior colliculus. The topography of projections from these sites to the inferior olive were evaluated with the anterograde transport of ^{3}H-L-leucine (0.3-0.4 μl, 150-170 μCi/μl) in 12 cats. Injections in the superior colliculus resulted in a dense distribution of silver grains in the caudal part of the contralateral medial accessory olive (MAO) with a similar but lighter ipsilateral deposit. After injections in the rostral part of the continuous cell distribution revealed with the HRP method, dense ipsilateral deposits were observed in the rostral MAO and in the rostro-medial portion of the principal olive. Cases with more caudal injections which included the ND and INC showed dense label in the rostro-medial MAO and light deposits in the rostro-lateral MAO ipsilaterally. In addition, there were accumulations of silver grains in the rostro-medial part of the principal olive and in the caudal part of the dorsal lamella of the principal olive again ipsilaterally. Injections which were centered in the rostral pole of the red nucleus and adjacent INC resulted in the labeling of the dorsomedial cell column bilaterally in addition to the rostral MAO distribution indicated in the previous case. In addition, in these cases, most of the caudal MAO, the two lamellae of the principal olive, and the medial portion of the caudal third of the dorsal accessory olive were labeled ipsilaterally. Animals with injections centered in the magnocellular red nucleus and extending to the rostral pole of that structure showed partial ipsilateral labeling of the dorsal lamella. These results indicate that the upper brainstem sites of origin as well as the areas of distribution of the projections to the olive are more extensive than hitherto demonstrated and present a topographic arrangement.

Supported by a grant of the Canadian Medical Research Council Group in Neurological Sciences at the University of Montreal.

197 **TOPOGRAPHICAL ORGANIZATION OF THE CEREBELLOTHALAMOCORTICAL PATHWAY IN CAT AND DOG.** **G. B. Stanton* (SPON: D. L. Robinson). Department of Anatomy, College of Medicine, Howard University, Washington, D.C. 20059.**

Results from isotope labeling and/or anterograde degeneration studies in the cat and dog indicate that projections from localized parts of the deep cerebellar nuclei terminate in patches in the ventrolateral nucleus of the thalamus (VL) which are distributed in a gross topographical organization. Retrograde labeling experiments with peroxidase (HRP) indicate a similar gross organization of the distribution of VL thalamocortical cells. Caudal parts of the fastigial nucleus terminate in ventromedial parts of VL and overlap to some extent with the terminals from dorsal parts of the dentate nucleus. In the cat, cells in this thalamic area were labeled from HRP injections in area 6 in the medial or rostrolateral precruciate gyrus. Monopolar stimulation in both areas produced muscle contractions of the contralateral face. Projections from ventral parts of the dentate nucleus terminate in the dorsal and medial parts of VL where labeled cells were found from HRP injections in hidden parts of the medial precruciate gyrus. Dentate projections to paralaminar portions of the mediodorsal nucleus were found which coincided with the distribution of HRP-labeled cells from injections in widespread parts of the pre- or postcruciate gyrus. Projections from the interpositus posterior terminated in fewer, more closely spaced patches in mid-ventral VL. This area contained cells labeled by HRP injections at the lateral end of the cruciate sulcus. No projections to paralaminar mediodorsal nucleus were seen following an isotope injection in the interpositus posterior. Peroxidase injections in several sites in the postcruciate gyrus produced labeled cells in ventrolateral VL, but no cerebellar projections to this area have been confirmed as of this writing. Comparison of these results with recent physiological maps of the cat motor cortex suggest there is a somatotopical organization of the cerebellothalamocortical pathway in which the face, trunk, and proximal forelimb are represented in the dentate nucleus; face and trunk are represented in the fastigial nucleus; and the distal forelimb is represented in the interpositus posterior.

This investigation was supported by the General Research Support Grant #5 SO1 RR 05361 of the General Research Support Branch, National Institutes of Health.

198 RECIPROCAL MODULATORY EFFECTS OF LOCUS COERULEUS AND RAPHE ON CEREBELLUM. Jean C. Strahlendorf and Charles D. Barnes. Dept. Physiology, Texas Tech Univ. Sch. of Med., Lubbock, TX 79409.

Previous work in our laboratory revealed that discrete stimulation (1-3 shocks, 25-500 μA, 0.1-0.2 msec) of raphe nuclei (pontis, centralis superior and inferior) (R) elicit an initial bursting or entrainment within 4-20 msec, followed by prolonged inhibition lasting 500 msec, and sometimes as long as 1600 msec in cerebellar cortex and fastigial nucleus. Consonant with raphe, locus coeruleus (LC) activation (3 shocks, 25-500 μA, 0.1-0.2 msec) produced excitation with subsequent inhibition in the cerebellum. Furthermore, conditioning stimuli to either LC or R 50-300 msec preceding test shocks to the other nucleus, suppressed characteristic cerebellar responses. C-T paradigm studies suggested at least 2 possibilities: that the interaction may occur between the 2 nuclei in the brain stem region, both inhibiting the firing pattern of the other or direct interaction on cortex and fastigial N. since R and LC terminate in Purkinje, molecular and granule cell layers. The purpose of this investigation was to electrophysiologically study the first premise, an interplay of 5HT and NE neurotransmitter systems within the brain stem region. Anatomical findings have revealed that the raphe dorsalis, CS and CI project to the ventrolateral part of LC, while projections between LC and raphe have recently been demonstrated, suggestive of a reciprocal innervation.

Raphe N. and LC were initially stereotaxically identified and verified by presence of characteristic pressor responses upon stimulation, in cats anesthetized with α choralose or decerebrated at precollicular level. Raphe stimulation (1 shock, 50-500 μA, -pulse, 0.1-0.2 msec) evoked transsynaptic spike activity within 6-10 msec in the locus coeruleus. Antidromic spikes identified by fixed latency and ability to follow up to 100-300 cycles/sec were recorded in LC following raphe activation. R stimulation (1 shock, 50-500 μA, 0.1-0.2 msec) produced transsynaptic spikes in LC nuclei 3-10 msec after introduction of the stimulus. Antidromic spikes were also elicited in LC 1-1.75 msec after raphe excitation. Mean conduction velocities were calculated for both areas. In addition, LC and raphe stimulation inhibited the firing pattern of spontaneously firing cells of the other for up to 350-400 msec, followed by rebound excitation. The data electrophysiologically verify the anatomical findings for a reciprocal innervation between these two areas and provide evidence of a means in which they may modulate each other's effect on the cerebellum. (Supported in part by NIH Grant HL 7289).

199 SPINOBULBAR AND SPINOCEREBELLAR PROJECTIONS IN THE RAT. Rand S. Swenson* and Anthony J. Castro, (SPON: Charles L. Webber, Jr.) Depts. Anat., National College of Chiropractic, Lombard, Ill., 60148 and Loyola Univ. of Chgo., Stritch Sch. Med., Maywood, Ill., 60153.

The normal distribution of spinobulbar projections was determined in adult Long-Evans, black-hooded rats using the Fink-Heimer staining technique. Various lesions of the spinal cord were placed at mid-thoracic levels. Animals were sacrificed 4 to 10 days postoperatively, and the brainstem and cerebellum were examined for axonal and preterminal degeneration. The major tract degeneration was found in the dorsal columns and in the ventrolateral portions of the brainstem. The ventrolateral tract gave rise to preterminal degeneration in the lateral part of the dorsal accessory olive, the subnucleus a of the medial accessory olive, the lateral reticular nucleus (particularly its rostral portions), and the nucleus reticularis ventralis, gigantocellularis and pontis caudalis. Degenerating fibers could be traced diverging from this bundle into the restiform body from which they were primarily distributed to the cerebellum with small inputs to the lateral vestibular nucleus and the subnucleus x of the vestibular complex.

At levels immediately caudal to the chief nucleus of V many degenerating fibers were seen to leave the ventrolateral region and arc dorsalward giving off fibers to the nucleus reticularis pontis oralis. This pathway forms the ventral spinocerebellar tract and occupies a position dorsal to the brachium conjunctivum where it proceeds caudally into the cerebellum. Ventrolateral fibers were also observed to project to the pontine gray, to the deep layers of the inferior colliculus bilaterally (crossing in the commissure of the inferior colliculus), and finally, the few remaining fibers at mesencephalic levels could be seen to join with the medial lemniscus.

Fiber degeneration was observed within the fasciculus and nucleus gracilis, and a few fibers could be traced leaving this region and coursing laterally, just beneath the dorsal surface of the brainstem. These fibers were traced to the external cuneate nucleus and the subnuclei x and z of the vestibular complex.

Degeneration within the cerebellar nuclei was heaviest within the fastigial nucleus with little observed within the interpositus and dentate. Considerable amounts of degeneration was found in the cortex of the anterior lobe and the posterior portion of the posterior lobe. Other areas receiving trace degeneration included the locus ceruleus, substantia nigra, periaqueductal gray, superior olive and the nucleus commissuralis. Differences resulting from lesion variances will be discussed.
(Supported by NIH Grant NS 13230.)

200 A POSSIBLE EXPLANATION FOR CEREBELLAR TREMOR. T. Vilis and J. Hore. Dept. of Physiology and Dept. of Ophthalmology, University of Western Ontario, London, Canada.

Trained Cebus monkeys can return their forearm, which has been displaced by a perturbation applied to a handle, back to the original position with little or no overshoot. However, if the function of the ipsilateral dentate and interpositus nuclei is impaired by reversible cryoprobe lesions, the same displacement triggers a prolonged series of oscillations at 3 to 4 Hz. We found, in three monkeys that the number, amplitude, and period of the oscillations was directly related to the degree of cerebellar cooling. Normally the return of the arm was initiated in part by short latency (15-25 msec) and long latency (30-80 msec) reflex responses in the agonist muscle. The return was usually actively terminated by EMG activity in the antagonist occurring 150 to 200 msec after the torque pulse. As the temperature of the cryoprobes was progressively lowered, the onset of this antagonist activity was progressively delayed causing the correction to overshoot by increasing amounts. This activity was also prolonged thus initiating a second cycle of oscillations.

Could these oscillations result from impaired stability of a servo mechanism through motor cortex? To answer this the activity of 74 precentral neurons, which responded to displacement of the arm were compared before, during and after cooling of the cerebellar nuclei. In 24 neurons an early excitation (onset 30 to 40 msec) was followed by a later inhibition (70 to 80 msec) for a perturbation in one direction, and early inhibition followed by late excitation for a perturbation in the opposite direction. Normally this late excitation appeared in advance of the EMG activity in the antagonist which acted to terminate the corrective movement. During cooling this response, in 17 of these 24 neurons, was delayed in this and subsequent oscillations so as to occur after the onset of EMG activity. The onset of this neural response occurred after the start of muscle stretch whereas in normal movement it was predictive i.e. it occurred prior to the start of muscle stretch.

A possible interpretation of these results is that after an arm perturbation an initial correction is initiated by segmental and suprasegmental stretch reflexes. The motor cortex, in advance of this movement correction generates a command to terminate this correction on the basis of predictive information provided by the cerebellum (the 70 msec cortical response). When this information is lacking, servo control through the motor cortex must rely on afferent information. This results in a descending command which comes too late in the movement thus causing overcorrection and subsequent tremor.
(Supported by the Canadian Medical Research Council PG-1)

201 THE ROLE OF CEREBELLAR AND BRAIN STEM NUCLEI IN VESTIBULAR COMPENSATION IN RATS: A 2-DEOXY-D-GLUCOSE STUDY. K. Walton and R. Llinás, Dept. Physiology & Biophysics, New York Univ. Med. Ctr., 550 First Ave., New York 10016.

Recovery from hemilabyrinthectomy in the rat has been used to investigate the role of the cerebellar system in motor learning. This vestibular compensation (VC), as indicated by the return of postural symmetry, the cessation of spontaneous nystagmus, and the return of normal eye position, occurs within 24 to 36 hours in normal animals. Compensation is irreversibly absent in animals in which the inferior olive (IO) has been chemically lesioned by the administration of 3-acetylpyridine (3-AP) and harmaline followed by niacinamide (see Llinás et al., Science 190: 1230, 1975) and is markedly delayed in animals with partial IO lesion. VC is lost if the IO is lesioned following compensation. In contrast, removal of the cerebellar cortex slightly retards, but neither prevents nor leads to, the loss of compensation. Thus, the olivo-cerebellar system, but not the cerebellar cortex, plays an essential role in both the acquisition and retention of vestibular compensation.

In order to obtain a global picture of the changes in neuronal activity underlying this learning process at brain stem and cerebellar nuclear levels, 2-deoxy-D-glucose was used to determine the distribution of glucose uptake at these sites in normal, uncompensated, compensated, and 3AP-decompensated animals. These studies showed that the asymmetries following hemilabyrinthectomy reflect an imbalance in the activity of the vestibular nuclei (VN) and that compensation results from the restoration of balance. In uncompensated and decompensated animals the VN ipsilateral to the lesion were significantly less active than the contralateral VN. In compensated animals, VN activity was the same on both sides, the level being close to or higher than normal. Thus, VC is accompanied by an increase in activity in the ipsilateral VN. This is mediated by several structures, as indicated by an elevation of their function above normal levels. For example, in addition to enhanced activity in the granular layer of the nodulus and uvula, compensated animals show enhancement in the ipsilateral cerebellar nuclei, the lateral reticular nucleus and in the contralateral IO, particularly the medial accessory subnucleus.

The present study demonstrates that the brain is capable of re-organizing its activity to recreate, by internal means, a meaningful equivalent of normal vestibular input. The actual distribution of this "engram" is reflected in the spatial distribution of radioactivity. (Supported by USPHS research grant NS-13742 from NINCDS)

CEREBRAL CORTEX

202 THALAMIC AND NON-THALAMIC SUBCORTICAL PROJECTIONS TO CAT'S FRONTAL EYE FIELD. C. Avendaño*, A. Llamas* and F. Reinoso-Suárez. Dept. Morfología, Inst. Invest. Oftalmol. Castroviejo, Fac. Medicina, Univ. Autónoma, Madrid 34, Spain.

Using minimal electrical stimulation, Schlag and Schlag-Rey (1971) identified regions in the frontal cortex of the cat from which oculomotor responses could be elicited. These regions corresponded to both the dorsal sector of the medial wall of the prefrontal cortex and to the depth of the praesylvian sulcus. Classical neuroanatomical procedures have not yielded conclusive results as to the subcortical projections to these areas. On the other hand, electrophysiological findings uphold the existence of reciprocal connections between them and the thalamic nucleus centralis lateralis (CL), the claimed thalamic locus for oculomotor control.

As a part of a broader study of the frontal cortex of the cat, we have explored the thalamic and non-thalamic subcortical projections to the frontal cortical areas of ocular movements by means of the retrograde transport of HRP technique. Small amounts (.1 to .3 µl) of a solution of HRP were injected in various portions of these areas in adult cats. After survival times of 44-46 hr. animals were perfused and brains processed to reveal HRP in the CNS.

Injections remained limited within the gray matter sparing the subcortical white matter, thus impregnating terminals only in restrained portions of the cortex. In all cases labelled neurons were found in the thalamic nuclei medialis dorsalis (MD), ventralis anterior (VA) and ventralis medialis (VM). Most injections gave rise also to labelling of neurons in the middle or two posterior thirds of the centralis medialis (CeM). In contrast with the above mentioned electrophysiological data, no labelled neurons could be observed in CL. Labelling in MD was topographically organized: injections in the medial wall of the hemisphere produced labelled cells dorsally in its lateral sector, whereas injections in the bottom of the praesylvian sulcus gave rise to HRP-cells also in the lateral sector, but ventrally and posteriorly.

Labelled neurons were also found in other subcortical regions. Conspicuously in the ipsilateral dorsal claustrum and basal magnocellular amygdaloid nucleus, occupying in these nuclei an intermediate position between those of neurons projecting to the motor cortex and of neurons projecting to the remaining prefrontal cortex. A few HRP-cells distributed sparsely in the medial hypothalamus and in the locus coeruleus complex.

These findings suggest that thalamic projections to the frontal area of ocular movements in cat organize topographically, similarly to those in monkey, and that projections to the same area from other subcortical levels resemble those reaching the remaining prefrontal cortex, although varying somewhat in their topography.

Supp. by Grant N° 78/77 from FDC-INP.

203 NEUROANATOMICAL BASES OF SHORT-TERM SPATIAL MEMORY IN THE RAT. James Becker*, John A. Walker*, David S. Olton, and Barbara G. O'Connell*. Dept. Of Psych., Johns Hopkins Univ., Baltimore, Md.

The frontal cortex and limbic systems have been implicated in spatial short-term memory. In order to dissociate between the roles played by these areas in spatial memory, post-operative retention of an eight arm radial maze task was measured in groups of rats with either frontal or limbic system lesions.

Prior to surgery all of the rats demonstrated good maze performance, choosing a mean of at least seven correct arms in the first eight trials. Rats were randomly assigned to groups and lesions were made in medial frontal cortex (MF), sulcal frontal cortex (SF), caudate nucleus (CN), dorsal medial nucleus of the thalamus (DMT), or the fornix (FX). Testing was resumed following one week of recovery.

After surgery, the normal and operated control animals showed good retention of the task. Immediately after surgery they were performing as well as they had pre-operatively. This high level of performance was maintained for the duration of the 20 sessions.

In marked contrast to the control group, the FX group had an immediate and severe deficit in performance. Choice accuracy dropped to the level expected by chance in the first five tests and never rose above this level for 50 post-operative sessions.

The groups of rats with damage to the frontal cortex system (MF, SF, CN, DMT) showed an immediate and severe deficit in performance. Choice accuracy decreased to chance level in the first five post-operative sessions. Performance subsequently recovered to normal levels in the second block of five tests. This behavior was maintained for the remainder of the 20 test sessions.

The recovery of function by rats with medial frontal damage was a result of the animals' experience on the maze, rather than time from surgery. The performance of rats with medial frontal damage that began retention testing three weeks after surgery was essentially the same as that of rats in group MF on all behavioral measures.

These data demonstrate that the frontal and limbic systems are differentially involved in short-term spatial memory. In addition, they demonstrate that recovery of function from frontal damage in the adult is dependant on experience.

204 RESPONSIVENESS OF S1 CORTICAL CELLS TO RECEPTIVE FIELD STIMULATION DURING DIFFERENT BEHAVIORS by John K. Chapin and Donald J. Woodward, U. Tx. Health Sci. Ctr. at Dallas, Dallas, Tx., 75235

Responses of single units in the S1 cortical area of awake freely moving rats were examined to investigate modulation of sensory input during different motor behaviors. Post-stimulus time histograms (PSTH's) were compiled following touch of the skin with a manually held probe, or after electrical stimulation through subcutaneously implanted electrodes in the forepaw. Unit activity was also correlated with a variety of spontaneous and evoked behaviors, including treadmill running. These were studied by computer analysis of films synchronized with EMG's. An aim of initial experiments was to determine behavioral conditions in which sensory transmission to the S1 cortex is enhanced or depressed. PSTH's produced either by probe activation of tactile receptive fields (TRF's) or by electrical stimulation were similar and included various combinations of: 1) two early peaks,(7-12 msec. and 14-40 msec. latency), 2) a following depression of firing (40-150msec.), and 3) a rebound facilitation following the depression. All phases of the response to just suprathreshold electrical stimuli, especially the longer latency components, were enhanced during a "freezing" behavior induced by holding the animal. Reduction or suppression of all components of the response was induced by active movements such as grooming, locomotion, and slow exploration.

An aim of later experiments was to test the modulation of natural sensory input during a variety of motor behaviors. About 60% of cells with TRF's covering the whole forepaw, especially those exhibiting prominent short latency PSTH's, fired in phase with the forelimb step cycle during locomotion. Yet many of these cells were found to be correlated with particular active movements of the forelimb in the absence of overt tactile stimuli, as observed in a variety of spontaneous and evoked behaviors. For example, cells phasic to step cycles often fired before footfall, ie. the expected sensation. Conversely, many cells not firing phasically during locomotion were found receptive to touch on the TRF with a manually held probe during the running sequence. These data suggest that certain inputs which are of special significance in the behavior may be selectively gated in. In addition it appears that cells with well defined TRF's may also fire in relation to central motor signals, suggesting a capacity for sensorimotor integration.

(This study was supported by grants NSF BNS 77-01174 to D.J.W.)

205 CENTRAL AUDITORY PROCESSING: PATTERNS OF RECOVERY, D.M. Daly, D.D. Daly, J. Pearson*. Dept. Neurology, UT Health Sci. Ctr., Dallas, TX. 75235.

Sparse acoustic stimuli (SAS) provide a measure of cortical auditory processing (Neurosci. Abstr. 2:5, 2:6, 1976). We report the results of serial testing in 2 patients with acute, resolving lesions of comparable extent in the left parieto-occipital region. Patients classified sets of SAS with systematic variations in the duration or extent of change in second formant frequency. Set 1: duration of rise yields |be⊢|wel; Set 2: duration of fall, |ge⊢|yel; Set 3: extent of change, |be⊢|de⊢|gel.

A 44 year-old male abruptly developed aphasia with severe comprehension defects and right homonymous hemianopsia. Computerized tomography confirmed an infarct in distribution of a posterior branch of middle cerebral artery. His native language was English; he also understood and spoke German and Spanish. Initially, with SAS presented to right ear he classified Set 1 without sharp boundaries; with SAS to left ear and binaurally he showed classes with vestigial sharp boundaries. With Sets 2 and 3 he reported great difficulty differentiating stimuli and had altered classes in all modes of presentation. Over 4 days visual defect resolved to superior quadrantanopsia; comprehension of speech improved and aphasia abated leaving primarily anomic deficits. He now showed sharp boundaries for classes in Set 1; these coincided with the earlier left and binaural vestiges. Classes for Sets 2 and 3 had not changed significantly.

A 45 year-old woman underwent craniotomy for a tentorial arteriovenous malformation. She was a fluent English speaker; she had never been able to understand or speak French or Spanish, but could read both languages. Postoperatively she had aphasia, difficulty in comprehending speech and right homonymous hemianopsia. As comprehension improved, hemianopsia contracted to upper quadrantanopsia. Five months later, aphasia testing revealed severe alexia and auditory comprehension defects although she spoke fluently with minimal anomia. Initially, for all sets classes were markedly altered; classes for Set 2 suggested inversion of perception. Two months later, classes for Set 1 showed sharp boundaries in the usual location. Set 2 classes were resolving; and with left ear-left hand testing a boundary had emerged. Ten months later, Set 1 classes remained intact. Classes for Set 2 continued to resolve with distinct boundaries for monaural SAS. For the first time she could differentiate among SAS of Set 3.

Despite differences in rate, the courses of recovery in these patients were similar. In both, perception of SAS in Set 1 was better preserved and resolved more rapidly than in Set 2. Initially, neither could differentiate among SAS in Set 3. At the last testings both classified Set 1 in the usual fashion. Both also classified Sets 2 and 3 consistently although each of them perceived SAS differently from the other. The alterations over time in their perceptions of SAS accompanied the resolution of their underlying pathologic processes. In contrast, in patients with static lesions such alterations have persisted unabated for more than 2 years. Some persons with difficulty in speaking and comprehending a second language have auditory perceptual domains different from those usually seen; the differences between these two patients may partly reflect disparate premorbid auditory perceptual domains.

These findings accord with experimental studies in animals and indicate that auditory cortex analyzes complex transformations on the basilar membranes. The apparently genetically-determined disparate perception of SAS may reflect alterations in cortical circuitry as well as significant variations among the cochleae of individuals or between cochleae in an individual.

206 EFFECTS OF RADIATION AT VARIOUS PRENATAL STAGES ON DEVELOPING RAT CORTEX AND CORTICOSPINAL SYSTEM. CONSTANCE J. D'AMATO AND SAMUEL P. HICKS, Department Pathology, University of Michigan Medical Center, Ann Arbor, MI 48109

Prenatal irradiation (Xrays) causes highly reproducible patterns of malformation and disturbed function of the nervous system. The degree of malformation is the result of a balance between damage done and the capacity for repair. Highlights of some experiments with 150R were: a deficient disordered cortex and clumsy locomotion on a narrow path but ability to jump from one platform to another in offspring irradiated on the 17th prenatal day (hereafter "17th day rats", etc.); a large subcortical ectopia, thin cortex, malformed spinal cord, synchronous hopping gait, and inability to run in 14th day rats; and only slightly abnormal brain, locomotion, and jumping ability, despite severe initial damage to cerebral vesicles, in 12th day (26 pairs of somites) rats.

To better study the development of the malformed cortices, corticospinal (CS) neurons were labeled with horseradish peroxidase from their cut axons in the cervical spinal cord in infant and mature rats. In normal mature rats, CS neurons formed a caudal band corresponding to areas, 3,4,6 separated by a gap from a rostral band in area 10. These were derived from a more extensive array of CS neurons in the infant including numerous CS neurons in the gap. Gap neurons normally lost their cord projections 2 weeks after birth. If the caudal band was ablated in infancy, gap neurons kept their cord projections into adult life, a kind of plasticity. Seventeenth day rats developed CS neurons in a recognizable layer V corresponding to the caudal band and gap, but not the rostral band. Some CS neurons had bifurcated apical dendrites, the polarity of others was askew. In 14th day rats, despite disorder of early cell migrations, CS neurons developed in a layer V of the cortex, irregularly distributed in the two bands and the gap. Some CS neurons were mislaid in the ectopia, but still sent axons to the cord. Possibly the persistence of gap neuron projections was caused by too few CS neurons being produced. In mature 12th day rats, CS neurons formed the two bands and gap nearly normally, but polarity of some CS neurons was abnormal. In 12th day rats 7 or 10 days old, the normal infant gap CS neurons were present.

Undoubtedly the abnormal CS system contributed to the movement abnormalities in the 14th and 17th day rats, but in addition to other cortical abnormalities these rats had striatal and commissural deficiencies, and 14th day rats had malformed spinal cord gray matter. (USPHS NS 10531)

207 MULTIPLE UNIT RECORDING IN AUDITORY CORTEX OF CATS DURING BEHAVIOR. Dept. of Physio., Univ. of Pa., Phila., PA 19104 Stephen Evanczuk * and George Gerstein.

Previous attempts to describe functional connectivity within the auditory cortex have met with only limited success due to technical and, possibly, physiological problems. In physiological preparations involving paralyzed animals where adequate control of stimulation and recording conditions is possible, the behavioral state of the animal is usually unsatisfactory. Since it is likely that the behavioral state of the animal is related to the degree of "activation" of the cortex, it would seem to be important(particularly in a study of intracortical functional connectivity) to record from an animal in a known behavioral state.

In this study, cats are trained in a two-response operant-conditioning paradigm (J.L. Orr et. al., J. Acoustical Soc. Amer., 62(5): 1269(1977) to respond to a pair of clicks for water reward. If the pair of clicks (separated by Δt=250 msec.) emanate from two different locations on the azimuthal plane (level with the cat's ears), the cat is required to lift one forepaw for a correct choice. If the pair of clicks appear to come from a single location, the correct response is lifting the other forepaw. Ablation studies (e.g., Neff, et. al., J Neurophys. 19: 500(1956) suggest that a functioning auditory cortex is necessary for solution of this task, particularly at small angular separations of the two sound sources. Throughout the behavioral session, a masking broadband noise is presented along with various tone bursts which are used as supplementary search stimuli for recording purposes.

Neuronal activity is recorded using an implant which allows independent microdrive control of several (independent) bundles of fine wires (10 μ, tungsten, factory-insulated with epoxy, ground to a 60° conical tip). The output of each wire is connected to a differential amplifier to attenuate correlated activity (noise). Computers are used to control the behavior, the data acquisition, and the analysis of spike trains. Preliminary data are discussed.

Supported by MH05606 and GM01994

208 SYNAPTIC REORGANIZATION IN THE ADULT NEOCORTEX. Robert E. Foster and Ford F. Ebner. Neuroscience Section, Brown University, Providence, RI 02912.

Does neocortex in adults undergo substantial synaptic reorganization after deafferentation as has been demonstrated in areas such as the septal nuclei or dentate gyrus? We have chosen layer I of neocortex to study this problem because 1)layer I consists of acellular neuropil with identifiable boundaries, 2)layer I is narrow enough to allow sampling for quantitative electron microscopy and 3)afferent axons from the dorsal thalamus terminate mainly in the outer 100μm while commissural afferents occupy the complementary inner portion of layer I. We examined layer I at various times after cutting the commissural fibers. Commissurotomy was performed in adult opossums (D. virginiana)and the animals were allowed to survive 6, 30 or 60 days. In order to test for thalamic fibers as one possible source of new synapses in layer I, thalamectomy was performed on some of the animals on survival day 30 or 60. An additional 6-day survival was allowed to produce the electron dense phase of anterograde degeneration. The aldehyde fixed brain was chopped into 500μm slabs and every third slab was saved for frozen section histology (Nissl and Fink-Heimer stains). The remaining slabs were processed for electron microscopy. We chose layer I of the preorbital cortex as our sample area since it receives a uniformly dense commissural projection deep to the input from the dorsal thalamus. Quantitative EM on layer I followed the procedures of Ebner and Colonnier (JCN, 179:263). Two results of these procedures are striking. First, the number of vesicle-containing profiles per unit area does not vary significantly from normal at 30 or 60 days after removal of the commissural fiber terminals. At these times the neuropil in the commissural input zone of layer I looks remarkably normal. Second, the thalamic fibers form a dense terminal degeneration field in the inner portion of layer I (Fink-Heimer technique) in those animals that underwent thalamectomy at the end of 30 or 60 day survival periods. This degeneration field is unlike any seen in "normal" animals after thalamectomy alone with a 6 day survival. Our results suggest that at least layer I in adult mammalian neocortex can undergo synaptic reorganization and that thalamic fibers contribute to this process. (Supported by NIH grant NS-13031 and NS 05561.)

209 DIFFERENTIAL DEPRESSANT EFFECTS OF N. RAPHE DORSALIS STIMULATION ON ELECTROCORTICAL ACTIVITY IN CATS. J. García Ramos. Physiol. Lab., Escuela Médico Militar. México 10, D. F. MEXICO.

It has been shown that N. Raphe dorsalis stimulation depresses the basal electrocortical activity, evoked potentials and strychnine spikes. Under certain conditions, however, evoked potentials and/or strychnine spikes could be enhanced over a depressed basal activity. The study of these conditions has shown that this occurred when the afferent impulses inducing the evoked potentials are relatively strong, or the direct cortical stimuli eliciting the local strychnine spikes are applied at a relatively high-rate, and when the basal electrical activity is constituted by low-voltage, high-frequency waves. The observations were made on unanesthetized curarized animals. The required basal cortical activity was obtained by constant stimulation of an afferent somatic nerve at a rate of 50 to 100 per sec, or by sound stimulation with white noise, between 60 and 80.

The cortical areas explored were the somatosensory, auditory, and an associative one such as the suprasylvian area. The obtained results were similar in all of them, thus supporting the idea that the liberated serotonin acts in a diffuse manner as a neurohumoral substance. It is suggested that a similar mechanism might be involved in the process of attention in which low relevant information would be blocked, leaving dendritic membranes in a condition under which high-value information could find priority for its processing by the brain.

210 PREFERRED TANGENTIAL ORIENTATION AND SPATIAL ORDER IN DENDRITIC FIELDS OF CAT AUDITORY CORTEX: A COMPUTER-MICROSCOPE STUDY OF GOLGI STAINED MATERIAL. E.M. Glaser, H. Van der Loos*, and M. Gissler*. Institute of Anatomy, University of Lausanne, CH1011 Lausanne, Switzerland.

Basal dendrites of pyramidal neurons and all dendrites of stellate neurons in layers IV and V of the primary auditory cortex (A1) of cat exhibit preferred orientations in the tangential plane. This was shown by means of a computer-microscope analysis of Golgi-Cox stained neurons in 100μm and 300μm thick sections. The 44 neurons we analyzed were harvested from 2 cats. The neurons were randomly selected, with the assistance of the computer, from an initial survey of 1115 stained neurons residing in optimally sectioned regions of A1. Neurons in the 300μm thick sections were studied with high power optics by means of 'obverse-reverse' computer microscopy, a technique that views a neuron from both sides of a double coverslip-section 'sandwich' (Glaser and Van der Loos, sub. for publ.) and then reassembles the acquired dendritic data by computer analysis. In this way it was possible to minimize the effects of truncation upon the dendritic trees. Only the apical dendrites of the pyramidal cells suffered truncation. Two techniques were used to represent the 3-dimensional structure of the dendrites: the 'dendritic stick' and the 'dendritic trumpet'. The former dismembers a dendrite into individual approximating chord segments of about 25μm length. The latter technique also uses approximating chord segments. In it a principal dendrite is considered as an entity and represented by its centroid, its moments about the dendrite origin, and the dispersion of its dendrites tangentially (azimuth), vertically (elevation), and radially (i.e., with respect to the neuron's soma). Both dendrite representations were examined by spatial Fourier and statistical analyses. Their results show that within the tangential plane there is a significant ($p<.05$) and consistent preferred orientation of the dendritic sticks in an approximately dorso-ventral direction (nearly perpendicular to the suprasylvian sulcus). This orientation was observed in the two cats analyzed and seems to be close to the orientations of the isofrequency contours observed by others in electrophysiologically obtained maps of this region. The dendritic trumpet analyses of pyramidal cell basal dendrites also show that they have a distinctly non-random vertical (normal to the tangential plane) distribution. Dendrites of stellate cells, on the other hand, have apparently a random vertical distribution.

Supported by a USPHS NIH Fogarty Senior International Fellowship to EMG, and by Swiss National Science Foundation Grant 3776 to HVdL.

211 SOME PARALIMBIC CONNECTIONS OF THE MEDIAL PULVINAR NUCLEUS IN THE MACAQUE. E. C. Gower* and M.-M. Mesulam. Neurological Unit, Beth Israel Hospital, Boston, MA 02215.

The medial pulvinar nucleus of the rhesus monkey thalamus has reciprocal connections with association cortex of the temporal lobe, inferior parietal lobule, and prefrontal cortex. Our recent observations suggest that in addition to these well known relationships, the medial pulvinar has an orderly set of connections with some medial and basal areas of the cerebral cortex: namely with the cingulate gyrus (areas LA and LC), medial frontal cortex (FL), caudal orbitofrontal cortex (13 and 14), the temporal pole (TG), and the parahippocampal gyrus (TF). These projections were investigated with cortical injections of tritiated amino acids (TAA) and horseradish peroxidase (HRP). In the present communication we will describe some connections between the pulvinar nucleus and the cingulate gyrus in the rhesus monkey.

After HRP injections in the anterior cingulate gyrus (LA), labeled perikarya were found in the medial pole of the pulvinar nucleus, a region directly caudal to the densocellular division of medialis dorsalis (MD dc). Injections of HRP or TAA in more central portions of the cingulate gyrus (at the junction of LA with LC) resulted in anterograde (TAA) or retrograde (HRP) transport of tracer into the central zone of the medial pulvinar. Furthermore, injection of TAA in the caudal cingulate gyrus (LC and retrosplenial area) resulted in the deposition of silver grain in the superficial aspect of the medial pulvinar. In each of these cases, a substantial concentration of transported label was seen in the pulvinar, even though other thalamic nuclei were also labeled, in some cases more heavily.

It is not yet entirely clear whether these connections of the pulvinar are related to specific limbic or paralimbic sectors of the cytoarchitectonically heterogeneous cingulate gyrus. However, in view of our evidence for additional connections with caudal orbitofrontal, medial frontal, temporal polar and parahippocampal areas, it is not unlikely that the medial subdivision of the pulvinar nucleus may be characterized by significant connections on the one hand with lateral sensory association cortices, and on the other with the medial paralimbic belt. These data, along with the evidence for its direct efferent projection to the lateral amygdaloid nucleus (Jones & Burton, Brain Res. 104:142, 1976) suggest that the medial pulvinar may participate in a multifaceted interaction between cortical association systems and the limbic lobe.

(Supported by NIH grants # 09211 and 06209, and the Benevolent Foundation of Scottish Rite Freemasoney, Northern Jurisdiction, USA).

212 DEGENERATION ARGYROPHILIA FOLLOWING LONG-STANDING DAMAGE TO THE HUMAN BRAIN. M.R. Grafe*, R.D. Schimpff* and C.H. Leonard. Depts. of Neuroscience and Pathology, Univ. of Florida, Gainesville, FL 32610.

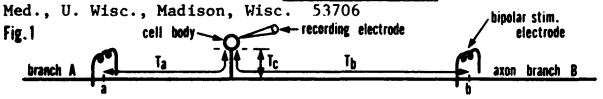
In the course of a long term study on the cortical connections in the human brain, we have previously reported a case in which degenerating fibers and terminal arborizations were demonstrated with modified Nauta techniques after a two year survival (Anat. Rec., 190:405-406, 1978). The appearance of the two year old degeneration was strikingly similar to that of degenerating fibers originating from a 3 week old lesion found contralaterally in the same brain. Loss of myelin was not apparent in tracts coming from either area of damage. Since modern axonal transport techniques cannot be used in the human brain, we have further investigated the effectiveness of degeneration argyrophilia as a method for studying human neuroanatomy. We report here two further cases in which extensive staining of degenerating elements is seen following long-standing damage. In the first case, contusive injury was received on the left side of the head six years prior to death. The entire temporal lobe suffered extensive damage. Degenerating fibers are seen in corticothalamic fibers to the medial geniculate, corticostriate fibers, and the stria terminalis. The second case had cerebrovascular damage which occurred 12 years prior to death, primarily affecting the right inferior parietal cortex. In the pons, the first area to be investigated, typical, well impregnated degeneration is found only in the right lateral parietopontine tract.

Light and electron microscopic examination suggests that in the human CNS little change in the appearance of degeneration occurs after the initial response to cell death. This contrasts with the peripheral nervous system where degeneration is cleared within a few weeks, or the CNS of experimental animals where degeneration is markedly reduced within a year. The extended time course of degeneration in the human CNS may be due to the nature of the lesion, the phagocytic response, or factors associated with the long human lifespan. Whatever the mechanism, the phenomenon of long-lasting degeneration argyrophilia provides a useful tool for the experimental neuroanatomist interested in the human brain. We encourage the cooperation of neurologists and pathologists who may have access to significant cases for study.

Supported by grants #NS 13516 to C.M.L. and an NSF pre-doctoral fellowship to M.R.G.

213 APPLICATION OF COLLISION TESTING TO INVESTIGATE PROPERTIES OF MULTIPLE ASSOCIATION AXONS ORIGINATING FROM SINGLE CELLS IN THE PIRIFORM CORTEX OF THE RAT. Lewis B. Haberly, Dept. Anat., Sch. Med., U. Wisc., Madison, Wisc. 53706

Fig.1



Collision between antidromic action potentials (APs) evoked at point a on axon A (fig.1) and antidromic APs evoked at point b on axon B will occur when the shock at point b follows that at point a at times $\leq$ an interval, $C_{a,b}$. The presence of collision can be detected by the failure of arrival of an AP at the soma following stimulation at point b. A collision interval $C_{b,a}$ can similarly be defined for stimulation at point b followed by point a.

If: T_a and T_b=latencies of antidromic activation of the cell body from points a and b respectively (fig.1).
T_c = conduction time in the axon proximal to the branch.
R_a and R_b = refractory periods for axon branches A and B.

Then: $C_{a,b} \simeq T_a + T_b - 2T_c + R_b$, and $C_{b,a} \simeq T_a + T_b - T_c + R_a$.

Combining equations for $C_{a,b}$ and $C_{b,a}$ reveals that:

$C_{a,b} - R_b \simeq C_{b,a} - R_a$, if separate axon collaterals of the same cell are antidromically activated as in fig. 1.

To establish that single unit recordings are obtained from cell bodies rather than axons, spontaneous or synaptically evoked APs can be collided with antidromic APs. Satisfaction of the well-known equations for such collision (within the limits of systematic error discussed by Fuller & Schlag, Br. Res., 112:283) provides strong evidence for a recording position near the soma.

Application of these methods to the piriform cortex has revealed that single cells give rise to association axons that are directed both rostrally and caudally. Three axonal branches were demonstrated for 2 units. Evaluation of T_c indicates that axonal branching occurred close to the soma in all cases (21 units).

Lesions were made at the recording sites for 13 units. The sites were located in layer IIa for 4 units, near the IIa-IIb border for 2, in layer IIb for 1, near the IIb-III border for 5, and in layer III for 1 unit. (Layers as defined by Haberly & Price, JCN 178:711). Conduction velocities (V_c) (calculated assuming straight axon trajectories) ranged from .45 to 1.25 m/sec for rostrally directed axons and .25 to .48 m/sec for caudally directed axons. For all units V_c in the rostral axon component exceeded that in the caudal. For most units T_a and T_b decreased slightly following spontaneous or evoked APs propagated in the opposite direction, indicating a possible increase in V_c.

Experiments were carried out in Dr. J.L. Price's lab, Dept. Anat. & Neurobiol., Wash U. Sch. Med., St. Louis. Supported by NIH grants #NS 09518 and NS 05612.

214 ABNORMAL SYNAPTIC CHEMISTRY IN ADULT RAT NEOCORTEX FOLLOWING PRENATAL TREATMENT WITH METHYLAZOXYMETHANOL. Michael V. Johnston* and Joseph T. Coyle. Dept. Pharmacology, Johns Hopkins Univ. School of Medicine, Baltimore, Maryland 21205.

The lateral neocortex of adult rats which had been treated prenatally on day 15 of gestation (DG) with 20 mg/kg of methylazoxymethanol (MAM), a potent nucleic acid alkylating agent, was examined with regard to the effects on presynaptic markers and postsynaptic receptors for component neurons. Treated rats gained weight normally but were microencephalic. The MAM treatment reduced the weight of the forebrain by 53% and of the lateral cortex by 67%, but the weight of the hindbrain was unaffected. Neocortical cytoarchitecture was strikingly altered; and cortical layers II and III were nearly absent.

The concentrations of the presynaptic markers for the GABAergic neurons - glutamate decarboxylase, endogenous GABA and [^{3}H]GABA uptake by P_2 fractions - were minimally altered in the MAM-treated cortex; however, the total amounts of the markers in the cortex were reduced by 60-70% ($p < .01$). The specific binding of [^{3}H]GABA to cortical membranes was similarly affected. In contrast, the concentrations of the presynaptic markers for the cholinergic neurons - choline acetyltransferase and endogenous acetylcholine - were increased 123% and 64% respectively ($p < 0.01$); however, the total amounts of these markers per cortex were depressed by 40% ($p < 0.01$). The specific binding of [^{3}H]quinuclidinyl benzilate to muscarinic receptors was reduced by 25% ($p < 0.01$) per mg cortex. The concentrations of the presynaptic markers for the noradrenergic terminals in MAM-lesioned cortex were markedly elevated with tyrosine hydroxylase activity increased by 336% ($p < 0.01$) and with [^{3}H]norepinephrine uptake by P_2 fractions and endogenous norepinephrine elevated by 130% ($p < 0.01$); however, the total amounts of the noradrenergic markers per cortex were only slightly reduced or unaffected. The specific binding of [^{3}H]dihydroalprenalol to beta-receptors was reduced by 29% per mg of cortex.

Since MAM primarily kills dividing cells, the resultant imbalance in the neurochemical markers in the adult neocortex reflects the selective vulnerability of distinct neuronal populations according to their time of mitotic activity in the fetal cortex. The cortical GABAergic neurons are severely affected whereas the cholinergic neurons are relatively spared after MAM treatment at 15 DG. The noradrenergic neurons, which cease dividing at 13 DG, appear to "hyper-innervate" the atrophic lesioned cortex although the total noradrenergic input remains comparable to control. MAM treatment is a promising tool for selectively lesioning the neocortex. (Supported by USPHS Grants MH 26654, RSDA KO2-MH 00125, and the National Foundation, McKnight Foundation).

215 DECORTICATION: WAS FLOURENS CORRECT? Bryan Kolb and Ian Q. Whishaw*. Dept. of Psychology, University of Lethbrdige, Lethbridge, Alberta, Canada, T1K 3M4.

Removal of the neocortex is known to produce a severe deterioration in the behavior of animals. The questions arise whether this can be alleviated by removing the tissue in infancy, thus allowing sparing of function or whether the effect is due to damaging some specific region.

The behavior of rats decorticated as adults was compared to that of rats decorticated at 7 days of age and to rats which received frontal lobe lesions. Following decortication the adult decorticates had severe difficulties in eating but would eat immediately after recovery from anesthesia if presented with a soft, easy to eat mash. The animals retained most of the normal movement patterns of locomotion, climbing, grooming, feeding and fighting. Nevertheless many behaviors were often not performed at the proper time and place. Thus hoarding, nest building, maternal behavior, social behavior and male sexual behavior were essentially abolished. The animals were never able to properly lick a metal water spout and were severely impaired at chaining together the components of grooming into a long behavior sequence and forepaw inhibition during swimming was abolished. The decorticate rats were also hyperactive in running wheels, but displayed good circadian rhythms and were able to develop a spatial reversal learning set in a Grice box, although considerably more slowly than controls.

Entirely removing the neocortex at 7 days of age had almost identical effects on behavior although grooming and swimming and sexual behavior were not as severely impaired. Removing the frontal lobe including the orbital frontal cortex, medial frontal cortex, and motor cortex produced the same behavioral effects as decortication whereas more posterior lesions did not. Lesions restricted to individual subfields of the frontal cortex (medial, orbital, motor) produced deficits on specific behaviors and the sum of the small lesions equalled the effect of the larger frontal lesion.

The results show that removing the neocortex in infancy does not allow sparing of function, although we have previously shown that removing the frontal cortex in infancy does. Further, the results imply that the motor deficits attributed to decortication in rats result from damage to the frontal cortex.

216 CORTICO-CORTICAL PROJECTIONS TO AREA 18 IN THE CAT: AN HRP STUDY. Lamont, P.* and M. Colonnier. Dept. Anat., Laval Univ., Quebec, P.Q., G1K 7P4.

Recent advances in HRP histochemistry (Rosene and Mesulam, J. Histochem. Cytochem., '78, 26:28) have significantly increased the sensitivity of the method and have permitted a better visualization of both retrograde and anterograde transport of the enzyme. This has led us to study the type and distribution of cortical cells projecting to small discrete patches of area 18.

0.1 to 0.25 ul of HRP was injected in area 18 of adult cats anaesthetized with fluothane. After a 24 to 40 hour survival period, the animals were perfused according to the protocol of Rosene and Mesulam. Frozen sections were cut at 50u and the HRP revealed with benzidine dihydrochloride.

Labelled axons can be followed from the injection site to ipsilateral areas 17 and 19, and to the contralateral area 18. HRP labelling is also seen in the Clare-Bishop area. In each of these zones, labelled cell bodies are seen, as well as a fine granular stippling of the background which is interpreted as being located in the terminal branching of axons after anterograde transport from cells in area 18, or along recurrent collaterals of the retrogradely labelled cells. Both labelled cells and terminal arborizations appear in clusters or columns which are quite strictly superimposed.

In area 17, both pyramidal and stellate cells are labelled. The pyramids are especially numerous in layers II and III but are also seen in IVA, V and VI. The many large stellates are concentrated in layer IVA. Fine anterograde labelling is practically absent from IVB, and densest in IVA and the lowest part of III. A prominent tangential plexus is seen in layer I. In area 19, labelled pyramidal cells are seen in layers II, III, V and VI but are much fewer in number. Fine anterograde labelling is virtually absent from layer VI, is most intense in IV, and only a few scattered grains are present in I. In the contralateral area 18, pyramidal cells are numerous but restricted to layers II and III. Discrete anterograde grains are nearly absent in V, and reach their maximum concentration in the upper part of IV and the lower part of III. Only isolated grains are seen in layers II and I.

The clusters or columns of cells are especially well-defined in area 17. The centers of the clusters are separated by intervals averaging about 0.5mm on transverse section, but which can average either 0.5 or 1.0mm in an antero-posterior direction, depending on the preparation. Even after small injections they extend over an area of at least 4 x 4mm (approximately 30 to 60 clusters). They contain numerous pyramids and stellates in the center of this area, but become smaller and consist only of a few pyramids more peripherally. The periodicity suggests that they relate to ocular dominance columns. Supported by MRC.

217 TEMPORAL PATTERN DETECTION IS NOT A FUNCTION OF ASSOCIATION CORTEX IN GENERAL. B.S. Layton,* A.W. Toga,* and S. Horenstein (SPON: R.G. Karis). Saint Louis University, Saint Louis, Missouri 63104.

The insular-temporal cortex (I-T) of the cat has been shown by anatomical or physiological methods to be multimodal, receiving auditory, visual and somatosensory projections as well as others from the posterior thalamic nuclear group, themselves multimodal. Colavita (Physiol & Behav, 18: 513-521, 1977) has shown that bilateral ablation of I-T results in inability to relearn a response to changes in temporal patterns of the form A-B-A to B-A-B independent of modality. This defect appears to be irreversible and does not occur after removal of large areas of primary sensory cortex (Colavita, 1977). The temporal pattern detection deficit cannot be explained simply as the result of indiscriminate loss of cortical tissue. It has not yet been established, however, whether loss of any heteromodal association cortex other than I-T might produce such a deficit. This investigation addresses the question of whether temporal pattern perception depends on the integrity of association cortex in general. The experimental design required the cat to detect changes in temporal patterns following bilateral symmetrical suprasylvian gyrus ablation. Adult cats were trained in a double-grill-box shock avoidance situation to detect a change in a continuous auditory temporal pattern from either soft-loud-soft to loud-soft-loud or the reverse. Each tone was 800 Hz and of about 900 msec duration. The tones were separated by about 100 msec of silence and the trials by 2000 msecs. The loud tone averaged 83 dB SPL and the soft 60 dB SPL. After achieving criterion performance of 18/20 correct detections on 2 successive days, large parts of the cortex of the suprasylvian gyrus were removed bilaterally in a single sitting. After a 2 week recovery period the animals were returned to the training situation and regained criterion level performance without delay. These results were compared to the performance of our own serially lesioned I-T cats and Colavita's simultaneously lesioned animals. The I-T cats showed no recovery of temporal pattern detection after weeks of postoperative training. Failure of suprasylvian gyrus ablation to interfere with this capacity demonstrates that temporal pattern perception is not a general function of association cortex. It appears somehow linked to the cells within the I-T region. The answer to the question of whether any other area of association cortex is similarly involved demands further research.

218 A NORADRENERGIC PROJECTION TO THE BARREL HOLLOWS OF MOUSE SOMATOSENSORY CORTEX.

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The noradrenergic (NA) innervation of mouse SI cortex was studied using the glyoxylic acid histofluorescence method, with particular attention to the distribution of NA fibers within the barrel field, a tangentially discontinuous specialization of layer IV. The demonstration of a NA projection to layer IV has been hampered by the difficulty in identifying cytoarchitectural features with fluorescence microscopy. This difficulty is obviated in rodent SI where the barrel field provides an unequivocal, in situ, marker for layer IV.

NA axons densely arborize in layers I,IV, deep V, and VI; the intervening layers have a markedly sparser innervation. The barrels are delineated by a striking autofluorescence restricted to the barrel hollows. Within the barrel hollows there are dense, ramifying nests of NA axons. The walls, in contrast, contain few fluorescent fibers. The barrel hollow is the principle site of interaction between thalamocortical (TC) afferents and the dendrites of stellate cells. The cohabitation of NA and TC afferents in the barrel hollows suggests that these two systems are intimately associated and may even share post-synaptic elements. The existence of functional noradrenergic innervation is supported by ultrastructural histochemical studies from our laboratory demonstrating numerous monoaminergic synapses in layer IV of young mouse cortex.

We find that in mouse SI cortex each of the zones that receives afferents from the thalamus - layers I,VI, the deep half of layer V, and the barrel hollows, subdivisions of layer IV - is also densely innervated by NA axons. We propose that the selective association between TC and NA afferents may be a general feature of cortical organization. Functionally, the postulated "regulatory" role of the NA system may occur at the level of the thalamic input to the cortex. (Support: USPHS NS-08153, NS-10920; H.G.W.L. supported by Training Grant GM-7309; F.L.R. supported by NIH Grant RR-5338 and fellowship NS-05790)

219 OXYGEN DISAPPEARANCE RATES IN THE GERBIL CORTEX UNDER HYPERBARIA.

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Oxygen disappearance rates were measured in the cerebral cortex of barbiturate-anesthetized Mongolian gerbils subjected to bilateral carotid ligation while breathing 100% oxygen at various levels of hyperbaria. The rates of disappearance were seen to be a linear function of initial tissue pO_2 below that of hemoglobin saturation but at higher values (pO_2 > 130) became pO_2 independent. These results are in agreement with those predictions made by the mathematical modeling of tissue oxygen consumption presented by Reneau (JAICE 15: 916, 1969) i.e., at lower pO_2 the desaturation of hemoglobin produces a slower rate of disappearance, but as more oxygen can be drawn from dissolved oxygen in tissue fluid as would be the case under hyperbaria, the rates of disappearance should be constant depending solely on the metabolic rate.

From the slopes of the oxygen disappearance curves, the metabolic rates were calculated by means of the mathematical modeling of Reneau. Since the measurements were made via an oxygen microelectrode with tip diameter of 1 μm, these measurements represent very focal metabolic activity. These metabolic rates were found to be depressed after chronic exposure to hyperbaria as would be expected from a possible toxic effect induced by long-term exposure to a pure oxygen atmosphere.

220 Identification Of Cerebral Cortical Afferent Terminals In The Pontine Nuclei Of The Rat Using Golgi, Electron Microscopic And Combined Golgi-Electron Microscopic Procedures.

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The massive input to the basilar pontine nuclei (BPN) from nearly all regions of the cerebral cortex has been reported with only relatively minor phylogenetic differences in a wide variety of mammals ranging from opossum to macaque monkey. Two recent publications from our laboratory have extended these earlier observations and demonstrated with autoradiographic procedures the pattern of organization in the projections to the pontine nuclei from sensorimotor (Brain Res. 145: 347-354) and visual (Brain Res. 143: 139-146) cortices in the rat. This information is significant in its own right but also provides a foundation for electron microscopic studies designed to clarify from a structural frame of reference the role played by such afferents in the overall synaptic organization of the BPN.

Two general categories of neurons have been described in the rat BPN processed according to routine rapid Golgi or Golgi-Kopsch protocols. These categories include 1) a local circuit neuron and 2) projection type neurons with axons coursing into the cerebellum via the brachium pontis. The latter category may include at least two sub-types, those with relatively spine-free dendrites (aspiney) and those exhibiting numerous spines along with a variety of other somal or dendritic protrusions (spiney cells). It was the intent of this study to focus on the spiney projection neurons and describe the morphological features of their synaptic interaction with cerebral cortical afferents.

Subsequent to unilateral decortication, two types of degenerating axon terminals were observed. One group was small (up to 1.3 μm), consistently contacted small dendritic or spinous profiles and exhibited all the typical features of electron dense degeneration. The other group was larger on the average (up to 2.2 μm), contacted both small and intermediate-sized dendrites and exhibited an initial lucent or filamentous reaction prior to becoming electron dense. When lesions were confined to sensorimotor cortex, primarily small dark boutons were observed while lesions restricted to visual cortices produced mainly the filamentous type of degenerating terminals. These findings suggest that at least two populations of cortical axons project to the pontine nuclei. Furthermore, when animals were processed according to combined Golgi-EM procedures, isolated spiney relay cells were contacted by both filamentous and dark boutons suggesting a convergence of these two cortical afferent systems.

Supported by NSF grant BNS 77-03263 to G. A. Mihailoff.

221 A LIGHT AND ELECTRON MICROSCOPIC STUDY OF LAYER I OF CAT PRIMARY VISUAL CORTEX AND ITS THALAMIC AFFERENTS.

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It is now known that primary visual cortex (area 17) receives input from more than one thalamic nucleus (Brain Res. 96:51,1975). We wished to expand on these findings in the cat by comparing cortical layers of termination and the ultrastructure of synaptic endings made by these thalamic regions. For light microscopy tritiated amino acids were iontophoretically or mechanically deposited in the dorsal lateral geniculate body (LGB) and the posterior thalamus. Layers I and VI of area 17 receive projections from the posterior nucleus (PN) and the pulvinar-lateral posterior nucleus (LP). However, layer IV and lower layer III receive input only from the LGB, corraborating numerous past studies. We further found that the extrageniculate projection to area 17 is visuotopically organized and reciprocal with the visuotopic organization of striatothalamic projections. The extrageniculate input to layer I is heavier than that to layer VI and was the focus of the electron microscopic studies. The ultrastructure of layer I shows a definite pattern in its composition. Adjacent to the pial surface is an organized astrocytic lamella of variable thickness made up of thick processes containing parallel glial filament bundles, microtubules, glycogen granules and mitochondria. Beneath the astrocytic lamella is an axonal plexus made up of both heavily and thinly myelinated axons. Proximal to the plexus is the general neuropil which forms the rest of layer I. The neuropil contains glial cell bodies but rarely a neuron. Its main composition includes numerous glial processes, a sparse number of scattered thinly myelinated axons, numerous dendrites and some unmyelinated axons. Occasionally the dendrites are seen with varicosities. Synaptic endings are seen throughout the layer extending from within the myelinated plexus to layer II. Apparently, they are exclusively of the asymmetrical type which contain round vesicles. The majority are small and end on dendritic elements which frequently contain a spine apparatus. However, there are also large boutons and it is not uncommon for boutons of any size to end on a dendritic trunk. We did not observe en passant contacts. Lesions were made of the thalamus with electrodes introduced through the opposite hemisphere. After lesions of the LGB some of the moderately to heavily myelinated axons found in the superficial plexus were degenerated. In contrast, after lesions of the posterior thalamus (which spared the LGB) the scattered, poorly myelinated axonal population was the one which degenerated. In these latter cases it was the small boutons in deeper layer I which showed evidence of degeneration.

(Supported by NSF Grant BNS 7507349)

222 IN VIVO STUDIES OF HYPOXIA ON CEREBRAL MITOCHONDRIAL NADH REDOX STATE AND EEG. Michael H. Mitnick, Dept. Physiology & Div. Neurosurgery, Sch. Med., Univ. of Pa. Phila., Pa. 19104

Using a television system microfluorometer suitable for in vivo studies, the effects of one to two minutes of hypoxia were noted for the intramitochondrial respiratory co-enzyme NADH and correlated with the EEG reaction to hypoxia. Optical monitoring of cortical areas was recorded on video tape for subsequent play back and data analysis, thus affording the opportunity to study the effect of the same hypoxic period on different geographic areas of cortex as well as the same spot over repeated hypoxic insults.

The results revealed three definite patterns of the brain's response to hypoxia. First, the NADH redox state response was heterogeneous both qualitatively and quantitatively over the surface of the cortex. Second, the NADH response was noted in almost all cases to differ, again both qualitatively and quantitatively, in the same field, with respect to the number of hypoxic exposures. Thus, as a consequence of the second or third hypoxic period, all showing similar blood gas changes, the NADH reduction was not constant. Lastly, the time from the introduction of hypoxia until the first discernable alteration of EEG Activity was noted decreased with subsequent hypoxic periods. For example, the second hypoxia, with identical blood gas changes to the first hypoxia, caused the EEG to begin to falter from 12% to 62% faster than the first hypoxic period.

The geographic heterogeneity might be reasonably expected as a result of various areas functional differences, cellular composition differences, regional variations of metabolic rate, etc. However the diversity of metabolic response with respect to the number of hypoxic periods and the apparent degenerate nature of the EEG response to hypoxia suggests the possibility of either a Delivery Lesion, where blood O_2 does not effectively reestablish transport to the mitochondria, or else a utilization lesion, where although intracellular Po_2 might be returned to control values, the bioenergetic machinery and/or ionic transport systems are unable to effectively function. These hypothesis are currently under study using the intracellular O_2 probe pyrenebutyric acid (PBA).

Supported by NINCDS 5F32 NS 05500.

223 DEGENERATION OF THE COERULEO-CORTICAL PROJECTION: ORGANIZATION AND IMMUNOHISTOCHEMICAL CHARACTERISTICS. J. H. Morrison, M. E. Molliver & R. Grzanna* Dept. of Cell Biology & Anatomy, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Immunofluorescent (IFL) staining using a homologous antiserum directed against rat dopamine-ß-hydroxylase (DBH) demonstrated an abundant noradrenergic (NA) innervation in all areas of neocortex. The distribution of NA axons is characterized by a geometric orderliness that exhibits a predominantly tangential organization*. We have utilized a variety of lesions to analyze this projection and to demonstrate more definitively the specificity of the IFL staining. The most frequently employed controls for IFL specificity (e.g., omission, absorption or replacement of the primary antiserum) do not exclude the possibility that the antiserum may cross-react with tissue proteins other than the specific antigen. In order to show that the antiserum is a specific marker for NA neurons, it is necessary to demonstrate the absence of staining following selective and complete destruction of the ascending NA projection. Hence, we have made a series of bilateral electrolytic lesions of the dorsal NA bundle in the midbrain and a series of bilateral microinjections of 6-hydroxydopamine (6-OHDA) at the identical site; brains from both groups were analyzed by means of glyoxylic acid induced histofluorescence (GAIF) or with DBH IFL. Both types of lesion resulted in a complete or nearly complete loss of GAIF in NA axons throughout the cortex; notably, dopamine axons in anterior cingulate cortex remain brightly fluorescent, indicating the high sensitivity of this method. Five weeks after electrolytic lesions, the IFL method reveals a pattern of DBH-immunoreactive axons identical to that seen in unlesioned rats. In striking contrast, 6-OHDA lesions result in complete disappearance of DBH-positive axons throughout the cortex, a result which demonstrates conclusively the specificity of the antiserum for NA axons. Electrolytic lesions demonstrate the persistence of immunoreactive (but enzymatically inactive) DBH in axons which appear morphologically intact although mechanically severed from their cell body. The loss of immunoreactivity following the 6-OHDA lesion suggests that the axonal degeneration may depend on anterograde transport of this neurotoxin. These results indicate potential pitfalls in the interpretation of IFL stains following lesions. For example, it has been necessary to utilize GAIF to demonstrate the interruption of NA axons following coronal cuts of the dorsal neocortex. Initial results reveal a drastic reduction in NA fibers in all cortical layers caudal to the lesion and support our proposition that the NA projection takes a tangential course through the grey matter. Consequently focal cortical lesions may result in noradrenergic denervation of distant cortical areas. (Support: USPHS grants NS-08153 & NS-10920).

*ref: J.H.Morrison et al, J. Comp. Neurol. 1978.

224 HEMISPHERIC SPECIALIZATION OF FACIAL RECOGNITION IN MAN BUT NOT IN MACAQUE. William H. Overman, Jr. and Robert W. Doty. Center for Brain Research, University of Rochester, Rochester, N.Y.14642

Among the various assymmetric functions of the human cerebral hemispheres one of the best documented is the superior ability of the right hemisphere for processing pictures of faces. One test requires the subjects to compare an unaltered photograph of a face with two composites of the same face which consist of the right half of the face plus its mirror image and the left half of the face and its mirror image. When asked to choose a composite which looks most like the unaltered face, normal right handed subjects on 70% of the trials choose the composite made from the right side of the face. This composite consists of features which fall primarily in the left visual field (LVF) when the normal face is viewed. Lesions of the right but not of the left hemisphere reduce the LVF bias to chance levels (Kolb, Milner and Taylor, in press, 1978). Twenty human subjects and six monkeys (Macaca nemestrina) were given this split-face test of facial recognition. The human subjects received 38 split-face comparisons (28 human faces and 10 monkey faces) in one test session while the monkey subjects received the identical split-face test trials at 3/day interspersed among 30 simultaneous matching to sample (SMS) trials which used human or monkey faces as stimuli. When viewing these photographs of monkey faces, both monkey and human subjects had a LVF bias no greater than chance (52% and 54%). However, when viewing human faces, the monkey subjects again showed no significant LVF bias (53%) while the human subjects had a LVF bias of 68%. This difference between man and monkey is statistically significant, $p < .01$. Two additional experiments indicated that monkeys perceive and react to projected colored photographs of faces as real faces. Monkeys displayed species-typical reactions to pictures of human and monkey faces but not to equally complex, non-facial stimuli. Inversion of the stimuli on a SMS task dramatically reduced recognizability of facial stimuli but not other classes of stimuli. Together these findings indicate that both monkey and human subjects perceive and react to photographs of faces in a similar manner but that man processes facial stimuli preferentially with one cerebral hemisphere while the macaque apparently uses either or both hemispheres.

(Supported by USPHS Grant NS 03606 from NINCDS).

225 THREE DIMENSIONAL ANALYSIS OF THE BRANCHING CHARACTERISTICS OF GOLGI IMPREGNATED PYRAMIDAL NEURONS IN THE HIPPOCAMPUS OF HUMAN IMMATURE BRAIN. Albert M. Paldino and Dominick P. Purpura. Dept. Neuroscience, Albert Einstein College of Medicine, Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Bronx, New York 10461.

A unique version of the computer microscope has been utilized to obtain three dimensional coordinate information of fiber networks from Golgi impregnated neurons. A microscope-closed circuit TV system, and its associated electronics, was interfaced to an Interdata 7/32 minicomputer to provide an arbitrary code and the X-Y-Z coordinates of preselected topological sites on the fiber network. A dynamic representation of the evolving fiber network was presented to a Tektronix graphics terminal to assist the operator during the data input phase and also in the detection of any asymmetries inherent in the fiber network. To date, twenty five pyramidal neurons from the hippocampus of an 18 to 20-week-old human fetus (natural abortion) were measured and analysed according to their axonal and dendritic branching characteristics. Polar angle and H-distributions of axonal and dendritic terminals were obtained. These distributions suggest, in part, that 1) the large majority of axonal terminals end at a level below (within 100 µm) their respective soma locations; 2) no dominant polar angle exists for dendritic terminals; and 3) most dendritic terminals are confined to a level within 100 µm above their respective cell body locations. Also, radial and cylindrical radius distributions for these terminals indicate that most axonal and dendritic terminals are confined to lie within a sphere whose radius is approximately 120 µm and which is centered at the cell body. Branching angle distributions were obtained which suggest that the average branching angle for both axons (75^o) and dendrites (72^o) were approximately equal. Finally, fission angle distributions indicate that the average fission angle (that angle formed between two daughter segments at a bifurcation) for both axons (87^o) and dendrites (88^o) were also approximately equal. It has been shown that this system is capable of generating computer tracings from the three dimensional coordinate data which, compared to the corresponding camera lucida drawings, possess a high degree of accuracy. Furthermore, any orthogonal projection of the neuronal network can be obtained. Additional studies involving progressively more developed neurons from older fetuses and infants will be discussed. The data that will be obtained bear on the quantitative analysis of neuronal branching patterns in conditions associated with cases of (profound) mental retardation having a developmental and/or chromosomal substrate.

226 ASSOCIATIVE FUNCTION OF THE ARCUATE ZONE IN THE MONKEYS' PREFRONTAL CORTEX. Linda Z. Podbros* and John S. Stamm, Dept. Psychol., SUNY, Stony Brook, NY 11794.

Functional dissociations have been identified among distinct zones within the monkey's dorsolateral prefrontal cortex (e.g., Goldman et al., Exp. Neurology (1970), 27, 291-304; Stamm, J.S., Neuropsychologia (1973) 11, 181-190). Ablations of cortex in the arcuate sulcus (ARC) have resulted in severe deficits on an auditory conditional position response (CPR) task. The present experiment is concerned with ARC functions on a CPR with visual cues and with differing spatial cue-response contingencies. Seven Macaques were trained in a Wisconsin Test Apparatus that contained a dark vertical testing panel and two food boxes recessed within the left and right walls of the compartment. Two task variations were used: for vertical CPR, the cue (white light) was presented at the top or bottom of the panel, with reward in the left or right box, respectively; while for horizontal CPR, the cue at the left or right side of the panel indicated reward in the opposite direction. Bilateral ablations were made to either the arcuate sulcus (ARC), the principal sulcus (PRIN) or to cortex inferior to the principal sulcus (IDL). With a multiple-stage procedure a total of 15 ablations were applied. Post-operative training to the criterion of 85 correct responses in 100 trials showed substantial performance deficits only after ARC lesions. The means of total errors for both tasks were: 137 for the ARC, 22 for the PRIN, and 45 for the IDL group. The deficits were significantly greater for the ARC than either of the other groups. Also, greater ARC deficits were found for the vertical than for the horizontal CPR. These findings support the unique role of arcuate cortex in the mediation of spatial-response contingencies in tasks where the cue is located in a direction different from that of the instrumental response, regardless of the modality of the cue. It is suggested that the proprioceptive stimuli elicited by the monkey's orientation to the cue serve as guidance for the appropriate instrumental response; and this associative process is disrupted by ARC lesions.

227 MEMBRANE PROPERTIES OF IDENTIFIED HUMAN CORTICAL NEURONS. D.A. Prince, R.K.S. Wong and A.I. Basbaum, Dept. of Neurology, Stanford University School of Medicine and Dept. of Anatomy, UCSF School of Medicine.

The use of the in vitro brain slice technique, together with intracellular labelling with horseradish peroxidase has allowed us to begin to examine the membrane properties of identified human neocortical neurons. Eighteen high quality stable intracellular recordings were obtained from slices of neocortical biopsies in 3 patients. In all cases removal of normal cortex was required to approach a remote lesion. Three neurons showed short duration action potentials (< 1 msec) and a steep increase in firing rate with increasing depolarizing current. One of these cells was labelled with HRP and appeared to be a spiny stellate interneuron. Fifteen neurons showed long duration spikes (> 1 msec); 4 of these were labelled and appeared to be pyramidal in type. The passive properties of 4 labelled pyramidal type neurons were as follows; membrane potential (Vm) 67.5 mV (64-70 mV); input resistance (R_{in}) 39MΩ (30-50 MΩ); time constant 36.7 msec (25-55 msec). Fourteen of the 15 cells with >1 msec duration spikes showed subthreshold nonlinear membrane properties which were both voltage and time dependent. During hyperpolarizing pulses V_m reached a maximum in 20 msec and then "sagged" to a less polarized level. During depolarizing current pulses there was an apparent increase in R_{in} which began about 20 milliseconds after the onset of depolarization. Such anomalous rectification has not been previously described in neocortex. Nine of 15 neurons in this group generated depolarizing afterpotentials (DAPs) following single spikes. DAPs could be evoked by 2 millisecond current pulses in some cells and reached a maximum amplitude of 10 millivolts, with durations of up to 20 milliseconds. These potentials disappeared when V_m was shifted in a depolarizing direction. In 3 instances DAPs appeared to be responsible for generation of a second spike during threshold depolarizations or the rebound from hyperpolarizing current pulses. Trains of evoked spikes were frequently followed by afterhyperpolarizations and increased conductance lasting as long as 1 sec. One neuron from the cortex of a two year old generated high threshold tetrodotoxin resistant spikes during depolarizing pulses. These properties of human cortical neurons are entirely similar to those described in a variety of other species. The presence of anomalous rectification, depolarizing afterpotentials, and post activation hyperpolarizations suggests a possible role of calcium conductances in the control of activity in these cells. The neocortical slice preparation will prove useful for further studies of differences in properties of pyramidal type cells and interneurons. Supported by NIH Grants NSO6477, NS12151, NS11614, and DA01949.

228 THALAMIC PROJECTIONS TO AREAS 5 AND 7 OF PARIETAL CORTEX IN THE CAT. Richard T. Robertson, Department of Anatomy, College of Medicine, University of California, Irvine, CA. 92717

Projections from the thalamus to areas 5 and 7 of parietal cortex were studied in cats by the method of retrograde transport of horseradish peroxidase (HRP). Visually guided unilateral or bilateral injections of 50 - 100 nl of 40% HRP in water or saline were placed 0.5 - 1.0 mm below the pial surface. Following survival times of 1 - 3 days, animals were sacrificed by perfusion with 0.5% paraformaldehyde and 1% glutaraldehyde. Standard histochemical processing using benzidine dihydrochloride as the chromagen demonstrated both retrograde labelling of cell bodies and anterograde labelling of axon terminals. HRP reacted and adjacent unreacted sections were counterstained with neutral red to identify the precise injection site and the thalamic nuclei containing retrogradely labelled cells.

Cortical area 5a, situated in the banks of the ansate sulcus and extending onto the anterior suprasylvian gyrus, receives prominent projections from the medial division of the posterior group of nuclei (Pom). Less prominent projections originate in ventral parts of the lateral posterior nucleus (LP) and the central lateral nucleus (CL). A very few labelled cells were observed in the ventral lateral nucleus (VL) and paracentral nucleus (PC). Cortical area 5b, situated just caudally to area 5a, receives a major projection from the rostral and ventral parts of LP. Additional, but substantially less impressive, projections originate in CL, VL, and Pom. The ventral anterior nucleus (VA) sends a light projection to area 5b on the suprasylvian gyrus, but not the marginal gyrus.

Cortical area 7, lying just caudally to area 5b, receives prominent projections from LP and the rostral pulvinar (Pul). In addition, retrograde labelling indicates light projections from CL, PC, and the ventral lateral part of the lateral dorsal nucleus (LD). VA projects lightly to area 7 of the suprasylvian gyrus, but not the marginal gyrus.

Supported by NSF grants BNS 76-08523 and 77-23361, and NIH grant NS 14267.

229 DIFFERENTIAL BEHAVIORAL EFFECTS OF RIGHT VS LEFT CEREBRAL INFARCTION: EVIDENCE FOR CEREBRAL LATERALIZATION IN THE RAT. R. G. Robinson Johns Hopkins University School of Medicine, Baltimore, Md. 21205.

Right middle cerebral artery ligation in the rat leads to a transient period of spontaneous horizontal hyperactivity as measured in a photocell chamber (Nature 255:332, 1975). We have reported that this hyperactivity can be blocked by post-operative treatment with the norepinephrine (NE) uptake blocker desmethylimipramine or by preoperative destruction of catecholaminergic neurons with 6-hydroxydopamine (Biol.Psychiat. 12:669,1977) and that there is a transient decrease in the number of fluorescent varicosities in widespread areas of brain during this hyperactive period (Brain Res.132:259,1977). The current experiments were carried out using cages which allowed free access to food, water and a running wheel. Following right middle cerebral artery ligation (N=14) as compared with sham operated control animals, there was a transient increase in the number of revolutions per 24 hours of the running wheel. If the animals were postoperatively placed in these cages as a novel environment, the hyperactivity was evident by one day after cerebral infarction and lasted 10 days, while if the animals were allowed previous access to the cages before cerebral infarction, activity did not exceed preoperative control levels until 10 days after infarction. Animals undergoing left middle cerebral artery infarction (N=18) did not show any of these changes in spontaneous activity regardless of whether they were exposed to the running wheels as a novel or a familiar environment. Gross and histological observation of the infarcted brains revealed that left middle cerebral artery ligation generally resulted in a slightly larger infarct (but still limited to frontoparietal cortex) than did right middle cerebral artery ligation, but that when comparable sized lesions were obtained, animals with left hemispheric infarcts were not any more active than sham operated controls. There were no significant differences in food or water intake between animals with right or left hemispheric infarcts. We are currently investigating whether these behavioral differences are the result of differential effects of cerebral infarction on catecholaminergic neurons on the two sides of the brain.

230 UNIT ACTIVITY IN THE VENTRAL PREFRONTAL REGION OF MONKEYS PERFORMING IN VISUAL AND SPATIAL DELAYED REACTION TASKS. Carl E. Rosenkilde and Joaquin M. Fuster. Brain Research Inst. and Department of Psychiatry, UCLA, Los Angeles, CA 90024.

Extracellular unit activity was recorded with microelectrodes from the ventral prefrontal region in two rhesus monkeys performing in delayed matching to sample (DM) and spatial delayed response (DR). At the start of a trial a cue light was projected on a single translucent response key. In DM, the light was green or red, and centrally located; in DR, it was white and located on the right or left. A press on the key by the animal extinguished the light and initiated a delay lasting 6 to 10 sec. After the delay, two keys were illuminated for choice. Choice of the key whose color (DM) or position (DR) matched that of the cue resulted in delivery of juice into the animal's mouth. The intertrial intervals were 40 sec. Sixty-two of 101 units were held long enough to obtain data for both tasks and were analyzed off-line with a PDP-12 computer.

Changes of firing frequency in 85 cells were related to task events. The most common changes were observed in 82 cells at onset or offset of the cue. Half of these cells showed differences in discharge dependent on the color or position of the cue. Altered activity during the delay was observed in 51 units, 41 of which showed differential firing in relation to characteristics of the (no longer present) stimuli. Some units showed inhibition or no change in firing during the delay of trials with incorrect choice, whereas they had exhibited activation of firing during delays of correct trials. Forty-three cells changed discharge rate in the post-trial period; typically this change began 0 to 4 sec after the choice. Analysis of firing after errors and after gratuitous delivery of juice showed that post-trial activity was dependent on reward in some but not all cells. Most cells showed altered activity in several phases of the trials and differential responses in at least one phase. A typical cell would display inhibition at cue offset, excitation throughout the delay after a specific cue, and reactivation in all post-trial periods.

These results indicate the involvement of the prefrontal region in visual analysis and reaffirm the importance of this area in delayed reaction behavior. The post-trial activity may serve to represent information on the reinforcing effects of the instrumental choice, or to erase mnemonic consequences from discharge during the delays. (Supported by NSF grant BNS 76-16984 and the Danish Medical Research Council.)

231 CONVERGENCE OF VISUAL- AND SOMATIC SENSORY-RELATED CORTICAL PATHWAYS IN THE LOWER BANK OF THE INTRAPARIETAL SULCUS OF THE RHESUS MONKEY. Benjamin Seltzer* and Deepak N. Pandya, V. A. Hospital, Bedford, MA 01730 and Harvard Neurological Unit, Beth Israel Hospital, Boston, MA 02215

In the course of an architectonic study of the parietal lobe of the rhesus monkey, a distinct architectonic zone has been identified in the lower bank of the intraparietal sulcus. This region is situated caudal to area 2 but rostral to area 19. In terms of both cyto- and myeloarchitecture, it differs from neighboring zones, notably area 7 (PG) of the inferior parietal lobule. Characterized by a broad, but cell-sparse, third layer with prominent IIIc cells, this sulcal zone has relatively lightly populated fourth, fifth, and sixth layers. At more caudal levels, however, the lateral portion is more densely cellular. With regard to myelination, this zone has clearly defined inner and outer bands of Baillarger, as well as a dense plexus of vertically-oriented fibers deep to the inner band.

An analysis of cortico-cortical connections, studied by both autoradiographic and silver impregnation techniques, demonstrates a specific pattern of projections to this architectonic region. Thus, it is the recipient of a major projection from visual-related cortex of the peristriate belt, especially the lateral surface of the preoccipital gyrus. It also receives afferents from parietal cortex, but only from a small area in the rostral inferior parietal lobule (area PF of Bonin and Bailey), a region which itself receives input from the lower sector of somatic sensory cortex (head, neck and face representations) in the postcentral gyrus.

The lower bank of the intraparietal sulcus thus receives converging input from both visual- and somatic sensory-related cortical areas. Furthermore, since the rostral inferior parietal lobule also receives vestibular input (Frederickson et al., 1966), this suggests a possible functional role for the intraparietal sulcus in the integration of kinesthetic input from head, face and neck with vestibular and visual information.

Supported by, NIH grant NS 09211 and V. A. Research Project #6901.

232 GOLGI-COX IMPREGNATED BARREL NEURONS IN THE RAT SmI CORTEX. D.J. Simons* and T.A. Woolsey. (SPON: M.A. Fishman). Dept. of Anat. and Neurobiol., Washington U. Med. Sch., St. Louis, MO 63110.

Layer IV of SmI neocortex in rodents is characterized by discrete cellular aggregates called "barrels". Each barrel is related in a one-to-one fashion to a single vibrissa on the contralateral face. A previous Golgi study of mouse cortex demonstrated that two major classes of cells can be distinguished according to a number of morphological criteria. Since there are differences in the cytoarchitectonic appearance of the barrels in rat and mouse, we wanted to compare the two species with respect to neuronal morphology. In addition, we were interested in determining how these data compared with physiological studies. The brains of adult rats were prepared by the Golgi-Cox technique and counterstained with a Nissl stain so that the barrels could be visualized. Thick sections (125 μm) were cut in coronal or tangential planes. The principal results are: 1.) As in mouse, two classes of cells can be distinguished; class I cells have spinous dendrites where as the dendrites of class II cells are smooth with beads. The somata of class II neurons are quantitatively larger than those of class I. 2.) The majority (about 80%) of cells of both classes have their dendrites restricted to the barrel in which their somata are located. The remainder of cells distribute their dendrites to two or more barrels and most of these neurons have their somata located in the septa between barrels. In mouse 85% of cells have their dendrites restricted to a single barrel. 3.) In our present material it appears that there are fewer smooth cells than spiny ones. These cells are found in equal number in the mouse. The data indicate a marked similarity among neurons in homologous cortical regions in two different species, the mouse and the rat. Further, the results in the rat are consistent with our physiological studies which show that in layer IV two types of units can be distinguished on the basis of bioelectric properties and that in layer IV a majority of units of both types respond to deflection of single whiskers only.

Supported by NS10244, EY01255, NS07057-02.

233 CORTICAL PROJECTIONS OF THE LATERAL DORSAL NUCLEUS IN THE RAT. T. Spiro, L.C. Massopust, and P.A. Young. Dept. Anat., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Many studies have shown that the cingulate and subicular cortices project to the lateral dorsal nucleus (LD) of the thalamus. However, reciprocal connections have not been described. Therefore, the cortical connections of the LD were studied using autoradiography. Discrete amounts (0.4 μl) of 3H-leucine (50 μCi/μl New England Nuclear) were injected at separate anterior and posterior locations in the LD. From both locations labeled axons emerged laterally from the thalamus, passed through the superior thalamic radiation, and entered the caudate-putamen complex. Once within the caudate-putamen complex a number of labeled fibers coursed rostrally and terminated in layers I and III of areas 23 and 24 in the ipsilateral anterior cingulate cortex. The remainder passed dorsally where they entered the fasciculus cinguli and projected to the ipsilateral posterior cingulate and subicular cortices. Terminations were observed in areas 29b and 29c of the posterior cingulate cortex. Within area 29b, silver grains were arranged in layers I and III at its border with area 29c. In the anteriorly injected animals, area 29c exhibited an input to layers I, III, and IV at the lip of the hemisphere. After injections in the posterior part of the LD, a stronger distribution was observed within the same laminae at the border of 29b and c. Projections were also observed in the lamina dissecans and externa of the pre- and parasubiculum. These results show that the LD has strong reciprocal connections with the anterior and posterior parts of the cingulate gyrus and the subicular cortex.

(Supported in part by USPHS grant FR 05388.)

234 EPd INFLUENCES VISUALLY DIRECTED BEHAVIOR. A.W. Toga,* B.S. Layton,* S. Horenstein, and D.G. Davenport.* Saint Louis University, Saint Louis, Missouri 63104.

Unilateral ablation of the entire ectosylvian region has been shown to result in specific changes in the pattern of visual choice on double simultaneous bilateral stimulation (DSS). This region has been shown to project to the homologous contralateral as well as the adjacent suprasylvian cortex. The goals of this study were to explore the relative influence of each component of the ectosylvian region on visually directed behavior and to consider the proposition that biased behavioral preference reflects hemisphere imbalance which can be altered toward normal by a contralateral lesion.

Eleven adult female cats responded to visual stimulation by entering either of two alleys of an automated equiangular Y-maze. Correct responses were rewarded by food. The subjects were trained to choose the illuminated alley when only one was lighted, but could select either compartment when both were lighted. The latter condition was DSS and presented randomly 20% of the time. Most animals demonstrated a hemiplane of response preference, the other side being referred to as subordinate. After preoperative learning and testing, one or more portions of either the ectosylvian region or the suprasylvian gyrus on the subordinate side was ablated. The postoperative performance on the same task was recorded after a suitable period of recovery. Then one or another portion of the contralateral ectosylvian or suprasylvian region was removed and the animal retested.

When a subject with unilateral cerebral damage responded at preoperative levels to single stimuli but changed its benavior upon DSS, it was regarded as having met the operational criterion for lateralized visual neglect.

In ten cases the lesions were confined to the intended regions and did not penetrate the optic radiations. In the eleventh, the second lesion extended into the deep white matter of the hemisphere resulting in an apparently hemianopic animal.

Within the temporal lobe, only lesions which included the posterior ectosylvian gyrus resulted in unilateral visual neglect. Further, if subsequent to its production the contralateral homologous cortex was ablated, the degree of neglect lessened and task behavior approached that of the original state. The functional mechanism thus appeared to seek equilibrium as the brain was restored to structural balance. This study then contributes to understanding of the role of the EPd cortex as an area involved in the regulation of visual behavior. Since it also receives projection from A_1A_{11} and the auditory thalamus, it may play a role in auditory-visual association.

235 EXCITABILITY CHANGES IN CORTICAL NEURONS FOLLOWING SLOW FREQUENCY ANTIDROMIC AND ORTHODROMIC ACTIVATION. E. Tzebelikos* and C.D. Woody. (SPON: E. Eldred) UCLA Med. Center, Los Angeles, CA. 90024

Effects of antidromic and orthodromic activation on the excitability of 63 neurons of the coronal pericruciate cortex were studied in awake cats. Both types of activation were accomplished by stimulating the pes pedunculi bilaterally with bipolar concentric electrodes (stereotaxic coord.: F 3.5, L 4.0, H 4.5) while recording intracellularly from the cortical unit. Single square pulses of 0.2 msec duration and 5 to 25V intensity were delivered at frequencies of 4-6/sec. The stimulation ordinarily produced facial movements such as eyeblink or nose twitch. Cortical cells were classified as antidromically responding if the spike latency measured at the peak amplitude of the induced discharge varied less than 0.05 msec. The cell was considered to be orthodromically responsive if otherwise activated. Other criteria for identifying antidromic discharge such as rates of following were not used because the procedures could have influenced unit excitability.

Unit excitability was measured separately as the threshold level of intracellularly injected current required for spike initiation (rectangular depolarizing pulses of 10 msec duration delivered at 100 msec intervals through the intracellular recording electrode). At least four such measurements were made during the period of stimulation of the pes pedunculi. When all measurements were consistently below or above the threshold initially measured for the cell, the excitability was considered increased or decreased, respectively. Neurons were studied for periods of 5 to 15 minutes. The resting potentials averaged 46+15mV. Mean threshold before stimulation was 0.64+0.20nA. The antidromic group included 28 neurons. In 9 of these cells excitability remained unchanged (0.69+0.27nA), in 11 it decreased (0.98+0.33nA), and in 8 it increased (0.32+0.20nA) during the stimulation period. In the orthodromic group (35 cells), 18 cells showed increased excitability (0.35+0.26nV) during the stimulation period, 5 decreased (0.92 +0.34), while 12 showed no appreciable change (0.70+0.30nA). 30 cells with resting potentials averaging 45+14mV, that were neither antidromically or orthodromically activated by the pes pedunculi stimulation formed a control group. Their mean threshold before stimulation was 0.65+0.34nA. During the period of the pes pedunculi stimulation this mean was 0.67+0.33nA. The effect on neural excitability of antidromic activation differed significantly from that of orthodromic activation (P = 0.02, Chi Square).

These direct observations of altered neuronal excitability compliment the assertion of Bindman et al. (J. Physiol. 1976) that high frequency antidromic activation of the pyramidal tract results primarily in decreased cortical excitability. Supp. by BNS 76-06886.

236 PROJECTIONS FROM THE ANTERIOR ECTOSYLVIAN GYRUS TO THE PERIRHINAL CORTEX. K. Yamaguchi and S. Horenstein. Saint Louis University, Saint Louis, Missouri 63104.

Projections from the anterior ectosylvian gyrus which have been commonly regarded as part of the secondary somatosensory system were studied by means of Fink Heimer, autoradiographic, and horse radish peroxidase methods. That the region was part of the somatosensory cortex was assured as previously described projections from it to the walls of the cruciate sulcus and sigmoid gyri (motor cortex), posterior sigmoid and coronal gyri (somatosensory I) and the suprasylvian sulcus were identified. Projections to the lateral wall of the posterior rhinal sulcus were found in each animal. We were unable to find an account of them in available literature. This projection appears to be direct and is represented within the perirhinal regions by a compact bundle of degenerating fibers stained by the Fink Heimer method which is found in the white matter deep to that part of the cortex which forms the lateral bank of the rhinal sulcus. It is distributed in columnar fashion, the fibers of termination appearing in the 2nd, 3rd, and molecular layers. Those in the third appear most dense. The course of this projection from the anterior ectosylvian lesion could not be traced. On autoradiography, grains were arranged in a columnar fashion in the superficial three layers of the cortex of the posterior rhinal sulcus, but were most dense in the third. Their cells of termination could not be identified. Injection of horse radish peroxidase into various perirhinal sites by stereotactic placement disclosed that only when the lateral bank of the posterior rhinal sulcus was injected at the plane of the posterior suprasylvian sulcus were cells of the anterior ectosylvian region filled with the reaction product. The cells of origin of the ectosylvian-rhinal projection appeared to be medium sized third layer and rare fifth layer pyramidal cells. We have found no studies describing the efferent projections of the lateral bank of the posterior rhinal sulcus of the cat. A similar region in the monkey is believed to have reciprocal connections with the entorhinal area (area 28). It would appear, therefore, that the secondary somatosensory cortex of the anterior ectosylvian gyrus has direct projections to the perirhinal cortex which may constitute a means of entry from it to the limbic system independent of other putative projections. Their functional significance is not currently known.

237 HISTOPATHOLOGY OF ELECTRICAL STIMULATION OF BRAIN: THE ROLE OF CHARGE DENSITY. Ted G.H.Yuen*, William F.Agnew, Leo A.Bullara* Deane B.Jacques, Robert H. Pudenz*. Neurological Research Laboratory, Huntington Inst.of Appl. Med. Res. Pasadena,CA 91105.

This report is a continuation of studies on the histopathological effects of chronic electrical stimulation of cat cerebral cortex surface using platinum and rhodium electrodes. The work comprises a part of a Neural Prosthesis Program whose ultimate aim is to develop techniques for supplementing or replacing lost function in persons with varied neurological deficits.

In previous brain stimulation experiments neural damage was assessed with respect to the relative significance of charge per phase (Q/ph), charge density per phase (QD/ph) and current density per phase (J/ph) when varied in 16 separate combinations. Although neural damage thresholds for 36 hour surface stimulations were roughly estimated, histological evaluation indicated a need for more definitive correlation between the extent of damage and responsible electrical parameters.

In the present study the relationship of QD/ph to neural damage has been investigated using surface stimulation of the parietal cortex of cats. Light and electron microscope studies were carried out on electrode sites of animals stimulated over a QD/ph range of 10 to 300 $\mu C/cm^2/ph$ for 36 hour (9 hr./day) stimulations. QD/ph variations were achieved by altering the electrode size (1.1 to 3.6 mm dia.) or Q(0.1 to 3.0 $\mu C/ph$). The neural damage threshold was observed at a QD/ph of approximately 20. Seizure activity appeared to be a function of numbers of neurons activated, i.e., when induced by using combinations of electrode size and QD/ph. For example, severe generalized seizures were observed with electrode sizes of 3.6 mm dia. (QD/ph 10) or 1.1 mm dia. (QD/ph 50-300) but were not observed with 1.1 mm dia. electrodes at QD/ph of 20 or 30. Work to date indicates that QD/ph-hours is the most closely correlatable parameter relative to neural damage.

Cerebrovascular permeability increases as indicated by Evans blue dye and horseradish peroxidase extravasation were generally limited to leptomeningeal vasculature even with the highest stimulation parameters. Histological changes were restricted to tissue 1-3 mm subjacent to stimulated electrodes and progressed in severity from mild to irreversible over a QD/ph range of 30 to 300. Ultrastructural alterations included increased sub-pial glycogen, astrocytic hypertrophy, and dendritic degeneration. With higher stimulations intracellular lipid and calcium hydroxyapatite crystals were present in degenerating neurons, astrocytes, axons and dendrites accompanied by marked phagocytic activity. Supported by Contract #NO1-NS-02275. Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD.

238 FRONTAL LESIONS IN THE FERRET: CHANGES IN SPATIAL ALTERNATION AND ACTIVITY LEVEL. Yvette Zatz*, Ausma Rabe, R.K.Haddad, Ruth Dumas*, M.H.Lee* and H. Wisniewski*. New York State Institute for Basic Research in Mental Retardation, 1050 Forest Hill Road, Staten Island, N.Y. 10314.

Ferrets with bilateral frontal lesions were tested for spatial alternation in a T-maze and activity in an open field, both before and after surgery. Preoperatively, using a massed trials procedure, the ferrets were trained to alternate left and right turns in the maze to a criterion of 8 correct choices in 10 trials on two consecutive days. They were allowed a few laps of milk as a reward for correct choices. The open field measure of activity, the number of squares crossed, was taken for 5 minutes daily for 3 consecutive days. Dorsolateral frontal lesions were produced by aspiration (n=10). For the sham-operated control group (n=9) the skull was opened to expose the frontal cortex, but no tissue was removed. After recovery from surgery, all subjects were retrained following the same procedures.

The frontal ferrets were markedly impaired in their spatial alternation performance and were hyperactive in the open field. In the T-maze, the frontal ferrets made more errors postoperatively than preoperatively ($p < .001$) and also made more errors than the sham-operated controls ($p < .0001$). In the open field, the frontal ferrets were more active postoperatively than preoperatively ($p < .02$) and they were more active than the sham-operated controls ($p < .01$).

Enhanced activity level is a well established consequence of frontal lesions in cats and monkeys (e.g., Warren and Akert,eds: The frontal Granular Cortex and Behavior, 1964), as are deficits in spatial and alternation learning (e.g., see Markowitsch and Pritzel, Psychol. Bull., 1977, 34, 817-837, for data on rats, cats, and monkeys). The deficit in spatial alternation and the enhanced level in activity which we have demonstrated in the ferret with frontal lesions are thus consistent with the findings on frontal lobe function in other animals.

CHEMICAL SENSES

239 CHARACTERIZATION OF RECEPTOR SITES FOR INTRAVASCULAR TASTE IN FROGS. Peter A. Balnave* (SPON: J.H. Teeter). Monell Chemical Senses Center, U. of Pa., Philadelphia, PA 19104.

Intracellular recordings from taste receptor cells and nerve recordings from the lingual nerve indicate that those receptor sites which give rise to intravascular taste obey the same physicochemical laws as those at the apical surface of the taste cell. The response of the taste cell to intravascular stimulation is primarily dependent on the capillary permeability of the stimulus and the clearance of the stimulus from the extracellular space. The permeability of the capillary wall may be radically increased by using a non-oxygenated perfusate (Landis, Am J Physiol 83:528,1928). By restricting percutaneous O_2 absorption and by using non-oxygenated perfusates, intracellular recordings to NaCl, LiCl, NH_4Cl, $CaCl_2$, CH_3COOH, quinine-HCl, sucrose and saccharin were obtained. Tentative evidence suggests that the taste response profile of a receptor cell as determined by stimulus flow over the surface of the tongue is similar to that determined by intravascular stimulation. Recordings from the lingual nerve to successive pulses of intravascular stimuli quickly adapt out (Bradley, Am J Physiol 224:300,1973); however, electrical stimulation of the hypoglossal nerve which causes fasiculation of tongue muscles brings back approximately 30% of the maximum normalized response (n=4) while massage of the tongue brings back approximately 50% of the maximum normalized response (n=6). It is suggested that muscular movement may help clear the extracellular fluid space in the tongue in a manner analagous to the formation of lymph (McMaster, Ann N Y Acad Sci 46:743,1946). The complete adaptation of receptor sites to successive intravascular stimuli may be explained by the short time course of receptor adaptation (Sato,Brain Res 34:385,1971; Smith, Neurosci Abstracts 248,1977) compared to the much longer clearance time of extracellular space. Stimulus interaction with intravascular taste receptor sites is constrained by access to and clearance from the extracellular fluid space, when these parameters are manipulated the intravascular receptor sites seem to follow those equations developed by Beidler (J Gen Physiol 38:133,1954) to characterize the apical taste receptor sites.

Supported by NINCDS Grant NS07068-02.

240 GUSTATORY RESPONSES TO WATER. Linda M. Bartoshuk, Marion K. Frank* and Carl Pfaffmann. Pierce Foundation and Yale Univ., New Haven, CT 06519 and Rockefeller Univ., New York, NY 10021.

The effects of water on gustatory receptors were studied in 248 single fibers from the chorda tympani nerves of rat, cat, hamster, and squirrel monkey as well as the glossopharyngeal nerve of rat. Neural responses to water were contingent on the nature of the preceding or adapting solution. That is, a particular fiber did not respond to water per se but rather responded to water following NaCl or water following HCl, etc. Four adaptation solutions were tested on each fiber: NaCl, sucrose, HCl, and QHCl (quinine hydrochloride). Sixty fibers responded to water following only one of the adapting solutions, 19 fibers responded following two, 4 fibers responded following three, and 1 fiber responded to water following all four adapting solutions.

The NaCl adapting solution is of special interest because saliva contains NaCl. Twenty eight fibers responded to water following NaCl. The proportion of these fibers varied across species and nerves.

Responses to water following NaCl were very sensitive to the adapting concentration of NaCl. Although such fibers were maximally responsive to water, they were also responsive to NaCl concentrations lower than the adapting concentration. For example, if the tongue was adapted to .3 M NaCl, then .03 M NaCl produced a "water" response. This suggests that removal of NaCl is the event required for stimulation of this response.

Fibers responsive to water following NaCl also tended to be responsive to QHCl in species and/or nerves sensitive to QHCl. This suggests that water following NaCl tastes like QHCl in these cases. That is, water may take on particular taste qualities because the neural signal it generates mimics the input normally produced by conventional taste stimuli.

The most dramatic correlation of responses to QHCl and water following NaCl in the present data occurred in glossopharyngeal fibers innervating circumvallate papillae in the rat. This suggests that water should taste like QHCl to the rat adapted to the NaCl in its saliva. Behavioral data (Morrison, G.R., Canad. J. Psychol. 21: 141-152, 1967) support this conclusion.

241 CENTRAL DISTRIBUTION OF CRANIAL NERVES V, VII, IX, AND X IN THE MONKEY. R. M. Beckstead and R. Norgren. Rockefeller Univ., New York, NY 10021.

The central distribution of primary afferent fibers in the trigeminal (V), facial (VII), glossopharyngeal (IX), and vagal (X) cranial nerves have been re-examined with the autoradiographic fiber-tracing method. Fiber-labeling in the principal and spinal nuclei of the trigeminus after subtotal injections of ^{3}H-proline in various parts of the trigeminal ganglion confirms earlier classical descriptions and further suggests that the trigeminal fibers which enter the ventrolateral portion of the nucleus of the solitary tract (NST) originate from cells in the ophthalmic segment of the ganglion.

Injection of the geniculate ganglion labels fibers of the VIIth nerve which both ascend and descend upon reaching NST. The ascending fibers distribute in a compact and circumscribed zone immediately dorsal to the spinal V nucleus as far rostral as the caudal pole of the principal trigeminal nucleus. The descending fibers distribute to the lateral NST rostral to the level at which the roots of the vagus join the solitary tract. For a short distance caudal to this level, sparse labeling is present only in a small part of NST which lies ventrolateral to the solitary tract and appears to correspond to the zone of NST which receives direct trigeminal afferents. No labeled fibers could be traced to the contralateral NST. A few labeled fibers of VII descend in the dorsomedial sector of the spinal V tract to distribute sparsely in the dorsomedial part of the spinal V nucleus and in a restricted zone in the lateral margin of the cuneate nucleus.

Fiber-labeling after injections of the ganglia of nerves IX and X suggest the following. Although, upon reaching the solitary tract, a few fibers in either IX or X ascend as far rostrally as had those of nerve VII, both have a much larger descending component which distributes to more posterior levels of NST. Many of the IXth nerve fibers appear to terminate in the lateral part of NST and the dorsolateral margin of the medial part. Only a few travel as far caudal as the commissural part of NST. Fibers of vagal origin, on the other hand, are abundant in the medial and commissural parts of NST. Moreover, only X appears to have a crossed projection in the commissural nucleus and caudal portion of the contralateral NST. Some of the vagal fibers in the commissural part of NST continue into the nucleus of the posterior commissure in upper cervical segments of the spinal cord. A few fibers of the vagus also appear to enter the area postrema. A significant descending component of IX and X in the spinal V tract is present only when the superior ganglion of either nerve is involved by the ^{3}H-proline deposit.

Supported by NSF grant BNS 76-81408 and NIMH grant 15125.

242 ACUTE OLFACTORY SENSITIVITY OF SOCKEYE SALMON TO CALCIUM IONS AND ITS SIGNIFICANCE FOR MIGRATIONS. David Bodznick* (SPON: J. Palka). Dept. Zool., Univ. Washington, Seattle, WA 98195

The ability to discriminate between natural fresh waters based on their odorant characteristics is essential for the homing migrations of adult Pacific Salmon (Oncorhynchus) and the lakeward migrations of sockeye salmon fry (O. nerka). Both physiological and behavioral experiments with sockeye salmon indicate that the calcium ions found in natural waters may be useful odorants for these discriminations.

The absolute concentrations of the major cations and anions in natural waters vary greatly among water sources and depend on the characteristics of the particular watershed. Of these ions, olfactory stimulation with calcium and only calcium elicited gross potential (olfactory EEG) responses from the olfactory bulbs of sockeye at concentrations below the natural water range. Responses were reliably observed to 5×10^{-6}M $CaCl_2$ and most animals responded differentially to calcium concentrations throughout this natural water range. In addition, both mitral cells and granule layer cells of the olfactory bulbs responded to $CaCl_2$ at concentrations as low as 10^{-6}M. Responses in each case were ion-specific and not a result of changes in total ionic concentration or osmotic pressure.

Behavioral tests of preference in a 2-choice Y-maze showed that unconditioned fish would discriminate between 2 water sources that differed only in calcium concentration.

It is proposed that salmon recognize individual natural waters not by a unique odorant in each water but rather by the characteristic combination of odorant qualities present, and that the calcium concentration is one of these identifying odorant qualities.

243 ADAPTATION AND CROSS ADAPTATION OF THE PERIPHERAL OLFACTORY AND GUSTATORY SYSTEMS OF THE CATFISH TO AMINO ACIDS. John Caprio and Jesse J. Robinson*. Dept. of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803.

Olfactory and gustatory systems of the channel catfish, Ictalurus punctatus, are highly sensitive to L-α-amino acids (Caprio, J. Nature 266:850, 1977). With continuous amino acid stimulation, the phasic gustatory neural response of the facial nerve is quickly adapting, whereas both olfactory neural and EOG responses exhibit a phasic response followed by a slowly declining tonic response level. Both systems also respond less to the second of two applications of an amino acid stimulus. The amount of reduction of the response to the test stimulus and the recovery of excitability depend on the relative efficacy, concentration and duration of the adapting stimulus, and the duration of the interstimulus interval (rinse time). However, the relationship between percent recovery of the response and rinse time is linear with a variable slope dependent upon the above factors.

In cross adaptation experiments, a continuously applied adapting solution of L-alanine (10^{-5}-10^{-4}M), the most effective taste stimulus, eliminates the taste responses to the other L-amino acids except L-arginine at tested concentrations up to and including 10^{-3}M. Conversely, L-arginine used as an adapting stimulus has minimal effect on the ordering of relative effectiveness of the other amino acids tested at 10^{-5} to 10^{-3}M. These and unit data (Caprio, J. and D. Tucker, Soc. Neurosci. 2:152, 1976) suggest that the peripheral facial taste system of the channel catfish is composed of taste cells containing primarily arginine specific binding sites which are innervated by arginine-best taste neurons. Other taste cells innervated by alanine-best taste fibers contain primarily alanine sites, but also have binding sites for other amino acids.

Olfactory cross adaptation experiments have shown that any of the more effective amino acids used as adapting stimuli will depress to varying degrees but not abolish the responses to the other amino acids. Presently, there is no evidence of specific olfactory receptors analogous to the arginine-best taste cells. (Supported in part by NIH Biomedical Research Support Grant S07 RR07039-06 awarded to LSU and a Summer Faculty Research Grant, both allocated by the Council on Research.)

244 AN EXTENDED OLFACTORY SENSITIVE PERIOD IN RATS. Catherine Cornwell-Jones. Dept. Psych., Princeton Univ., Princeton, N.J., 08540.

Three days of differential olfactory exposure before, but not after sexual maturation, influenced the odor preferences of Sprague-Dawley rats. Three days before testing, animals were placed in either cedar or pine shavings in isolated olfactory environments. On the fourth day, rats were given two odor preference tests in an apparatus which allowed animals to smell but not taste or touch stimulus shavings.

Pine-housed neonates 11 days old and juveniles 32 days old preferred the odor of fresh pine to fresh cedar shavings. Housing in cedar induced a preference for cedar odor in pups and tolerance in juveniles. Exposure did not influence scores of sexually mature males 66 days old on cedar vs. pine.

Cedar-exposed neonates showed a statistically greater preference than pine-housed pups for the odor of cedar nest shavings in which rats had been housed against fresh pine odor. Cedar-exposed juveniles and adults also preferred cedar nest to pine odor, but their averages did not differ significantly from pine-exposed averages. Thus, exposure induced differences in neonatal responses on both odor choices, and in juveniles responses on fresh cedar vs. pine, but failed to significantly influence adult responses to either set of odors.

A comparison of averages across age groups indicated that preference scores of cedar- but not pine-exposed rats changed significantly with age. The decline in the effects of olfactory experience therefore reflects a change with age in the behavior of cedar- but not pine-exposed rats.

Receptor adaptation cannot account for most of the differences observed. Exposure-induced receptor fatigue would have reduced, rather than increased preference for cedar nest odor in pups, and resulted in tolerance rather than preference for natural cedar odor. Habituation to familiar odors could explain the exposure-induced tolerance of juveniles for natural cedar odor, but not their preference for cedar nest odor. The data therefore implicate changes in central rather than peripheral neural substrates.

245 ROUTES AND TERMINATION PATTERNS OF THE CENTRIFUGAL AFFERENTS TO THE MAIN OLFACTORY BULB FROM THE RETROBULBAR AREA AND PIRIFORM CORTEX IN THE HAMSTER. Barry J. Davis, Foteos Macrides and William M. Youngs. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The routes and termination patterns of projections to the main olfactory bulb (MOB) from the anterior olfactory nucleus (AON), hippocampal rudiment (HR) and piriform cortex (PC) were studied in the hamster with the autoradiographic technique. Injections of tritiated amino acids centered in pars externa of the AON revealed a heavy projection to the contralateral MOB via the bulbar limb of the anterior commissure (AC). These afferents were found to terminate most heavily in the superficial half of the granule cell layer (GRL), producing a ring of autoradiographic grains around the circumference of the GRL. Injections in the more caudal subdivisions of the AON revealed bilateral projections to the MOB via the AC. The AON fibers passed ventromedially from the AC to enter the caudal part of the MOB, and dorsally around the accessory olfactory bulb to enter the more anterior part of the MOB. Striking differences were seen in the termination patterns of the centrifugal projections from variods subdivisions of the AON. Injections in the lateral or caudal peduncle involving primarily pars lateralis, pars ventralis or pars posterior produced a heavy terminal pattern of grains bilaterally over the superficial half of the GRL, whereas the heaviest terminal labelling after injections centered in pars medialis was seen bilaterally over the deep half of the GRL. Injections in pars lateralis also revealed heavy terminations in the periglomerular region of the ipsilateral MOB. Such terminations were found bilaterally only after injections which involved pars posterior and pars ventralis. Injections in the HR and PC revealed projections to the ipsilateral MOB. The HR fibers traveled diffusely in the medial and dorsal sectors of the peduncle, entered directly into the GRL at caudal levels of the MOB, and coursed around the accessory olfactory bulb into the anterior part of the MOB. Like the projection from pars medialis of the AON, the centrifugal projection from the HR was found to terminate predominantly in the deep half of the GRL. The centrifugal projection arising in the PC was found to reach the MOB primarily via the AC and to a lesser extent via the deep portion of the lateral olfactory tract (LOT). The LOT component coursed ventrally and dorsolaterally into the MOB. The routes into the MOB taken by the AC component paralleled those taken by the projections from the AON. Anterior PC injections produced heavy terminal labelling over the superficial half of the GRL whereas the posterior PC appeared to terminate preferentially in the deep half of the GRL. These findings suggest that various subdivisions of the AON, the HR and the PC exert direct but heterogeneous influences on MOB function. (Supported by grants BNS75-07652 and NS12344)

246 DISTRIBUTION OF CANDIDATE TRANSMITTER AMINO ACIDS IN THE PIRIFORM CORTEX AS AFFECTED BY TRANSECTION OF THE LATERAL OLFACTORY TRACT. D.A. Godfrey, C.D. Ross, J.A. Carter*. Dept. Pharmacol. and Dept. Anat. and Neurobiol., Wash. U. Sch. Med., St. Louis, MO. 63110

Quantitative histochemical mapping procedures have been applied to study the distribution of GABA, glycine, glutamate and aspartate in the olfactory system of male Sprague-Dawley rats. In four rats, lesions (knife cuts made by Dr. Joseph L. Price, Dept. Anat. and Neurobiol., Wash. U. Sch. Med.) transected the lateral olfactory tract (LOT) just behind the olfactory bulb on the right side. The distributions in the piriform cortex of GABA, glutamate and aspartate were examined seven days later. (Glycine levels were too low to warrant detailed study.) The results are summarized in the table, with amino acid levels in mmoles/kg dry wt, mean ± SEM for the four rats:

Region	Control side	Lesion side
	GABA	
LOT	4.7±0.5	4.8±1.0
Layer IA	13.1±0.8	13.7±1.4
Layer IB	16.0±1.5	16.4±2.3
Layer II	17.6±0.6	16.2±1.8
Layer III	16.8±0.7	14.4±1.3
	Glutamate	
LOT	21±1	7±1
Layer IA	48±2	30±3
Layer IB	61±2	53±2
Layer II	66±2	62±2
Layer III	57±2	56±1
	Aspartate	
LOT	4.8±0.6	1.8±0.3
Layer IA	12.2±1.0	7.3±0.3
Layer IB	15.6±0.8	12.7±1.0
Layer II	16.1±0.7	14.2±0.8
Layer III	14.3±0.1	12.8±0.7

Both glutamate and aspartate levels were significantly reduced in the LOT, which contains the axons projecting to the cortex from the olfactory bulb (65% decrease), and in layer IA of the piriform cortex, which contains the terminals of these axons (40% decrease). These results support the possibility that glutamate and aspartate are preferentially associated with mitral and/or tufted cell projections to the piriform cortex.
(Supported by American Cancer Society grant BC4S and USPHS grants NS-08862 and NS-08000).

247 AFFERENT SOMA POPULATIONS WITHIN THE GENICULATE GANGLION. Maximo M. Gomez. Dept. Anat., Bowman Gray Sch. Med., Winston-Salem, NC

This study was designed to distinguish specific subpopulations of geniculate ganglion (GG) soma that project via facial nerve branches on the basis of number, location and cytoarchitecture. One each of the greater superficial petrosal (GSP), chorda tympani (CT) and distal VIIth nerves was cut and desheathed in separate rats. The GSP was cut at its exit from the petrotympanic fissure, the CT was cut in the infratemporal fossa and all of the distal VIIth nerve was cut at the stylomastoid foramen. Dry flakes of horseradish peroxidase (HRP)were allowed to dissolve in tissue fluid surrounding the central stumps of cut nerves and reapplied every 6-8 hours over a total incubation period of 18-24 hours. After fixation, the GG were removed, reacted en bloc with diaminobenzidine (or Hanker-Yates reagent) and H_2O_2, and serially sectioned in Parlodion at 20um. All soma in each GG were counted and classified as either HRP positive or negative. Incubation of the GSP in three rats resulted in the labeling of a mean of 365±18 (S.E.) cell bodies which is 20% of the mean total of 1787±82 soma. Labeled cells are primarily located towards the apex of the GG, near the origin of the GSP, with a few cells scattered throughout the entire GG; nearly all are 15-25um in cross-sectional diameter with a few in the 30-40um range. CT incubation yields a mean of 506±35 HRP positive soma (N=3) or 28% of the 1799±80 GG cells. These cells are mostly located at the medial margin of the GG, along the base of the GG adjacent to the VIIth nerve, with some cells throughout the GG. The diameter of CT cells is similar to that of GSP soma with a few more large cells labeled. Distal VIIth nerve incubation labels an average of 838±56 (N=2) or 44% of the 1899±52 GG soma. They are located along the lateral side of the GG near the VIIth nerve. Labeled cells are scattered in the GG, including a small accumulation of cells well out along the GSP and some cells in the nervus intermedius. Combined percentages of HRP positive cells thus accounts for 92% of GG soma. Although from these data the tongue appears to receive a heavier gustatory innervation than the palate, the near total bilateral overlap of GSP fibers in the palate results in an equivalent density of innervation for the lingual and palatine fields. The GSP, however, contains 4.5 times more sensory fibers than labeled soma while the number of CT sensory fibers is about equal to the cells labeled from CT incubation. The excess of fibers over labeled soma in the GSP series may be due to axonal branching or to the presence of non-GG fibers elevating the GSP sensory fiber count. The above data also defines the number of potential information channels available for peripheral sensory coding and central input from the palatine and lingual gustatory fields.

(Supported in part by NIH grant NS10389.)

248 Effect of Intranasal Irrigation of Mitotic Inhibitors on Olfactory Behavior and Biochemistry in Mice. J. W. Harding and J. W. Wright* Washington State University, Pullman, WA 99164.

Mice were intranasally irrigated daily with 100 microliters of normal saline, hydroxyurea (10 mM), and ethidium bromide (2 mm), or weekly with colchicine (1 mm). The mice which were pretrained to find buried food pellets (45 mg) or amyl acetate (5 λ of 1:40,000) scented sugar cubes, were tested daily in order to monitor olfactory mediated behavior. Both colchicine and ethidium bromide had dramatic effects on behavior - by day 6, none of the colchicine treated animals were capable of finding amyl acetate sugar cubes; by day 8, 20% or less of the ethidium bromide treated animals could find buried food pellets or sugar cubes. There was no change in the performance of the saline animals while the hydroxyurea animals exhibited only a modest and temporary decline in olfactory capabilities. Following 21 days of treatment the experiment was terminated. The behavioral results for the different experimental groups corresponded well with both bulb weights (saline - 94.2% of untreated control; colchicine - 59.5%; ethidium bromide - 63.2%; and the hydroxyurea - 95.9%) and the levels of the chemoreceptor neuronal marker, carnosine synthatase, (saline, bulb - 99.6% untreated control; epithelium - 97.6%; colchicine, bulb - 9.9%, epithelium - 3.4%; ethidium bromide, bulb - 7.6%, epithelium - 4.5%, hydroxyurea, bulb - 104.0%; epithelium - 108.0%). Additionally, the in vitro measurement of ^{3}H-thymidine incorporation showed a significant decrease in total incorporation by olfactory epithelium for all inhibitors tested. (colchicine - 69% of control; ethidium bromide 46%, and hydroxyurea 48%). Together the above results suggest that a functional mitotic process is necessary for continuous olfactory capabilities and the maintenance of stable population of chemoreceptor neurons.

249 UNIT ACTIVITY IN THE IPSILATERAL AND CONTRALATERAL OLFACTORY PATHWAY IN THE PIGEON. Larry V. Hutchison and Bernice M. Wenzel. Dept. Physiol., UCLA Sch. Med., Los Angeles, CA 90024.

Continuing neurophysiological study of central projections of the olfactory bulb (OB) in the pigeon has confirmed and extended identification of higher-order ipsilateral projection areas, viz., caudal neostriatum (NC), hippocampus (Hp), parahippocampal area (AHP), and the dorsomedial thalamus, both anterior (DMA) and posterior (DMP) subdivisions. Stimulation of one olfactory nerve (ON), after section of the contralateral ON, with 8-12 v pulses (single or train), 0.5-1.0 ms duration, at 0.1-1.0 Hz resulted in evoked responses with properties characteristic of polysynaptic connections and in significant modification of spontaneous unit activity (± 25% change in rate). The majority of affected units in NC, DMA, and DMP were enhanced by stimulation, while a smaller number of cells showed depression. All cells recorded to date in Hp and AHP showed enhancement.

A Fink-Heimer study of OB projections had shown degenerating fibers passing through the anterior commissure (ca) to the contralateral forebrain. Degeneration patterns showed that major direct OB efferents terminate in the contralateral parolfactory lobe (LPO), nucleus accumbens (Ac), and components of the paleostriatal complex, viz., paleostriatum primitivum (PP), paleostriatum augmentatum (PA), and the intrapeduncular nucleus (INP). Our pilot electrophysiological work had provided confirmatory evidence of paleostriatal connections. More extensive confirmatory data can now be reported from the study of unit activity in various contralateral sites. Response properties characteristic of monosynaptic stimulation were recorded in PP, PA, and LPO. Unit activity in PA was enhanced by contralateral ON stimulation, whereas units in LPO generally showed depression, results consistent with our previous findings in the same structures ipsilaterally. Cell populations in PP near the ca, which previous work had shown to be consistently inhibited by ipsilateral ON stimulation, showed only excitation during contralateral ON stimulation. Peak firing rates at a constant latency, consistent with the peak latency of the evoked potential recorded through the same electrode, have been observed in those areas where analysis has been completed. Tetanic stimulation of contralateral ON (20Hz-20sec) typically produced prolonged posttetanic changes in unit activity in projection sites.

Also confirmatory of anatomical findings, results to date show that contralateral ON stimulation enhances firing rates of cells in ventral hyperstriatum and AHP. (Supported by USPHS grant NS 10353 to B.M. Wenzel and NINCDS postdoctoral fellowship NS 05896 to L.V. Hutchison).

250 GUSTATORY DISTRIBUTION OF THE RAT GLOSSOPHARYNGEAL NERVE. Jerry W. Lawson. Dept. of Anatomy, Bowman Gray Sch. of Medicine of Wake Forest University, Winston-Salem, NC 27103.

It is known from degeneration experiments that the glossopharyngeal nerve innervates taste buds in the circumvallate and foliate papillae of the rat. Summated electrophysiological responses from the rat glossopharyngeal nerve indicate a greater sensitivity to quinine, a higher threshold for NaCl, as well as a longer latency to response onset and maximum. Zotterman has asserted that quinine sensitivity is a property of small diameter gustatory afferents, so a study of the rat glossopharyngeal was undertaken to determine the distribution of axon diameters. Male, Sprague-Dawley rats were perfused intravascularly with Karnovsky's glutaraldehyde-paraformaldehyde fixative and the glossopharyngeal nerves were dissected unilaterally on each animal and sectioned at the emergence from the posterior lacerated foramen. The nerves were post-fixed in osmium, dehydrated, embedded in epon and sectioned for electron microscopy. Montages of photomicrographs of nerve cross sections were reconstructed and axon counts, as well as diameters were determined. There were 1895 ± 41 (S.D.) (N=3) myelinated profiles and 1423 ± 125 unmyelinated profiles for a total of 3318 ± 92 axons or 57% myelinated and 43% unmyelinated profiles. The diameters of the 3 nerves were equivalent for the myelinated axons with averages from 2.7 ± .83 um to 2.8 ± 1.0 um with a range from 0.7 to 6.7 um. The unmyelinated profiles in these montages ranged from 0.4 to 2.3 um in diameter. The chorda tympani nerve in the rat contains a total of 1016 axons which include 60% myelinated and 40% unmyelinated profiles. Therefore, the proportion of myelinated and unmyelinated profiles in the glossopharyngeal nerves is nearly equivalent. Neuronal somata were counted from serial paraffin sections of 2 petrosal ganglia. These contained 6196 and 5616, respectively, which should correspond to the total number of sensory neurons of the glossopharyngeal nerve; however, there are, in addition to the oral distribution, the Herring nerve to the carotid body, the tympanic branch to the ear and a branch to the pharynx. Two morphological differences exist between the lingual glossopharyngeal and chorda tympani nerves: 1. The glossopharyngeal nerve contains about 3 times as many total axons as the normal chorda tympani; 2. The gustatory distribution of the glossopharyngeal nerve overlaps bilaterally to the midline circumvallate papilla and ipsilaterally to the foliate papilla, while the chorda tympani is ipsilateral to the fungiform papillae. The differences in the gustatory sensitivities between the rat glossopharyngeal and chorda tympani nerves cannot be ascribed to differences in axon diameter. Sympathectomy and intracranial deefferentation studies are in progress to specify the sensory axon population of the glossopharyngeal nerve. (Supported in part by NIH Grant NS 10389).

251 SEXUAL AND AGGRESSIVE BEHAVIOR IN MALE HAMSTERS AFTER LESIONS OF THE CORTICOMEDIAL AMYGDALA. Michael N. Lehman, Golda A. Kevetter, and J. Bradley Powers. Neurosci. Prog. and Neurosci. Lab. Bldg., Univ. Michigan, Ann Arbor, MI 48109.

Olfactory input to the main olfactory bulb and vomeronasal organ input to the accessory olfactory bulb are essential for normal mating and aggressive behavior in male hamsters (Winans & Powers, Brain Res., 126: 325; Murphy, Brain Res., 113: 95). Since neurons of the main and accessory olfactory bulbs project to adjacent but separate nuclei of the corticomedial amygdala, and since this is the major projection of the accessory system, we studied mating and aggression in male hamsters before and after bilateral electrolytic lesions of the corticomedial amygdala (n=14) or sham operations (n=4). Each male's mating behavior was observed in a neutral arena with an ovariectomized, estrogen and progesterone primed female hamster. Aggressive interactions were recorded after introducing an unfamiliar male hamster into the test male's home cage.

Sham animals showed no postoperative behavioral changes, but aggressive responses were severely diminished in 13 out of 14 lesioned males and were essentially eliminated in 11 of these animals. Mating behavior deficits in lesioned animals ranged from a total absence of copulation to no observed change, and the degree of deficit was positively correlated with the amount of damage to the rostral part of the corticomedial nuclear group. Males with lesions of the rostral corticomedial amygdala also spent significantly less time during postoperative mating tests investigating the anogenital region (but not the head or flank region) of the female. Males with damage confined to the caudal corticomedial amygdala, like sham operates, showed little impairment of either mating behavior or anogenital investigation of the female. In summary, the results suggest that the rostral nuclei of the corticomedial amygdala are relatively more important than the caudal nuclei in facilitating mating behavior. Aggressive behavior deficits showed no comparable localization; lesions in either the rostral or caudal part of the corticomedial amygdala led to similar deficits.

Since the major outflow of the corticomedial amygdala to the hypothalamus is by way of the stria terminalis, we also studied the mating behavior of male hamsters before and after bilateral electrolytic lesions of the stria terminalis (n=7) or sham operations (n=11). Although all stria terminalis lesioned males showed some increase in latency to ejaculation, 6 out of 7 lesioned males continued to mate postoperatively; stria terminalis lesions were thus not comparable to lesions of the rostral corticomedial amygdala. This suggests that non-strial efferents from the rostral corticomedial amygdala play a major role in the mating behavior of male hamsters.

252 SUBPOPULATIONS OF FUNGIFORM TASTE BUDS IN THE RAT. Edward H. Lubarsky* and Inglis J. Miller, Jr. Dept. of Anatomy, Bowman Gray Sch. of Med. of Wake Forest Univ., Winston-Salem, NC 27103

It has not been established whether taste buds function primarily as individuals or in groups, but lateral depression and disparity in response magnitudes from equal numbers of fungiform taste buds in different regions suggest interaction among neighboring taste buds. This report identifies groups of fungiform taste buds on the rat tongue which degenerate following the surgical section of each of the 5 major divisions of the chorda-lingual nerve in the tongue. Our designation of these divisions include 2 posterior branches, D and E; a midregion branch, C; and 2 anterior divisions, A and B, which distribute to the tip. Section of one or more of these divisions was accomplished in 15 Sprague-Dawley rats either unilaterally or bilaterally. The animals were allowed 10 days of post-surgical survival, sacrificed, perfused with Bouin's fixative and complete serial sections of the tongues were prepared in paraffin and stained with H & E or FitzGerald's silver technique. Microscopic examination was performed to determine the number and location of normal and degenerating taste buds on each tongue. The anterior branches, A and B, terminate in an overlapping distribution to fungiform taste buds on the rostral 15mm of tongue surface. An average ± S.D. of 35±15% (N=5) of total fungiform taste buds degenerate when A is severed, but 29% of these are located on the anterior 4 mm of the tongue tip. When B is sectioned, an average of 41 ± 13% (N=3) of fungiform taste buds degenerate, but only 13% are located on the anterior 4 mm. An average of 4 taste buds remain on the tongue tip with ipsilateral section of the entire chorda tympani nerve and these are innervated by axons which cross the midline. The midregion branch, C, distributes to 11±1% (N=3) of taste buds which lie anterior to the intermolar eminence 13-20 mm from the tongue tip. Posterior branches D and E innervate 8±3% (N=5) of fungiform taste buds located lateral to the intermolar eminence as well as 5-10 taste buds in the first 2 trenches of the foliate papillae. Degenerative changes ranged from a total absence of taste bud cells to a reduction in the size of buds and cell numbers. Silver-stained preparations reveal the presence of nerve fibers in partially degenerated taste buds due to the overlapping of axons from the unsevered nerve bundles. If the axons which are responsible for trophic maintenance of the receptors are the ones which mediate taste perception, then the major divisions of the chorda-lingual nerve correspond to regionally identifiable subpopulations of taste buds supplied by overlapping groups of facial gustatory neurons.

(This research was supported in part by NIH Grant NS 10389).

253 ODOR DISCRIMINATION AND MEMORY IN KORSAKOFF'S PSYCHOSIS. Robert G. Mair*, Cythnia Capra*, William J. McEntee, and Trygg Engen*. Neuro. Service, V.A. Hospital, Davis Park, Providence, RI 02908.

Korsakoff's psychosis is characterized by severe memory impairment, associated with consistent patterns of diencephalic and brain stem lesions including the mediodorsal thalamic nucleus in a high percentage of cases. We have recently reported evidence linking the memory impairment of Korsakoff's psychosis with decreased central noradrenergic activity. Patients with this disorder have been reported to be unable to discriminate between odors and to show a deficit in supra-threshold scaling suggesting impaired sensitivity to weak odors. We compared absolute olfactory sensitivity in 10 Korsakoff patients with matched normal controls using signal detection methods and found no impairment in their sensitivity to threshold level stimuli. We then proceeded to measure odor quality discrimination and memory by presenting separate blocks of similar and dissimilar odor pairs at time delays up to 30 seconds. The choice of similar and dissimilar odor pairs was based on extensive ratio scaling and recognition memory experiments with normal subjects. It was found that the patients had difficulty distinguishing between similar, but not between dissimilar odors, although matched normal controls performed at a higher level with both sets of stimuli. Furthermore, the Korsakoff patients showed no decline in odor memory performance over the time intervals tested. This latter finding is in marked contrast to the performance of the same Korsakoff patients on auditory and visual memory tasks. Treatment of Korsakoff patients with catecholamine agonist drugs which have improved performance on other memory tasks, had no effect on odor memory. These results suggest that Korsakoff patients are impaired in their ability to discriminate between two odors, but not in their ability to detect odorants. The failure of catecholamine agonists to improve performance and the equivalent results observed across time delays up to 30 seconds, suggest that the odor discrimination deficit does not directly result from the Korsakoff's memory impairment.

254 AN APPROACH TO CHEMICAL NERVE SECTION IN THE MOUSE OLFACTORY PATHWAY. F.L. Margolis and M. Grillo*. Department of Physiological Chemistry and Pharmacology, Roche Institute of Molecular Biology, Nutley, NJ 07110.

The use of a neurotoxic agent to selectively destroy or block the formation of certain cell types has been a powerful tool for the analysis of developmental and functional relationships in the nervous system. Intranasal irrigation with 0.17 M $ZnSO_4$ solution has previously been shown to have a long-term effect on the morphology and biochemistry of the olfactory bulb and mucosa, as well as on food-finding behavior (Brain Res. 140, 271 [1978]). We have now extended these studies to evaluate the effects of intranasal irrigation with a large number of compounds. Initially, we monitored the effect on food-finding behavior. Many compounds (3-acetylpyridine, kainic acid, xylocaine, pronase, tyrothricin, Ellman's reagent, etc.) had little or no effect on food-finding behavior. However, intranasal irrigation with vinblastine SO_4 (10 mM) had effects similar to 0.17 M $ZnSO_4$ on behavior, olfactory bulb weight, S-100 protein, olfactory marker protein (OMP), synthesis and transport of carnosine (STC) and carnosinase activity (C'ase). The use of lower concentrations of vinblastine SO_4 (VB-SO_4) caused a transient reduction in some of these parameters. Other agents such as Triton X-100, colchicine, N-ethyl maleimide and dimethyl-(2-hydroxy-5-nitrobenzyl)-sulfonium bromide at appropriate concentrations resulted in a rapid loss of those biochemical parameters associated with olfactory neurons (OMP, STC), but much smaller, rapidly reversed, changes in non-neuronal parameters (S-100, C'ase). The olfactory neuron specific markers return to control levels at 4-6 weeks after treatment. Essentially, identical biochemical patterns were seen in bulb and mucosa. Food-finding behavior followed a similar time course. Histological evaluation of these materials is not yet completed. However, the time course of the biochemical changes (for several of the treatments) is almost identical to that seen after surgical section of the olfactory nerve (Brain Res. 132, 11 [1977]). These data suggest that the olfactory neurons are especially susceptible to environmental insults and that these treatments may function as a chemical nerve section.

	0.5% Triton Intranasal Olfactory Mucosa (% Control)				10 mM VB-SO_4 Intranasal Olfactory Mucosa (% Control)			
Days	OMP	STC	S-100	C'ase	OMP	STC	S-100	C'ase
0	100	100	100	100	100	100	100	100
2	12	10		120	13	6		54
7		11	70	75		1	24	5
14	7	16		93	7	1		7
30	46	50	93	108	6	5	16	2
42	72	80			5	8		

255 MODULATION OF OLFACTORY BULB EVOKED POTENTIALS BY STIMULATION OF CENTRIFUGAL INPUTS. Diane P. Martinez* (Spon: W.J. Freeman). Dept. of Physiol.-Anat., Univ. Calif., Berkeley, CA 94720.

Averaged evoked potentials (AEPs) from the main olfactory bulb of lightly anesthetized rats were recorded in response to electrical stimulation of the lateral olfactory tract (LOT), deep pyriform cortex (PC), the anterior limb of the anterior commissure (AAC), and the primary olfactory nerve (PON).

The LOT and AAC AEPs recorded at the surface and deep to the mitral cell layer were observed to be consistent with results reported by many authors. Stimulation of the region just deep to the PC activated centrifugal fibers from the cortex resulting in a long latency AEP (10-12 msec), initially surface positive but without a clear potential turnover. Part of this AEP could be due to input relayed through the anterior olfactory nucleus, although direct projections to the bulb from the cortex have been demonstrated anatomically. The PON AEP consisted of a damped sine wave superimposed on a nonoscillatory baseline shift. The initial peak of the oscillation and the baseline shift were negative at the surface and positive deep in the bulb. In some cases the PON compound action potential could be distinguished immediately following the shock artifact. With deep anesthesia the baseline shift of the AEP disappeared and the oscillatory component became more damped. The baseline shift was only recorded with PON stimulation and appeared to result from activation of periglomerular cells.

No potentials could be evoked in the bulb from stimulation of the nucleus of the horizontal limb of the diagonal band (HDB), a structure known to send centrifugal input to the granule cell and glomerular layers of the bulb. However, tetanization (200 per sec for 20 secs) of the HDB immediately prior to stimulation of the PON had a marked effect on the oscillatory PON AEP. The AEP became less damped, continuing to oscillate for longer periods, with an increase in the amplitude of the baseline shift. In contrast, tetanization of the AAC resulted in suppression of the PON AEP without a decline in the amplitude of the mitral cell compound action potential. While the AAC interconnects the two olfactory bulbs and is not a true centrifugal input, the HDB lies in the medial forebrain bundle where limbic, hypothalamic, and reticular systems project. This suggests that the modulating effect exerted by the HDB on the bulb controls arousal or background activity by acting at the level of the periglomerular cell population. Supported by MH 06686.

256 AMYGDALA RESPONSE TO ELECTRICAL STIMULATION OF VOMERONASAL NERVE. Michael Meredith. Worcester Foundation for Experimental Biology Shrewsbury Mass. 01545.

The vomeronasal organ and accessory olfactory bulb (AOB) in the golden hamster have been; 1) implicated in sensory control of mating behavior(Winans and Powers 1977); 2) shown to be sensitive to odor components of hamster vaginal discharge (Meredith 1977) and have been, 3) shown by experimental anatomical methods to project to the medial and posterio-medial cortical nuclei (PMCN) of the amygdala (Davies et al 1978). We have used electrical stimulation of the vomeronasal nerves (VN) to trace the functional connections through AOB to the amygdala, using bipolar and monopolar recording. The nerve was stimulated rostral to the main olfactory area to avoid activating inputs to the main olfactory bulb (MOB). When recording from the amygdala, VN stimulation was again used in preferrence to AOB stimulation to avoid activating MOB output fibers. Simultaneous recordings in AOB and PCMN give response latencies of 35-45 ms for the negative component of the AOB evoked potential, recorded at the level of the second order cells. The variation is due to differences in conduction distance. The surface N_1 component of the PMCN evoked potential has a latency of 8 ms from the AOB potential. A deep-negative, surface-positive component (P) follows N_1 in the PMCN with an additional latency of 5 ms. It reverses deep to N_1 and fails to follow 3/s stimulation. This suggests that the cells generating N_1 project to and activate cells in the deeper layers of the amygdala. Ongoing experiments are designed to identify these cells as belonging either to layer 3 of PMCN or to the deeper basomedial nucleus. Microelectrode recordings at the level of the maximum deep negative component corresponding to P show a relatively narrow layer of cells driven by VN stimulation.

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Supported by Van Ameringen and Monell Foundations and by NINCDS grant NS 14453 and NINCDS fellowship 1F32 NS 05849.

257 THE LOCALIZATION AND FUNCTION OF SH GROUPS IN FROG OLFACTION. Muriel H. Nathan and Sarah S. Winans. Dept. of Anatomy, University of Michigan, Ann Arbor, Michigan 48109.

A replication of Getchell and Gesteland's (1972) electrophysiological study of the effect of N-ethyl maleimide (NEM), an SH group blocker, on the responses of the olfactory receptors to odor stimulation was undertaken. The results corroborated that the application of 4mM NEM for three minutes to the olfactory epithelium abolished the odor-evoked electro-olfactogram (EOG), whereas rinsing the epithelium with ethyl n-butyrate, an odorant, during the NEM application prevented the inactivation of the EOG. Getchell and Gesteland hypothesized that ethyl n-butyrate protected its receptor sites in the olfactory epithelium from NEM binding, and thus, preserved its receptor potential.

To determine the possible NEM binding sites within the olfactory epithelium, histological localization of SH and SS groups in 6 micron serial sections of the frog nasal cavity was accomplished using the APM (N-(4-aminophenyl)maleimide) dual staining technique (Sippel, 1978). Light microscopic examination of the sections revealed the nuclei, olfactory vesicles and cilia within the epithelium had a high content of SH groups. SS-rich granules, which may correspond to granules previously reported in supporting cells (Graziadei, 1971), were found in the apex of the epithelium. Larger granules in the Bowman's glands were also high in SS content. Treatment of the sections on slides or the epithelium in vivo with 4mM NEM required at least 4 hours to effectively block APM staining of SH groups.

Since a 3 minute application of NEM abolished the EOG but did not block APM staining of SH groups, tritiated NEM (2uCi/nostril) was applied to the olfactory epithelium for 3 minutes to determine the extent of NEM binding necessary to inhibit the EOG. The results of this experiment will be compared to odorant sites localized by washing the epithelium with unlabeled NEM mixed with an odorant followed by a rinse with H^3-NEM. This will indicate the feasibility of mapping specific receptor sites in the epithelium using maleimides.

258 INDICATIONS OF CHOLINERGIC INNERVATION OF TASTE BUDS. W.T. Norfleet*, F.M. Matschinsky* and D.A. Godfrey. Dept. Pharmacol., Washington U. Sch. Med., St. Louis, MO.

Circumvallate, foliate, and fungiform papillae in the tongues of male Sprague-Dawley rats were examined for choline acetyltransferase (ChAc) and acetylcholinesterase (AChE) activity through radiometric assay of samples dissected from freeze-dried microtome sections. The fungiform papilla was contrasted with epithelium surrounding this structure. Portions of circumvallate and foliate papillae close to the surface of the tongue where taste buds are absent were compared to deeper sections in which buds constitute a large portion of the papilla volume. Also, submucosal tissue directly adjacent to these two types of papillae containing buds and tissue more remote from the papillae were analyzed. The results are summarized in the following table with ChAc activity in μmoles/kg dry wt/min, AChE in mmoles/kg dry wt/min, mean ± SEM for "n" animals:

		ChAc	AChE
Circumvallate Papillae	Papillae w buds (n=7):	22.3±3.7	10.5±1.3
	Papillae wo buds (n=5):	1.0±0.4	2.1±0.3
	Nearby tissue (n=2):	14.3, 22.2	6.2, 11.2
	Distant tissue (n=1 or 2)	1.3, 8.8	1.0, ---
Foliate Papillae	Papillae w buds (n=6):	40.8±6.2	22.4±1.3
	Papillae wo buds (n=5):	-0.2±1.2	3.5±0.3
	Nearby tissue (n=1 or 2):	7.6, ---	14.6, 15.8
	Distant tissue (n=1 or 2):	1.1, ---	6.8, 1.3
Fungiform Papillae	Papillae w buds (n=2):	0.0, 3.9	1.9, 2.7
	Nearby epithelium (n=2):	0.6, 1.2	0.5, 0.3

Both ChAc and AChE were found in circumvallate and foliate papillae containing taste buds and in submucosal tissue adjacent to these structures. However, enzyme activity was low or absent in more distant submucosal tissue and in fungiform papillae. The results are consistent with cholinergic innervation of the taste buds of circumvallate and foliate papillae. This innervation may be part of an efferent neural system analagous to auditory and olfactory cholinergic pathways.

259 IXth NERVE TASTE RESPONSES DEPEND UPON AXOPLASMIC TRANSPORT
Bruce Oakley, Lee B. Jones*, Joyce S. Chu* and Mark A. Hosley*. Div. Biological Sciences, Univ. Mich., Ann Arbor, MI 48109.

We have examined impulse discharges and axoplasmic transport in intact and treated IXth nerves of the Mongolian gerbil (Meriones unguiculatus). Previously, we reported that following transection of the gerbil's IXth nerve, the summated impulse discharge to taste solutions declined in 1-4 hr to 0-25% of the initial response magnitude (Berland et al., In: Olfaction and Taste VI, J. LeMagnen and P. MacLeod, eds., Information Retrieval Ltd., Arlington, 1977). Control experiments indicated that impulse propagation mechanisms along the transected nerve were not disturbed. In addition, we found that the rate of taste response loss in transected IXth nerves was nerve length dependent, suggesting that a flow of axonally transported material normally sustains the taste response mechanism. To test this proposition, we injected into the petrosal ganglion of the IXth nerve 0.04 µl (1 µCi) of either ^{3}H-leucine or a complete mixture of ^{3}H-amino acids. Subsequent liquid scintillation counting of portions of the tongue and approximately 1.2mm serial segments of the IXth nerve indicated that labeled material was transported down the IXth nerve to the foliate and vallate taste papillae of the tongue. Following nerve transection, transport continued in the distal portion of the nerve, as predicted from the electrophysiological observations of the nerve length dependency of the taste response decline. The intact IXth nerve could be cooled for 15-60 min with a 3-10°C metal probe. This caused an accumulation of labeled material proximal to the cooled segment. Furthermore, taste responses also declined in intact nerves after such cooling. Thus, local cooling of the IXth nerve impaired both axoplasmic transport and taste response mechanisms.

We conclude that the integrity of the physiological taste response mechanism is maintained by materials supplied to the IXth nerve axon terminals by axoplasmic transport. The precise role of such transported material in taste function remains to be elucidated.

Supported in part by NIH grant NS-07072.

260 BEHAVIORAL EFFECTS OF CUTTING THE EFFERENT NERVES TO THE HAMSTER VOMERONASAL ORGANS.
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The nasopalatine nerve (NP) has been shown to control stimulus access to the vomeronasal organ (VNO). Stimulation of this nerve activates a vascular pump in the organ which draws odor laden fluids from the nasal vestibule into the lumen of the VNO where the sensory epithelium is located. Removal of the afferent outflow from the VNO and the olfactory epithelium regularly eliminates male mating behavior. Presumably by interfering with the perception of odor cues from receptive females. Therefore we postulated that cutting the NP should also interfere with mating behavior by preventing stimulus ingress to the VNO.

In addition to the usual measures of mating behavior (ie. no. of mounts and their respective latencys) we were also interested in measuring male responses to the odor of estrous hamster vaginal discharge (HVD) using a standard two bottle preference test. This preference is regularly exhibited by normal males in the absence of other hamsters. The 12 males selected for study had a strong preference for HVD and were known to have mounted a receptive female during a single 5 minute mating test. Two groups, of six males each, were formed so that both had equal preoperative preferences for HVD. The NP of the experimental animals was cauterized through a small hole in the hard palate. Control males were treated similarly except that the NP was not cauterized. After a 4 day rest period all animals were examined for mating and HVD preferences. Four alternating mating and preference tests were spaced over a period of 14 days.

Three of the six experimental animals failed to show any postoperative preference for HVD. The remaining three animals had reduced preferences. In only 9 of the 24 individual trials was there any preference for HVD. In one of the experimental animals which continued to show a postoperative preference, histological examination revealed an incomplete nerve section on one side. Control animals prefered HVD in 21 of the 24 trials. There were no significant differences between the two groups on the mating tests. Further experiments are underway to determine if NP cuts coupled with deafferentation of the olfactory mucosa will eliminate both the preference for HVD and male mating behavior.
(Supported by NINCDS grant NS14453).

261 EFFECT OF PHOTOPHASE ON PRIMARY TASTE RECEPTORS.
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Richter thought receptor changes could account for altered taste preferences[1], but Pfaffmann did not show any electrophysiological differences between normal and adrenalectomized rats[2]. In insects, however, taste receptors do change. Butterfly larvae receptors have a lessened impulse frequency when their adequate stimuli had been present in ingested food prior to testing[3]. In satiated Blowfly adults, receptors show reduced impulse frequencies[4]. Fewer taste sensilla produce impulses during diapause in the Blowfly[5], and in the Locust following feeding, when sensillum tip resistance is high[6]. We now report similar multiple changes associated with photophase in Musca domestica.

A micropipette with stimulus and electrolyte (1/2 M sucrose and/or 1/3M LiCl) placed over a taste hair allowed the display of impulses and slow potentials passed through a Grass P16 preamplifier. Male flies, deprived 24-48 hr had been kept in briefly interrupted (1/2 hr/day) darkness or in the fall cycle of sunlight (through window glass) since the late pupal stage.

Under the regimen of darkness, receptor functions were reduced. Fewer sensilla and fewer cells in each produced impulses; impulse frequency was lower; impulse discharge was more brief.

These effects of light were offset by ingestion. By 72 hr of deprivation, responses were up, in spite of the darkness. With a 24-hr access to food (0.1M sucrose), lighted animals showed the reduced activity pattern: fewer sensilla responded, and with fewer impulse types, lower frequencies, and brief discharges.

In addition to spike potentials, slow oscillations recurred at about 1-2/sec and slightly increased the non-spiking excursions. Under each regimen, oscillations coincided with high receptor function but were absent from animals with low receptor function.

The observation that taste receptors change in relation to photophase as well as ingestion, is indicative of a broadly based regulatory system for taste receptor action.

(1) Richter, C. Endocrin. 24:367, '39; (2) Pfaffmann, C. et al. J. Comp. Physio. Psych. 43:320, '50;(3) Schoonhoven, L. Proc. Kon. Ned. Aka. Weten. C. 72:491, '69; (4) Omand, E. Am. Zool. 9:594, '69; Comp. Bioch. Physio. 38A:265, '71;(5) Stoffolano, E. J. Geron. 28:35, '73; (6) Bernays, E. et al. J. Exp. Bio. 57:745,'72.

Supported by NIH 1-R01-NS-12040.

262 NEURAL RESPONSES TO AMINO ACIDS IN THE RAT.
Thomas C. Pritchard* and Thomas R. Scott. Dep't Psychol., U. Delaware, Newark, DE 19711.

The role of gustation in selecting nutrients from the environment would seem to require sensitivity to complex nitrogenous molecules such as amino acids (a.a.'s). Although the environment more commonly provides nitrogenous compounds in peptide chains, individual a.a.'s have been shown to be effective taste stimuli in humans and lower animals. In this study we determined the neural effectiveness of 12 l-a.a.'s relative to .1 M NaCl and to each other across a range of concentrations. Responses were recorded from the whole chorda tympani nerve of adult male albino rats, stimulated by 40 ml of solution delivered to the anterior half of the tongue in 4 sec. The concentration of each a.a. ranged, usually in half log molar steps, from that which evoked no increase in whole nerve activity to the intensity at which the neural response reached asymtote or at which the solution in 25°C DH_2O was saturated. This included approximately ten concentrations of each stimulus, and 3-5 applications were made of each concentration. Trials of NaCl were interspersed throughout the stimulus series to verify stability of the neural response. The relative effectiveness of the a.a.'s, as defined by the response elicited by the highest concentration, correlated +0.89 with their solubility in water. Thus, such a definition of responsiveness is more a measure of how concentrated the solution could be made than a test of taste sensitivity to various molecular species. Perhaps a better measure is the concentration at which a threshold response was elicited, threshold being arbitrarily defined as a 10% increase from spontaneous activity level. By this definition, the order of a.a. effectiveness was histidine > cysteine HCl > lysine > arginine > methionine > alanine > leucine > isoleucine > glycine = threonine > proline > tryptophan. This sequence correlated +0.88 ($p<.001$) with that of human thresholds for the same stimuli. (supported by NIH grant NS 10405).

263 CENTRIFUGAL EFFERENTS TO THE OLFACTORY BULB IN THE RHESUS MONKEY. Douglas L. Rosene, Lennart Heimer, and Gary W. Van Hoesen. Harvard Neurological Unit, Beth Israel Hospital, Boston, MA 02215, Dept. of Anatomy, University of Virginia, Charlottesville, VA 22904, and Depts. of Anatomy and Neurology, University of Iowa, Iowa City, IA 52242.

In humans, olfaction is often ignored or viewed as a somewhat vestigial sensory modality and comparative neuroanatomical studies indicate that primate olfactory centers do not share in the phylogenetically progressive development of non-olfactory limbic structures. Nevertheless in the presumably microsmatic rhesus monkey, olfactory bulb efferents are widely distributed to the same telencephalic structures as in macrosomatic rodents. Utilizing injections of the retrograde tracer horseradish peroxidase (HRP) into the olfactory bulb of the rhesus monkey we now report that centrifugal efferents to the olfactory bulb originate from a surprisingly widespread group of central structures.

Ipsilateral to the HRP injection heavy labeling was observed in all subdivisions of the anterior olfactory nuclei (AON) except the pars externa. Contralaterally the pars externa was heavily labeled while lighter labeling was observed in the AON. The lateral transition cortex between the pars lateralis of the AON and the primary olfactory cortex (POC) was labeled bilaterally while the medial transition cortex (MTC) between the pars medialis of the AON and the induseum griseum was labeled only ipsilaterally. Previously the MTC has been identified by others as part of the anterior hippocampal rudiment.

In the POC labeled neurons were seen ipsilaterally in both layers II and III. In adjacent entorhinal cortex labeled neurons were found in the superficial layers of the most rostral, transitional subdivision of lateral entorhinal cortex. In the amygdala labeled neurons were found throughout the anterior amygdaloid area as well as superficially in the cortical amygdaloid nucleus. Labeled neurons were also found in both the vertical and horizontal limbs of the diagonal band, the nucleus basalis, the lateral and dorsal hypothalamus, the ventral tegmental area of the midbrain, the dorsal and medial raphe and the locus coeruleus.

Both the quantity and wide distribution of these centrifugal efferents suggest that pathways for centrifugal efferent modulation of the olfactory bulb in the primate may be comparable to macrosmatic mammals. Thus while the primate olfactory system does not show a progressive development, there is little anatomical basis for regarding olfaction as a vestigial system.

Supported by NIH grants NS 09211, 06209, 10972, and the Benevolent Foundation of the Scottish Rite Freemasonry, Northern Jurisdiction, USA.

264 EVIDENCE FOR A CENTRAL ORIGIN OF CHOLINERGIC STRUCTURES IN THE OLFACTORY BULB. C.D. Ross, D.A. Godfrey, A.D. Williams* and F.M. Matschinsky*. Dept. Anat. and Neurobiol. and Dept. Pharmacol., Washington U. Sch. Med., St. Louis, MO. 63110.

The distributions of choline acetyltransferase (ChAc) and acetylcholinesterase (AChE) activities in the olfactory bulbs of 4 male Sprague-Dawley rats were studied one week after knife cuts were placed at locations caudal to the bulb on the right side (lesions made by Dr. Joseph L. Price, Dept. Anat. and Neurobiol., Washington U. Sch. Med.). In rats A and B, the lateral olfactory tract (LOT) was cut near the junction of the anterior olfactory nucleus and piriform cortex. In rat B, the lesion additionally extended through cortical layers II and III. In rats C and D, a cut through the olfactory peduncle separated the olfactory bulb from the rest of the brain, completely in rat D, but leaving a slight amount of dorsally located tissue intact in rat C. The table presents the ChAc activities in olfactory bulb layers on the lesion side as a percentage of those on the control side (+ difference significant at p=0.05; *difference significant at p=0.005). AChE results were similar, but less dramatic.

Layer	Rat A	B	C	D
glomerular	92	64*	12*	0.5*
ext. plexiform, superf.	93	59*	15*	0.4*
ext. plexiform, deep	88	103	12*	1.3*
mitral	93	80	16*	0.8*
int. plexiform	75+	45*	8*	2.1*
granular	79	54*	12*	2.2*

The percent loss of enzyme activity was related to the extent of the lesion. The virtually complete loss of enzyme activity following the lesion in rat D suggests that all cholinergic elements in the bulb derive from regions caudal to the bulb. The small decrease in enzyme activity following the LOT lesion (rat A) suggests that few, if any, of the cholinergic axons from these central locations are located in the LOT. In rat B, the decrease in enzyme activity was greater laterally than medially in the olfactory bulb, correlating with the lateral location of the lesion. In rat C, the residual ChAc activity in the ventral part of the bulb was similar to that in rat D, while the activity in the dorsal part was much higher. These results extend the concept of a cholinergic centrifugal pathway to the olfactory bulb by suggesting that the axons of this pathway are located somewhere deep to the LOT and may account for all cholinergic synapses in the olfactory bulb. (Supported by NIH grant NS-08000).

265 PATTERNS OF MITRAL AND TUFTED CELL PROJECTIONS TO LOCALIZED REGIONS OF THE RAT OLFACTORY CORTEX AND OLFACTORY TUBERCLE. John W. Scott and Russell L. McBride*. Dept. Anat., Sch. Med., Emory University, Atlanta, Ga. 30322.

Studies of the neuronal connections of the olfactory bulb (OB) have not found a topographic representation of the OB on the olfactory cortex (OCx) although some reports have described different origins for the projections to the medial and lateral olfactory tubercle (OTb). Recent retrograde transport studies of the OB projections with horseradish peroxidase (HRP) have disclosed a preferential tufted cell projection to the OTb and perhaps to anterior OCx (Brain Res. 1977, 129, 152; J. Comp. Neurol. 1977, 172, 1). Those reports emphasized labeling of small clusters of cells from all parts of the OB after localized injections into OCx or OTb.

This study tested whether larger groupings of labeled mitral/tufted cells would be seen if the OB was examined in regularly spaced frontal sections after small HRP injections into OCx or OTb. HRP was injected via an orbital approach. After a 24 hour survival, the brains were fixed and processed with the tetramethylbenzidene technique. The positions of labeled cells from every third 40μ thick section were plotted with a camera lucida. The distance of each cell from the top of the OB was measured along the mitral cell body layer. This distance was plotted along with the distance from the frontal pole of the OB onto two dimensional maps of the OB surface. The data were analyzed by dividing these maps into ten equal longitudinal strips (based on suggestions that the afferents to the OB are organized in longitudinal zones: Brain Res. 1973, 52, 115; 1974, 70, 506) and by testing with chi square against the hypothesis that mitral tufted cells from all parts of the OB project with equal probability to each localized part of the OCx and OTb. Of the 10 bulbs with enough labeled cells to apply this test, 7 show significance with $p<.001$. These distributions also differ significantly from each other, showing that they do not result solely from non-uniform distributions of mitral/tufted cells in the OB. Several bulbs show obvious clusters of labeled cells generally oriented in longitudinal zones. In some cases, there is a restriction to either anterior or posterior regions of the OB.

While we do not yet propose a topographic organization in this projection, we do have statistically reliable evidence that the OB does not project uniformly to each local part of the OCx and OTb.

This material is based upon work supported by the National Science Foundation under Grant No. BNS77-24171.

266 EFFECTS OF pH ON OLFACTORY RESPONSES TO AMINO ACIDS IN THE FRESHWATER EEL. Wayne L. Silver and Don Tucker. Dept. of Biological Science, Florida State University, Tallahassee, Florida 32306.

Averaged neural activity recorded from small bundles of primary olfactory nerve and the underwater electro-olfactogram (EOG) were used to study the effects of pH on the olfactory responses to amino acids in the freshwater eel. Amino acids were dissolved in well water and introduced into the stimulator at 10^{-4}M. Solutions were diluted at least to 66% of the applied concentration as determined by photodensitometry of dye solutions. The pH of the stimulus solutions was adjusted with HCl and NaOH. Both the neural response and EOG appeared to be independent of pH over a relativity wide range between 3.0 and 9.5 regardless of the amino acid tested. At pHs above 9.5 the response magnitudes declined. In most cases the EOG became positive around pH 11.0 although the neural response, while diminished, was still present. Responses recorded at pHs below 3.0 differed for different amino acids. For L-gln, the neural response declined while the EOG increased; for L-cysh, low pHs had no effect on either response type; for L-ala and L-ala methyl ester both the neural response and EOG increased at pHs below 3.0. At low pH the initial neural response was often followed by an off response, but no off response was seen for the EOG. The pKs of the amino acids tested are around 2.0 for the carboxyl group and between 8.0 and 10.0 for the amino group. L-cysh has a third pK at 10.3.

The neural response to well water alone was independent of pH between 2.5 and 10.0. There was a decrease in response magnitude at pHs below 2.5 and above 10.0. At pHs around 2.0 only an off response was observed. The EOG response to well water increased at pHs below 4.5 and decreased around pH 10.0. At pHs above 10.5 only a positive EOG was seen. No EOG off response occurred at low pH.

pH effects on the olfactory bulb induced-wave response to amino acids in the rainbow trout appeared to be similar to those seen on the neural response in the eel. The response was independent of pH between 3.0 and 10.0. At high pHs there was a decrease in the bulbar response magnitude. (Supported by NIH grant NS-08814).

267 IS AN INTACT ACCESSORY OLFACTORY SYSTEM NECESSARY FOR DEVELOPMENT OF ADEQUATE MATERNAL BEHAVIOR IN THE RAT? Pauline J. Singh, Scott Manaker*, A. Marie Tucker* and Myron A. Hofer. Dept. of Psychiatry, Albert Einstein Col. of Med., Montefiore Hosp., Bronx, N.Y. 10467.

Schwartz & Rowe (1977) have reported serious deficiencies, such as cannabalism, in maternal behavior of primiparous rats after bilateral olfactory bulbectomy (BOB). However, this could be due to lack of function of 1) the main olfactory system, 2) the accessory olfactory system, or 3) both. Fleming and Rosenblatt (1973) found that irrigation of the nasal cavities with $ZnSO_4$ solution (which results in a nonfunctional main olfactory system only) did not interfere with the development of maternal behavior in virgin female rats. Thus it was hypothesized that denervation of the accessory system was primarily responsible for the deficiencies noted by Schwartz & Rowe.

In order to test this, 19 female rats and their litters were observed from the day the litter was born (Day 1) through Day 16. Nine females underwent vomeronasal nerve section (VN) which renders the accessory system nonfunctional; 6 were surgical controls (SC); and 4 were normal controls (N). Histology is being done to verify VN. Surgery was performed before mating occurred. No significant differences were found in litter size and pup mortality. Pup weights and temperatures were recorded daily, and no consistent differences were found among groups indicating that nursing behavior was not seriously impaired by the nerve section. Home cages were checked daily for changes in nest location and number of times pups were found out of the nest, and, again, no significant differences were found. Retrieving tests were conducted on Days 4, 7, 10, and 13. No consistent differences were found among groups in number of pup retrievals, number of times the mother nosed or licked the pups, and number of times all pups were returned to the nest by the end of the test. A number of other behavioral items (such as self-grooming, climbing or rising, and digging or burrowing in shavings) were recorded to get an indication of whether or not the mothers were disturbed. No significant differences were indicated.

These data indicate that an intact accessory system is not necessary for the development of adequate maternal behavior. The results of Schwartz & Rowe are due to 1) a nonfunctional main system, 2) nonfunctional main and accessory systems, either one alone being sufficient for adequate maternal behavior, or 3) secondary effects of BOB such as heightened emotionality and aggression. Further experiments are being done to clarify this issue.

268 BEHAVIORAL CORRELATES OF GUSTATORY INFORMATION PROCESSING IN THE HAMSTER CENTRAL NERVOUS SYSTEM. David V. Smith, Joseph B. Travers and Richard L. Van Buskirk. Dept. Psychol., Univ. Wyoming, Laramie, WY 82071.

Several approaches have been taken toward the behavioral classification of gustatory stimuli. Operant conditioning techniques were used by Erickson (Olfaction and Taste, 1963, 205) to examine taste similarities among stimuli in rats. Morrison (Canad. J. Psychol., 1967, 21, 141) also employed operant procedures to generate taste quality profiles based on the generalization of a learned task from a conditioning stimulus to several others. The taste aversion that occurs following association of a stimulus with gastrointestinal illness provides another tool for determining gustatory similarities (Nachman, J. comp. physiol. Psychol. 1963, 56, 343; Tapper & Halpern, Science, 1968, 161, 708). The reduced consumption of various stimuli following conditioned taste aversion was used by Nowlis & Frank (Olfaction and Taste VI, 1977, 241) to generate taste quality profiles for the hamster, which were then compared to single fiber sensitivities in the chorda tympani nerve. The present study provides a comparison between gustatory responses in the central nervous system to a variety of stimuli and taste quality profiles developed from the generalizations of hamsters following conditioned taste aversions to each of these compounds. These generalizations were measured by counting the number of licks to each stimulus following aversive conditioning. This approach allows the comparison among all the stimuli in a single test session. Hamsters were conditioned to avoid one of 10 stimuli, chosen to represent a range of taste experiences, at concentrations equal to those used in the neurophysiological analyses. The generalization of each compound to every other stimulus was measured as the percentage of reduction in the licking rate following aversion. These cross-generalization measures were used to generate a similarity score for each pair of compounds, based on the similarity in their generalizations to the other stimuli. These similarity profiles were then compared to the results of several types of analysis of neural responses from cells in the nucleus tractus solitarius (NTS) and parabrachial pons (PB pons) of the hamster. Cross-correlational profiles for each stimulus across all the others were directly comparable to the behavioral similarity profiles. There was very good agreement between these measures for both the NTS and PB pontine neurons, with the exception of the cross-correlations involving quinine from cells in the PB pons. Classification of these NTS and PB pontine cells into best-stimulus categories allowed an examination of the contribution of these neural channels to the neural representation of gustatory quality.

This research was supported by NINCDS Grant NS10211 and Research Career Development Award NS00168.

269 BIOCHEMICAL BASIS OF THE SYNERGISTIC TASTE EFFECT OF MSG AND 5'-RIBONUCLEOTIDES. Kunio Torii* and Robert H. Cagan. Monell Chemical Senses Center, Univ. of Pa. and Veterans Admin. Hosp., Philadelphia, PA. 19104

Monosodium glutamate (MSG) evokes a taste sensation that is characterized as "unique" or "distinctive"; it is called *umami* in Japanese, which translates as "savory." The remarkable synergistic effect of certain 5'-ribonucleotides in mixtures with MSG is well documented. The taste intensity of such a mixture is greater than the sum of the tastes of the two components. Neither the site of action nor the biochemical mechanism of glutamate taste has been known, although various possibilities were suggested [R.H. Cagan (1977) In *Chemical Senses and Nutrition*, ed. by Kare and Maller. Academic Press, N.Y., pp. 343-359]. In particular, attention was called to the synergistic action as an important criterion to guide biochemical studies.

We have measured directly the binding of L-[^{3}H]glutamate to preparations from bovine circumvallate (CV) (taste) papillae and from tongue epithelium (EP) devoid of taste buds as a control. Differential centrifugation of homogenates of EP and of the sidewall epithelium from CV resulted in a sedimentable fraction. Binding was assayed using L-[^{3}H]glutamate as a ligand with a rapid Millipore filtration method [J.M. Krueger & R.H. Cagan (1976) *J. Biol. Chem.* 251: 88-97].

Substantial binding occurs to CV, while the amount bound to EP is very low. The K_D for L-glutamate was estimated to be 17-20 mM. A remarkable enhancement of binding of L-[^{3}H]glutamate was caused by 5'-GMP. Scatchard analyses showed that the K_D for L-glutamate remained unchanged in the presence of GMP while the maximal binding capacity increased from 10 nmol/mg protein to 60 nmol/mg. The enhancement shows a degree of specificity for the nucleotide indicating that the effect is not merely a nonspecific perturbation. GMP, IMP, and UMP each caused enhancement. On the other hand, CMP and AMP were not effective, and none of these five nucleotides showed any stimulation of the low level of glutamate binding to EP. Guanine, GDP, GTP, adenine, ADP and ATP were ineffective in enhancing glutamate binding to CV.

We postulate (*i*) that the synergistic effect between MSG and 5'-ribonucleotides occurs peripherally at the taste receptor membrane, and (*ii*) that the mechanism of the effect is an increase in the number of available glutamate binding sites caused by the ribonucleotide. This may be related to the phenomenon of ligand enhancement of binding seen with the alanine taste receptors of the catfish [R.H. Cagan (1977) *Soc. Neurosci. Abstr.* 3: 77, Abstr. No. 222]. In the present case, it is possible that the ribonucleotide causes exposure of "hidden" glutamate binding sites.

[Supported in part by NIH research grant NS-08775 from NINCDS.]

270 RESPONSIVENESS OF DOG OLFACTORY RECEPTORS TO THE FATTY ACIDS BUTYRIC, MYRISTIC, OLEIC AND LINOLEIC ACID, AMYL ACETATE, DIMETHYLBENZYLCARBINYL ACETATE AND ANISOLE. Don Tucker and Sadao Kiyohara*. Biol. Sci., Florida State Univ., Tallahassee, Fla. 32306, U.S.A.

Olfactory nerve twig preparations were used for electrical recording of action potential responses from the axons of olfactory receptor cells. The overlapping impulse activity, due to the large numbers of about 0.2 μm diameter axons contained in the smallest possible nerve twigs, was quantified with the leaky-integrator technique to yield a moving average of the firing rate of a small population of the olfactory receptors. The stimuli were delivered to a nasal breathing chamber with a continuous flow of air by the naris at 100 cc/sec. Concentrations were varied over the range 10^{-3} to unity of vapor saturation at 20°C.

Amyl acetate elicited the largest response and was used as the standard for comparison; only two other odorants were available at any time in the olfactometer. Breathing patterns were so variable under Nembutal anesthesia that tracheostomies were performed and air was pulled through the ipsilateral nasal cavity (contralateral nostril plugged). The response dependence on nasal flow rate was least for amyl acetate, responses tending to reach concentration-dependent plateaus at the limit of 100 cc/sec.

Response-concentration curves, at 32 cc/sec, ranged highest to lowest in the order amyl acetate, anisole, butyric a., dimethylbenzylcarbinyl acetate (DMBCA), linoleic a., myristic a, and oleic acid. Nitrogen gas was used in the odor-saturated line to avoid air oxidation of the sensitive unsaturated acids. The magnitude of response to DMBCA was small, although in turtles the maximal values for amyl acetate and DMBCA are about equal. The butyric a. response relative to the amyl acetate response was noticeably greater than in turtle and rabbit. However, responses to the large fatty acid molecules were small. The butyric acid response dependence on flow rate was so strong that the response was approaching that to amyl acetate at 100 cc/sec.

A response to either a residual contaminant in the olfactometer or an odor derived from the front of the dog's nose appeared as an increasing function of nasal flow rate. The flow-rate response, appearing before application of a stimulus, made measurements of small response ambiguous. For example, linoleic a. response measured from the level of flow rate response was much smaller than if measured from the no-flow baseline level. The linoleic a. response may have been accelerating similar to the butyric a. response as a function of flow rate. Also, use of butyric a. caused responses to other stimuli, including that responsible for the flow-rate response, to increase. This effect was interpreted as an increase in accessibility to the olfactory receptors caused by reflex changes in nasal dimensions.

Supported in part by NIH Grant NS08814.

271 X-IRRADIATION INDUCED GRANULOPRIVAL OLFACTORY BULB: PRELIMINARY BEHAVIORAL AND ANATOMICAL FINDINGS. C. P. Walters* and R. G. Struble* (SPONSOR: J. Altman). Lab. Devel. Neurobiol., Dept. Biol. Sci., Purdue Univ., West Lafayette, IN 47907.

Low-level X-irradiation is known to kill mitotic and migrating precursors of neurons. By starting X-irradiation schedules of the olfactory bulbs of male rats soon after birth and continuing until 17 days postnatally, a dramatic loss of small neurons (granule cells) can be obtained with little if any effect on mitral or tufted cell numbers. Behavioral and anatomical data from the granuloprival main bulb preparation were obtained.

The main bulb of the X-irradiated animals is reduced in volume by about 80% relative to control bulbs. All of the subdivisions of the main bulb (e.g. external plexiform layer, internal granular layer) are affected, with the possible exception of the nerve layer. Granule cells are greatly reduced in number, while there is a normal but more tightly packed complement of mitral and tufted cells. Golgi-Cox analysis of the mitral cells suggests abnormalities of cell shape and dendritic field.

The behavior of the X-irradiated animals was indistinguishable from controls on tasks that presumably involve olfactory orientation and/or detection: initial homing to cage shavings; finding buried food; learning an olfactory mediated "Y" maze; reacting to the odor of a cat; mating; and acquisition of a conditioned food aversion.

In contrast, where the olfactory components are more subtle and may require greater integration with non-olfactory information, testing reveals group differences. X-irradiates, as compared with controls, show decreased preferences in food selection tests; take longer to eat a novel food in a novel environment; do not modify their behavior after a noxious tone in an open field; and decrease activity at a slower rate in a novel environment, while they are more activated by novel visual and visual-tactile objects in that environment.

In sum, there are gross anatomical differences between X-irradiated and control animals' main bulb. Surprisingly, there are minimal differences on tasks that may be directly olfactorily mediated. Conversely, the two groups differ when the tasks are not obviously olfactorily mediated. Further, the behavioral effects resulting from neonatal X-irradiation are not similar to reported effects of bulbectomy, arguing against a mass effect and for a selective cell loss hypothesis.

272 POSTNATAL NEUROGENESIS AND REGENERATION OF THE VOMERONASAL EPITHELIUM FOLLOWING AXOTOMY IN GARTER SNAKES. Ruu-Tong Wang, Louis Guida* and Mimi Halpern. Dept. of Anatomy & Cell Biology, SUNY Downstate Medical Center, Brooklyn, N.Y. 11203.

Previous morphological observations of the vomeronasal (VN) epithelium of adult garter snakes (Wang, Kubie & Halpern, Neuroscience Abstract, Vol. 3, p. 85) revealed heterogeneity in cell morphology particularly in both the undifferentiated (Ud) and bipolar (Bp) cell layers suggestive of a continuous process of cell proliferation, differentiation, maturation, aging and cell death. This report confirms the dynamic nature of the epithelium. The VN nerves of garter snakes (Thamnophis sirtalis & T. radix) were bilaterally transected. ^{3}H-thymidine was injected intracardially 24 h prior to sacrifice in animals surviving 1 to 28 days. ^{3}H-proline was injected into the VN organs 24 h prior to sacrifice in other animals surviving 4 to 16 weeks. One VN organ was removed and processed for electron microscopy. The head containing the remaining VN organ was processed for autoradiography.

Following axotomy ultrastructural changes are observed in Bp neurons on postoperative day 2. Degeneration marked by retrograde necrosis and cell loss in the Bp cell layer commences on postoperative day 4, reaches a climax two weeks following axotomy and declines drastically throughout an eight week period. On the other hand, one week following axotomy, the Ud cell layer expands to occupy a portion of the former Bp cell layer. Ud cells continue to increase in number and, by 4 postoperative weeks, occupy two-thirds of the original cell column.

Studies of ^{3}H-thymidine uptake reveal that following VN nerve lesions, the number of labeled Ud cells increases over control (unoperative) levels and this increase is proportional to the length of the postoperative interval.

Eight weeks following surgery and continuing into the 16th postoperative week signs of neuronal maturation are observed among the Bp cells at the apex of the cell column. These neurons sprout a distal process (dendrite) oriented toward the luminal surface of the organ, and a central process destined to terminate in the accessory olfactory bulb (AOB). Injection of ^{3}H-proline into regenerating VN organs reveals that these axons transport labeled macromolecules. Label is found in the AOB in animals surviving eight weeks following axotomy.

These studies suggest that Ud cells are a source for postnatal replacement of neurons in the VN organ. The mitotic activity of Ud cells presumably for the purposes of neuronal replacement under normal conditions is low, but can be enhanced by axotomy. Apparently, after axotomy the VN epithelium regenerates through postnatal neurogenesis.

(supported by NIH grants NS12152 and SO7RR05401)

273 INTRACELLULAR RESPONSES AND CHARACTERISTICS OF TASTE BUD AND LINGUAL CELLS OF THE MUDPUPPY. Charles H. K. West and Rudy A. Bernard. Dept. Neurophysiology, Univ. of Wisconsin, Madison, WI 53706 and Dept. Physiology, Michigan State Univ., East Lansing, MI 48824.

Due to their exceptionally large size, the cells of the mudpuppy tongue are advantageous for intracellular recordings. Therefore, this animal was selected to investigate certain aspects of taste reception such as taste bud cell specificity, electrotonic coupling, and correlation of physiological responses with the different anatomical types of cells. Intracellular recordings of membrane potentials of mudpuppy lingual cells were made with micropipette electrodes. Three types of cells were distinguished by their responses to chemical stimulation. Surface epithelial (SE) cells outside of taste buds responded with large membrane potential and resistance changes to a variety of stimuli representing the four taste qualities. Salts and acids evoked particularly large and rapid potential changes, and $MgCl_2$, acids and quinine greatly increased the membrane resistance. One type of taste bud cell (TB-1) was characterized by large depolarizations to K-salts, and the other type of taste bud cell (TB-2) characteristically hyperpolarized to $MgCl_2$, acid, and sugar solutions. Membrane resistance changes accompanying TB-1 and TB-2 cell responses were relatively small compared to those of SE cells. Electrotonic coupling was observed between pairs of SE and TB-2 cells but not for pairs of TB-1 cells nor cells of different types. After recording cell responses, dye marking with Procion navy blue dye allowed verification of results in situ and histologically. From the identification of cells in section, it is hypothesized the TB-1 and TB-2 cells identified physiologically here correspond to the morphologically defined light and dark cells respectively. Responses of TB-1 cells imply a taste receptive function; whereas, TB-2 cell responses suggest secretory, supportive, and/or receptive function.

274 TASTE NEURON RESPONSE PROFILES: NO EVIDENCE FOR TYPES. D.C. Woolston* and R.P. Erickson. Dept. Psychology, Duke University, Durham, NC 27706.

Frequently in taste neurophysiology the possibility of types of neurons corresponding in some sense with the "primary" taste qualities of Henning has been entertained; recently types of gustatory neurons have been proposed by Frank according to which of the classical "primary" stimuli single neurons give their best response (ISOT VI: 241, 1977); considerable variation occurs in response profiles of neurons of the same type. This variation and previous research by Erickson (Psych. Rev. 75: 447, 1968) and associates suggest that there may be instead a more or less continuous distribution of neuron profiles. To resolve this issue, the responses of 50 single neurons (recorded from the gustatory nucleus tractus solitarius in anesthetized female Sprague-Dawley rats) to as many as 32 chemical stimuli were analyzed by multidimensional scaling and hierarchical cluster analyses. No evidence for clusters of highly similar neuron response profiles was found by either method; similar analyses of the stimuli showed no clear evidence for stimulus types, but the possibility of complex stimuli clouds this issue. The results question the relevancy of the term "primary" for neural coding in taste, and suggest that not all neuron populations need be subdivided into types for an understanding of their organization and function. It is concluded that, just as in audition and color vision where stimulus dimensions are better understood, taste neurons can profitably be depicted in a coherent neuron-stimulus space. In this taste space, the neurons are evenly distributed along stimulus dimensions, as auditory neurons are spaced in an ungrouped fashion along the frequency dimension. (Supported by grants from the US Army and NSF).

275 RESPONSES OF OLFACTORY RECEPTORS IN FETAL AND NEONATAL RATS. R. A. Yancey* and R. C. Gesteland. Northwestern Univ. Dept. of Biological Sciences, Evanston, IL 60201.

The relations between sensitivity to olfactory stimulation and developmental age of olfactory receptor neurons were investigated in rat fetuses from day 11 to term and in neonates from birth to 20 days of age. The olfactory receptors are first seen in fetuses about 12 days old. Responses of the olfactory organ as measured by electro-olfactograms (EOGs) and of single receptor neurons measured by recording action potentials extracellularly are clearly evident between two and four days after the receptors first differentiate from neural anlagen tissue. Responding cells in day 14 to day 17 fetuses appeared non-selective, showing excitation to all stimuli presented (amyl acetate, n-butanol, eugenol, and valeric acid). After day 17 cells were selective in their responses. This is the time when synaptic connections between the receptors and the cells of the olfactory bulb are established. Typically cells were excited by one or more substances and inhibited or not affected by others. They generally were not excited by all four. Fetal EOGs were like those evoked by odors in neonates and adults except that the peak amplitudes were low between days 14 and 17. This may be due to low receptor cell density, shunting of the small tissue mass by bathing saline, or reduced response capacity. Recording from single receptors was remarkably easy in fetuses 16 or more days old. In most preparations only a brief search was required to isolate units with either a platinized metal microelectrode or a pipette with a tip diameter of 3 microns filled with 3M NaCl made up in a 1.5% by weight gelatin solution. The activity of single cells was usually followed for less than 30 minutes, although some units lasted for more than an hour. Responses to stimulation were repeatable during this period. EOG amplitudes were stable for periods exceeding 5 hrs in the best preparations. Commonly the log of the EOG amplitude varied linearly with the log of the stimulus concentration over a range of 0.004 to 0.4 times saturation. Slopes varied from 0.38 to 1.25 and were functions of the stimulus substance and the particular preparation. Fetal olfactory receptors from day 17 on are much like their post-partum counterparts both with respect to the patterns of stimulus-evoked activity and to the irregular, bursting spontaneous activity. For these experiments the olfactory epithelium was quickly exposed after ligation of the unbilical cord and removal of the fetus from the anesthetized (pentobarbital, 35 mg/kg) mother. The fetus was immediately transferred to an experimental chamber maintained at room temperature with an atmosphere of moisture-saturated 95% O_2-5% CO_2. Supported by NSF Grant No. BNS 75-02339.

276 A THEORETICAL MODEL OF THE FLY NERVOUS SYSTEM IN FEEDING. Jacob Zabara and Elizabeth Omand* (SPON: A.R.Freeman)Department of Physiology & Biophysics, School of Dentistry, Department of Physiology, School of Medicine, Temple Univ., Phila., PA 19140.

It has been postulated that, in addition to sensory input, neural autorhythmicity is an important element of the central excitatory state (CES) which culminates in feeding behavior†. We describe by this model some possible relationships between the sensory and autorhythmic activities of the fly's nervous system. In this limit cycle representation, every major element of the feeding system may oscillate at a different phase or lag time. The minimum of the cycle corresponds to the satiety state and the maximum to the fully developed feeding behavior leading to ingestion. Sustained ingestion completes the cycle as satiety supervenes.

The gain of the system relates to the development of the CES. The fully developed CES represents a state of autorhythmic activity upon which is superimposed sensory input activity. We assert in this model that a corresponding region of the brain neuropil is involved in the development of the CES. A single oscillation of the limit cycle begins with an increase in the autorhythmic activity of this neuropil, which then initiates food searching behavior. Ingestion is consequent to direct chemoreceptor stimulation (labellar nerves), producing an increment in the CES. The increment is a partial function of prior receptor potentiation. The CES and receptor activation show a parallel and either can indicate the gain of the system. Other cyclic phenomena (e.g. photophase) can interface with feeding through the action of the CES.

By this model, the overall excitational state of the neuropil can be summarized as follows:

$$S_i = \int_{i=o}^{i=n} \frac{de}{dz} \cdot Z_i \, dz,$$

where S_i = the central excitatory state (CES)
Z_i = the equivalent synapse
e = excitation
$\frac{de}{dz}$ = the excitability factor

This model, which represents an integration of receptor dynamics with centrally mediated processes, will be discussed in relation to pertinent mammalian studies. (NIH Grant RO1-NS-14209-A).

†Omand, E., Comp. Bioch. Physio. 38A: 265, '71; and Dethier, V.G., The Hungry Fly, pp. 460-476, Harvard, Cambridge, '76.

COMPARATIVE NEUROBIOLOGY

277 HETEROLATERAL INFLUENCE OF LIGHT UPON RETINAL SHIELDING PIGMENTS POSITION IN THE CRAYFISH. B. Barrera-Mera, G.Y. Berdeja-García* and J. Cibrian-Tovar*. Depto. de Fisiología, Fac. Med., U.N.A.M. & Rama de Entomología, Col. Postgraduados S.A.R.H., Chapingo, México, D.F.

The strong tendency to maintain the same frequency and phase angle of the electroretinogram (ERG) of both left and right eyes during circadian oscillations in Procambarus bouvieri (Barrera-Mera, 1978 in Press) as well as the bilateral diminution of the ERG voltage seen after sustained (60 min) photic stimulation of either eye (Barrera-Mera and Abasta, Brain Res. Bull. 3: 101-106, 1978) suggested that bilateral input of light activates the neuroendocrine system of the sinus gland in both eyestalks and thus modulates the crayfish retinal sensitivity. We have found that eye glow area (EGA) diminution, due to the light adaptation position of retinal shielding pigments (RSP), can also be heterolaterally induced (Fig. 1 Ac). This response, better observed during the rest phase of ERG circadian oscillations, has a relative long latency (15-20 min), is proportional to the intensity of the heterolateral photic stimulation and is suppressed by surgical bisection of the supraesophageal ganglion (Fig. 1 Bc).

The equal migration toward the light adaptation position of the distal (d) RSP on both sides in intact animals (Fig. 2 A) but not in splitbrain animals (Fig. 2 B) contrasts with the mobilization of proximal (p) RSP. Since the ERG, EGA size and dRSP follow a similar time course we believe RSP mobilization is an important modulating influence on the retinal sensitivity due to the action of light adapting hormone.

Fig. 1

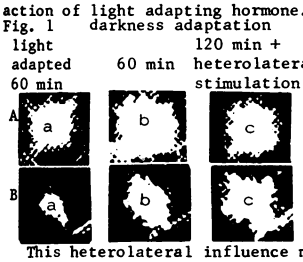
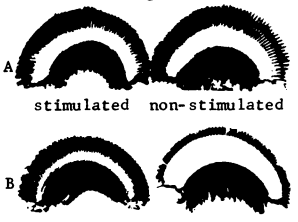


Fig. 2



This heterolateral influence mediated by the position of the peripheral effectors dRSP, may be the synchronizing process for the entrainment of the left and right ERG during circadian changes. This interesting model seems to be the first for which the coupling mechanism of symmetrical circadian pacemakers of apparently similar hierarchical importance has been found.

278 OLFACTORY PATHWAYS IN THE CHANNEL CATFISH, ICTALURUS PUNCTATUS. Andrew H. Bass. Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109.

Secondary olfactory pathways in channel catfish were studied by autoradiographic and Fink-Heimer methods. Seven animals were injected with 100-200 nl of ^{3}H-proline in the olfactory bulb and allowed to survive 7-12 days. The olfactory peduncle was cut in 3 animals who survived 3-10 days.

The present analysis utilizes the nomenclature of Nieuwenhuys (J. Hirnforsch. 6:171, 1963). The olfactory bulb projects to the ipsilateral subpallium, pallium, preoptic area, hypothalamus, and contralateral olfactory bulb. As the medial olfactory tract (MOT) courses along the ventromedial surface of the subpallium, a sparse terminal field appears lateral to the ventral subpallial nucleus (Vv). Fibers course dorsomedially to terminate in a capsular fashion about the dorsal subpallial nucleus (Vd). An additional terminal field appears lateral to Vd. The lateral olfactory tract (LOT) courses along the ventrolateral aspect of the pallium to terminate in an expansive ventrolateral zone. The latter comprises rostral and caudal (Dp) divisions. Dp receives the largest, densest olfactory input in the pallium. The area dorsal and medial to Dp also receives olfactory input. This area (Dc) contains a dorsal region receiving a sparse olfactory input, and a central area receiving a denser input. Continuous with Dp, an additional terminal field, the intermedial nucleus of the subpallium (Vi), extends ventromedial.

Olfactory fibers proceed caudally to terminate in the nucleus preopticus periventricularis anterioris. Olfactory fibers also terminate in a caudal medial region of the hypothalamus. All terminal fields are bilateral. The MOT crosses in the anterior commissure (AC) and gives rise to an interbulbar commissure also. The LOT crosses in the AC and the habenular commissures.

These findings generally confirm those in the bullhead catfish (Finger, JCN 161: 125, 1975). Additional subpallial and preoptic inputs are described.

This work was supported by Rackham Dissertation Grant funds, Univ. of Mich., and by PHS Grant 1 R01 EY02485 to R. Glenn Northcutt.

279 BASAL METABOLISM PREDICTS SIZE OF VERTEBRATE CNS. Robert J. Blumenschine*, Jonathan W. Mink*, and David B. Adams. Dept.Psych. Wesleyan Univ., Middletown, CT 06457.

We have found that in most vertebrates between two and ten percent of the total basal metabolism is used by the CNS. The relationship is practically linear for the following animals: perch, trout, sharks, frogs, python, turtles, chicken, pigeon, sparrow, swallow, emu, shrew, bats, rabbit, horse, zebra, elephant, various rodents, ungulates, carnivores, primates, and cetacea. Whole body metabolism was available for most animals, but CNS metabolism was available only for a few mammals and was calculated for the other animals from rates of CNS metabolism and brain and spinal cord weights. On a log-log scale, 105 individual determinations correlated .96 to a regression equation with a slope of .95.

Data for a few vertebrates do not lie close to the regression line. Humans use a remarkably high 20% of their resting metabolism for the CNS. The whale's low percent (0.15%) may be due to a recent phylogenetic increase in body size which has not been matched by brain size, since smaller cetacea (porpoise and dolphin) are similar to other vertebrates. The low values for domestic livestock (pig, cow, sheep, horse) may be due to domestic selection for increased body size without selection for increased brain size, since other domestic animals (cats, dogs) show predicted values.

If the above exceptions,humans,whale,and domestic livestock, are removed from the regression equation,then the remaining 88 determinations show a correlation coefficient of .98 and a slope of .99. The slope of unity means that the equation is a linear one,unlike the logarithmic equations which have been used traditionally to predict body metabolism or brain size from body size.

The direct linear relationship of CNS and body metabolism makes possible an improved method for predicting CNS size of any vertebrate. Previous methods using non-linear equations, have required separate factors for poikilotherms and homeotherms. The new equation is based on the linear relationship of CNS and body metabolism for all vertebrates. It is complicated only by the calculation of CNS metabolic rate which is known to vary as an orderly function of temperature, temperature acclimation,and neuronal packing density. The latter,in turn,is proportional to body size.

The data suggest a new interpretation for the dramatic increase in ratio of brain to body weight which occurred in the phylogenetic transition from poikilothermy to homeothermy. By increasing their resting metabolic rates by an order of magnitude, homeotherms were able to afford CNS metabolism which was also an order of magnitude greater. The increase came mostly in CNS size rather than in CNS metabolic rate which increased a smaller amount up to the level which would be expected for a poikilotherm at 37°.

280 ORGANIZATION OF THE THALAMUS IN THE LONGNOSE GAR, LEPISOSTEUS OSSEUS. Mark R. Braford, Jr. and R. Glenn Northcutt. Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109.

Following cytoarchitectonic analyses, graphical reconstructions, and the study of various afferent fiber systems with degeneration and/or autoradiographic methods, the thalamus of gars has been divided into three major zones. The pars dorsalis and the pars ventralis form longitudinal zones, whereas the pars tuberculi posterioris lies caudoventral to them (cf. Bergquist, Acta Zool., 13, 1932). The pars dorsalis consists of three major cell groups: a rostral complex comparable to DM-DL of Schnitzlein, which receives a retinal input, and two caudal groups --the dorsal posterior nucleus, which receives a tectal input, and the central posterior nucleus. Between the pars dorsalis and the pars ventralis lies a slightly migrated group, nucleus intermedius, a portion of which receives a retinal input. Whether this group is part of the dorsal thalamus or the ventral thalamus, or constitutes a separate zone (see Bergquist) is unclear without further data. The pars ventralis consists of a prominent, laminated, periventricular group, the ventromedial nucleus, which receives a cerebellar input, and a smaller migrated group, the ventrolateral nucleus, which receives a retinal input. The pars tuberculi posterioris lies dorsal to the hypothalamus and rostral to the tegmentum. In gars, as in the actinopterygian fishes generally, the pars tuberculi posterioris is relatively much larger and more complex than in any other group of vertebrates. Its major constituents include nucleus tuberis posterior (which receives an input from the olfactory bulb), the preglomerulosus complex (which receives an input from the telencephalon proper), nucleus thalami posterior, and nucleus supramammillaris. No true nucleus glomerulosus is present in gars. The so-called nucleus geniculatus lateralis receives a retinal input but appear to be a superficial pretectal nucleus rather than a part of the thalamus.

This work was supported by PHS Grant 1 R01 EY02485 to RGN.

281 THE CROCODILIAN MIDBRAIN TEGMENTUM: A KEY TO UNDERSTANDING THE AVIAN THALAMUS. Steven E. Brauth, Cheryl A. Kitt & John L. Ferguson, Dept. Psychology, Univ. of Md., College Park, Md. 20742

In birds, two paths descend from the thalamus: a thalamocerebellar path originating from the nucleus spiriformis medialis (SpM), and a thalamo tectal path originating from the nucleus spiriformis lateralis (SpL) (Karten & Finger, 1976; Brecha, Hunt & Karten, 1976). However in the mammal there are no direct paths from the thalamus to either the cerebellum or the superior colliculus. In an attempt to interpret these major differences in avian and mammalian thalamic organization, neuroanatomical tracing techniques were employed to determine the origin of midbrain or thalamic afferents to the cerebellum and tectum of a crocodilian, Caiman crocodilus. Fossil evidence suggests that Crocodilians may closely resemble the ancestors of birds.

Two nuclei: nucleus circularis (Cr) and the interstitial nucleus of the posterior commissure (nICP) were identified in the midbrain tegmentum of Caiman each of which projects upon lobus medialis of the cerebellum. Also a nucleus, the dorsal nucleus of the posterior commissure (nDCP) was identified in the midbrain tegmentum of Caiman which projects to the tectum. Like SpL of birds, nDCP receives projections from the paleostriatum as shown by autoradiographic tracing of anterograde projections of the paleostriatum (nucleus basalis of Kuhlenbeck) in Caiman. These pathways allow the basal ganglia to influence the tectum in both birds and crocodilians. Thus, cell groups exist in the midbrain tegmentum of Caiman, which, based on afferent and efferent connections are comparable to posterior diencephalic nuclei in avian forms.

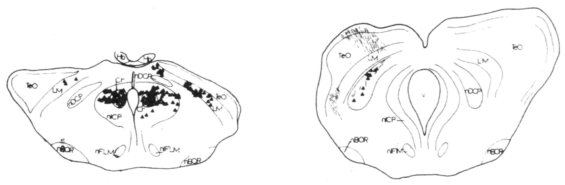


Figure on the left represents the pattern of label in Cr and nICP after an HRP injection in the lobus medialis of cerebellum in Caiman. On right, cells in nDCP are labeled after tectal HRP.

282 EFFECTS OF SERIAL LESIONS OF TELENCEPHALIC COMPONENTS OF THE VISUAL SYSTEM IN PIGEONS. Nellie M. Bugbee*, William Hodos, and Tatiana Pasternak. Dept. of Psych., Univ. of Maryland, College Park, MD 20742

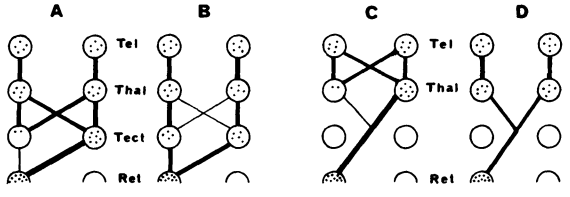
Pigeons were trained to discriminate three types of stimuli in a quasi-random sequence: color stimuli (yellow vs. green), intensity stimuli (0.8 log unit difference) and two sets of pattern stimuli (vertical vs. horizontal bars and triangles with apex up vs. apex down). Following this training, one group of birds received lesions in the visual Wulst, which is the telencephalic component of the thalamofugal visual pathway. In a second group, lesions were made in ectostriatum, the telencephalic component of the tectofugal pathway. Postoperatively, both groups were retrained to criterion. As previously reported, birds with visual Wulst lesions were only mildly impaired while birds with ectostriatum lesions showed moderate to severe impairment on intensity and pattern problems.

After postoperative reacquisition of the discriminations, the ectostriatum-lesion group received lesions of the visual Wulst and the visual Wulst-lesion group received lesions of ectostriatum. In general, the effects of ectostriatum lesions in pigeons with prior visual Wulst lesions resulted in a postoperative return to chance performance followed by a protracted period of retraining to criterion. Thus, ectostriatum lesions seem to produce the same effects in birds with prior visual Wulst lesions as in birds with visual Wulst intact. In contrast, the effects of visual Wulst lesions in pigeons with prior ectostriatum lesions resulted in a profound and seemingly permanent impairment of pattern discrimination performance. Color and intensity discrimination were initially impaired but improved after retraining. Thus, the visual Wulst lesions produced markedly different effects in birds with prior ectostriatum lesions as compared to birds with ectostriatum intact. In a third group, simultaneous lesions of visual Wulst and ectostriatum were made. The performance of these birds was equivalent to that of pigeons with visual Wulst lesions that had been made after ectostriatum lesions.

These data indicate an order effect of visual Wulst lesions; i.e., the relative sparing of pattern discrimination following visual Wulst lesions depends upon an intact ectostriatum. No order effect was seen after ectostriatum lesions. Since the destruction of ectostriatum after visual Wulst lesions produces no greater impairment than lesions of ectostriatum alone, ectostriatum does not appear to serve as a "surrogate visual Wulst" in birds with visual Wulst lesions.

283 A POSSIBLE MECHANISM FOR THE CHANGE OF CENTRAL NERVOUS SYSTEM PATHWAYS OVER EVOLUTION. Ann B. Butler. Dept. Anat., Georgetown Univ., Washington, D.C. 20007.

From recent studies on visual system pathways, some common patterns can now be recognized within various groups. Figures below show general patterns of retinotectofugal projections in non-mammals (A) and mammals (B). While most non-mammals have predominantly crossed retinotectal projections, the tectum sends substantial projections to both the ipsilateral and contralateral thalamus. In mammals, however, the retinotectal projection is substantial ipsilaterally (Hubel et al,'75; Graybiel,'76; Harting and Guillery,'76; Tigges et al, '77) as well as contralaterally, and the subsequent tectothalamic projection is almost completely ipsilateral. Thus, similar net input from each retina to thalamus and telencephalon is achieved in both groups but via quantitatively differential development of different parts of the pathways. Taking the condition of predominantly crossed retinal projections to be ancestral among vertebrates, it is hypothesized that the increase in ipsilateral retinotectal projections in mammals resulted in a quantitative change in the post-synaptic pathway in this system--a decrease in contralateral tectothalamic projections--and this is referred to as trans-synaptic evolution. The same phenomenon may account for the presence of bilateral thalamotelencephalic projections in sharks, birds, and lizards (C) but not in mammals (D). This phenomenon, combined with sprouting of new pathways seen in response to deafferentation of a structure (Steward, '76), as would be the effect of decreased contralateral tectothalamic projections in mammals, can be envisioned to have occurred over evolution, resulting in changes of neural interconnections throughout the brain. Supported by NSF Grant BNS77-26022.



284 REFLEX CONTROL OF POSTURAL MUSCLE STIFFNESS IN HERMIT CRAB. William D. Chapple., Physiology Section, Biological Sciences Group, University of Connecticut, Storrs, CT 06268.

Phasic mechanoreceptors in the ventral epidermis of the hermit crab abdomen reflexly excite motoneurons innervating the ventral superficial muscles (VSM), the major group of abdominal muscles supporting the shell during standing and walking. Mechanical stimulation activates three motoneurons on each side of each segment. Muscle tension is elevated by the initial high frequency burst of the motoneurons and slowly declines during the after-discharge. The VSM are composed of four parallel groups of muscle fibers, each with characteristic sarcomere lengths.

Isometric length-tension curves of the VSM are complex. As the muscle is extended there are several tension plateaus, which suggests that in different types of muscle fibers, maximum tension is developed at different extensions. Constant velocity lengthening of the VSM and epidermis shows that the passive tension is high compared with the increment of force produced by a train of stimuli. In addition, tension is a linear function of velocity throughout stretch. In a muscle composed of muscle fibers with different length-tension curve maxima, nonlinearities during lengthening due to the short range elasticity of one muscle type may not be present. Such muscles would maintain specific positions by the reflex increase in muscle stiffness.

285 SPERM RELEASE EVOKED BY ELECTRICAL STIMULATION OF THE BRAIN OF THE GOLDFISH, CARASSIUS AURATUS. Leo S. Demski. School of Biological Sciences, Univ. of Kentucky, Lexington, KY 40506.

Stimulation sites were tested on 60 dorsoventrally directed electrode tracks in 9 fish (14-20 cm standard length). Semen release was evoked at 41 sites and 28 of these were identified histologically with Prussian blue. In general techniques used previously on sunfish (Demski, Bauer and Gerald; J. Exp. Zool., 191: 215-232) were employed. The main difference was that except for the first fish (2 stimulation sites) the anesthetic level (0.15%) was half that used with sunfish. The "standard procedure" of testing at each .1mm was used for 16 points and the technique of "maximizing the response" by moving the electrode up and down was used for 12 sites. Since there appear to be no significant differences in the results obtained using the two methods, the data from each have been combined. Several low threshold sites (15-50 μA) were found in and just dorsal to the nucleus glomerulosus complex while moderate to high threshold points (51-300 μA) were scattered throughout this region as well as the preoptic area, dorsal hypothalamus, subhabenular region and basolateral medulla (see table below). Allowing for species differences in brain anatomy, the results in goldfish appear comparable to those obtained earlier in sunfish. Thus, it can be suggested that similar sperm release mechanisms may exist in many teleosts.

Regional distribution of stimulation sites from which sperm release was evoked

Region stimulated	Threshold (μA)				
	15-25	26-50	51-100	101-200	201-300
Preoptic and adjacent areas	0	0	5	4*	1
Hypothalamus-near postoptic commissures	0	1	1	1	0
Subhabenular region	0	0	1	0	0
Tegmentum-near the n. glomerulosus complex	4	3	0	1	1*
Basolateral medulla-cerebellar to midvagal lobe levels	0	2	1	0	2

*one site was tested using the higher anesthetic level

286 BIVALVE HEART MUSCLE CELLS: RESPONSE TO IONTOPHORETICALLY APPLIED ACETYLCHOLINE. Ellen J. Elliott* (SPON: R.M. Steinman). Dept. Zool., Univ. Md., College Park, MD 20742.

The response to iontophoretically applied acetylcholine (ACh) was observed by intracellular recording from heart muscle fibers of three species of bivalves: Mytilus edulis (mussel), Mercenaria mercenaria (clam), and Crassostrea virginica (oyster). These species were chosen as representatives of the different types of response to bath-applied ACh (excitation, inhibition, and mixed excitation-inhibition) which have been reported for bivalve hearts. Hearts were bathed in an artificial sea water (ASW) solution in which $MnCl_2$ was substituted for $CaCl_2$ in order to abolish spontaneous contractions. Heart fibers were impaled with 80-100 MΩ microelectrodes filled with 2M KCl-1M Kcitrate or with 3M Kcitrate. The mean resting potentials recorded were -47, -50 and -44 mV for Mytilus, Mercenaria and Crassostrea, respectively. 20-50 MΩ iontophoretic microelectrodes were filled with 1M AChCl. In Mytilus, iontophoretic pulses of ACh caused a rapid depolarization which in a few cases was followed by a slower and smaller hyperpolarization. In Mercenaria, ACh caused only a relatively slow hyperpolarization. In Crassostrea, ACh always produced a biphasic response, consisting of a depolarization followed by a hyperpolarization. The two types of response were distinguished by differential sensitivities to antagonists and by different ionic dependencies. The depolarizing response was blocked completely by 5×10^{-5} M d-tubocurarine or hexamethonium, while the hyperpolarizing response was much less sensitive to these two drugs. The hyperpolarizing response was blocked completely by 10^{-7} M methylxylocholine, to which the depolarizing response was much less sensitive. The depolarizing and hyperpolarizing responses were both accompanied by apparent increases in membrane conductance, as determined by passing short pulses of current through an extracellular suction electrode. In Na-free ASW (substituted with Tris or glucoseamine) the depolarizing response disappeared completely and reversibly. In K-free ASW the hyperpolarizing response increased and in elevated-K ASW (22, 45 and 67 mM) it decreased progressively. The highest K concentration often caused reversal of the hyperpolarizing response, even though the resting membrane potential was significantly depolarized. In Cl-free ASW (substituted with isethionate and sulfate), the hyperpolarizing response was unchanged. These results suggest that the depolarization results from an increase in Na conductance and the hyperpolarization from an increase in K conductance. These two types of ACh response are similar in terms of pharmacology and ionic mechanism to two ACh responses seen in ganglionic neurons of Aplysia and other gastropods. The presence of a biphasic ACh response is also similar to the situation found in some gastropod neurons.

287 PHYLOGENY OF ENTERIC SEROTONERGIC NEURONS. J.T. Goodrich and M.D. Gershon (SPON: C. Noback). Dept. Anat., Col. P&S, Columbia Univ. New York, N.Y. 10032.

Serotonergic neurons have been identified in the enteric nervous system of humans, sub-human primates, rodents and chicks. These neurons have a high-affinity uptake system for serotonin which is Na^+- and energy-dependent. The neurons can be grown for long times in organ culture and so are intrinsic to the gut. These enteric neurons can synthesize serotonin (5-HT) from L-tryptophan and contain immunocytochemically demonstrable tryptophan hydroxylase. The high-affinity uptake of 5-HT permits visualization of these neurons by radioautography. These neurons have been found to develop early in ontogeny. Nevertheless a phylogenetic study of their evolution has not yet been done. Such a study would provide insight into whether these neurons in mammals represent a well established neuronal system common to all vertebrates. Baumgarten et al., have reported serotonergic neurons in the lamprey gut. However, since modern cyclostomes have diverged considerably from the ancestral form that gave rise to higher vertebrates, it is necessary to demonstrate enteric serotonergic neurons in another cyclostome species before one can consider them to be characteristic of cyclostomes generally and not just a specific feature of the lamprey bowel. The present study was undertaken to investigate the other major class of cyclostome, Myxine glutinosa (Hagfish). Serotonergic neurons were identified by radioautography. Both strips of muscularis externa with attached myenteric plexus and diced whole tissue were incubated with ^{3}H-5-HT (0.9uM) with excess (9uM) norepinephrine added to prevent 5-HT uptake by catecholaminergic mechanisms. Additional controls were done by adding either a 100-fold excess of cold 5-HT to rule out non-specific binding or Lilly 110140 (fluoxetine), a potent 5-HT uptake inhibitor, fifteen minutes prior to the addition of ^{3}H-5-HT. Radioautography revealed uptake of ^{3}H-5-HT into axons in both the muscularis externa and in the submucosal region. This uptake was prevented by the addition of cold 5-HT or Lilly 110140 prior to the addition of ^{3}H-5-HT. Occasionally, labelled cell bodies could be seen in the muscularis externa and submucosal regions. We conclude that serotonergic neurons are present in the enteric nervous system of these primitive vertebrates and the cell bodies are intrinsic to the gut. Since enteric serotonergic neurons are found early in ontogeny and phylogeny and have been retained in evolution of both birds and mammals, it seems likely that they are a general feature of vertebrates and are probably critical to enteric function. Supported by NIH grants #NS 12969 and GM-02042.

288 TECTAL AFFERENTS IN GOLDFISH AS REVEALED BY RETROGRADE HRP LABEL. B. G. Grover* and S. C. Sharma.
Dept. Ophthalmology, New York Medical College, N.Y.C. 10029.

The optic tectum in fish has long been considered a primary site of sensory motor integration. However, recent studies in several teleosts failed to confirm the existence of tectocerebellar and tecto-oculomotor pathways. The only efferents to the optic tectum in fish that have been studied with modern techniques are those from the retina and telencephalon. Detailed information is necessary before the role of the fish tectum can be understood. We have used retrograde transport of HRP to determine the centers which project to the tectum in goldfish.

Following injections of HRP into the tectum, labelled cells are found in the telencephalon, several diencephalic-pretectal nuclei, the contralateral tectum and a number of tegmental centers. The diencephalic-pretectal areas include the area pretectalis, nucleus pretectalis, nucleus dorsomedialis and nucleus dorsolateralis.

HRP labelled cells were found in the rostral dorsolateral tegmentum, in the torus semicircularis and the nucleus isthmi. A large group of cells caudal and rostral to the nucleus glomerulosis also projects to the tectum. A number of HPR labelled cells were found in the group of efferents that exit the lateral tectum. Some of these cells appear to have one process that reaches the tectum and another that continues down the ipsilateral ventral rectus of the tectobulbar tract (TTB). Cell bodies were also found in the lateral reticular formation of the pons, in the vicinity of the TTB. A few small cells in the area of the dorsal oculomotor complex were found to contain HRP. The tectum also receives a heavy projection from the nucleus lateralis valvula; it is possible that the axons of these cells were mistakenly identified as the dorsal tecto-oculomotor and tecto-cerebellar tracts by early workers.

Fibers of passage through an injection site often pick up HRP. We have traced fibers through the intertectal commissure into the contralateral tectum and out into the contralateral torus semicircularis, where they form terminal fields. These fibers apparently originate from cells in the torus semicircularis on the injection side.

HRP-containing fibers are found in the TTB as far caudal as the gustatory lobe. These fibers probably represent tectal efferents because labelled cell bodies were not found at these levels.

Supported by N.I.H. EY 01426 and EY 05137.

289 GANGLIOSIDE PATTERNS IN VERTEBRATE RETINA AND BRAIN: REGIONAL AND PHYLOGENETIC VARIATION. Louis Irwin and Carol Irwin* (SPON: G. Adelman). Dept. Biochem., Shriver Center, Waltham, MA 02154.

Gangliosides are sialoglycosphingolipids which separate chromatographically according to molecular complexity into patterns that vary phylogenetically. Since regional differences in ganglioside patterns have been reported for the mammalian CNS, particularly differences between the retina and the brain, the observed phylogenetic variations could be due to the gross differences in brain morphology between evolutionarily diverse groups. To examine this possibility, we have analyzed ganglioside patterns from the retina and three morphologically distinct brain regions (medulla, midbrain, forebrain) in each of five species (goldfish, bullfrog, lizard, chick, rat) from different vertebrate classes. Gangliosides were extracted with chloroform:methanol (2:1, v/v), filtered through a Unisil column, eluted with chloroform:methanol: water (10:10:3), resolved by thin layer chromatography on Silica Gel G, and visualized with resorcinol reagent. The full range of ganglioside complexity typically seen in mammalian brains was present in all five species, indicating widespread distribution among the vertebrates of a full set of enzymes for ganglioside synthesis and breakdown. However, the ganglioside patterns varied in detail uniquely for each species, suggesting species-specific differences in the quantitative expression or activity of identical enzymes and/or gene duplication of functionally related enzymes. In contrast to the phylogenetic differences in ganglioside pattern, different neural samples from the same species always showed similar ganglioside patterns despite gross morphological differences. Even the retina differed in ganglioside pattern less from homospecific brain samples than from heterospecific retinal samples. Thus, differences in brain morphology alone cannot account for the phylogenetic variation in ganglioside patterns. (Supported by NSF Grant BNS 77-20575).

290 ARCHICORTICAL FORMATIONS IN DOLPHIN BRAIN. Myron S. Jacobs*, Peter J. Morgane and Willard L. McFarland. Dept. Path., NYU Coll. Dent., New York, NY 10010; Worcester Found. Exp. Biol., Shrewsbury, MA 01545; NIH, Bethesda, MD 20014.

Our studies of the archicortex (Acx) in the cetacean brain have distinguished a number of interesting and critically different features as compared to brains of other mammals. The archicortical formations merge with those of the paleocortex (Plcx) bilaterally in the unci and anteriorly in the septal area. In the temporal sector the archicortical formations are most definitive as in higher mammals with expanded, temporalized hemispheres. Anteriorly they merge with the periamygdalar area of the Plcx and laterally border on the entorhinal area (periarchicortex,Periacx). The dentate area (FD), characterized by small granule cells, is highly reduced, attenuates rapidly posteriorly and is confined to the temporal sector. The cornu ammonis about the shallow hippocampal sulcus (HS) is also distinctive. Areas h5 and h4 are less pronounced than in primates. Area h5 contains only scattered polymorphic cells in the hilus of FD. The medium sized pyramids of area h4 organize into superficial hyperchromatic and deep lighter staining zones. The more cellular areas h3 in the floor, and h2 and h1 along the medial wall of HS show increasing separation into two cell zones towards the subiculum (Sb), the superficial layer being broken up into nests of hyperchromatic larger pyramids. The Sb cell plate is broader and its smaller cells radially oriented. Although cytoarchitecture of the Sb and presubiculum of the Periacx is not as definable as in man, their architectonic transition is marked by a steplike notch in the cellular plate. In the retrosplenial sector, the Acx extends over the splenium as the fasciola cinerea (FasCin) and is less definitive. The hippocampal plate, reduced to 7-10 cell layers, shows only rudimentary characteristics of areas h5-h1. The Sb is less cellular, but its transition with the Periacx is clear. In the supracallosal sector the Acx is maximally reduced. The FasCin continues forward as the indusium griseum (IndGr) which consists only of scattered small hippocampal pyramids. Anteriorly, areas of the hippocampal rudiment (HR) become obliterated. Bilaterally, the fiber bundles of the taeniae tectae (TT) separate the IndGr from the slighly more cellular Sb. The transition of Sb with limbic cortex is still evident. In the subcallosal sector the HR expands into subcallosal, geniculate and medial olfactory gyri. The TT fuse to form a zonal layer over the subcallosal gyri. In summary, the cetacean Acx, especially its inferior part, is temporalized and better developed than in retrosplenial, supracallosal and subcallosal regions. It exhibits regressive features that render morphological criteria somewhat less definitive than in other mammalian brains. (Supported by NSF Grant # BNS 77-08660).

291 DEOXYGLUCOSE MAPPING OF THE VISUAL SYSTEM IN THE GARTER SNAKE. John L. Kubie and Theresa O. Allen*. Dept. of Physiol. and Dept. of Psych., Univ. of Penn., Phila., PA 19104

The (^{14}C)-2-deoxyglucose (2DG) method of neural mapping has revealed dramatic patterns of activation in the mammalian visual system (e.g., Hubel, Wiesel & Stryker, Nature 269:328, 1977). We have attempted to extend this method to a reptilian visual system.

Unanesthetized garter snakes (Thamnophis sirtalis parietalis) with one eye removed or patched were restrained and exposed to a pattern of moving vertical stripes for five minutes preceding delivery of a pulse of 2DG (14 μCi/100 g body weight, sc). Control animals were kept in darkness for the 60 to 84 minute 2DG incorporation period. Brains were processed according to the technique of Kennedy, Des Rosiers, Jehle, Reivich, Sharpe & Sokoloff, (Science 187:850, 1975).

X-ray autoradiographs revealed contralateral activation of all brain structures known to receive direct retinal input, with the possible exception of the nucleus of the ventral supraoptic decussation (Halpern & Frumin, J. Morphol. 141:359, 1973). These included the nuclei of the lateral geniculate and pretectum, the basal optic nucleus, and the superficial layers of the optic tectum. The optic nerve and tract were unilaterally activated, indicating that some fiber systems can be traced with the 2DG method. Some evidence was seen for activation by secondary visual projections: anterior dorsal ventricular ridge activity appeared to be greater contralateral to the stimulated eye.

A number of structures were bilaterally activated independent of visual stimulation. Most of these structures (including olfactory bulb, habenula, interpenduncular nucleus and posterior colliculus) correspond to structures exhibiting high activity in resting, awake rats (Schwartz & Sharp, J. Comp. Neur. 177: 335, 1978). Bilateral activity was also seen in the accessory olfactory tracts, the habenula-interpeduncular tract, the nucleus sphericus, and the anterior dorsal ventricular ridge.

(Supported by NIH grant 2-R01-NS-10617 02A2 and NIMH grant I T32 MH 15092).

292 THE SYMPATHETIC NERVOUS SYSTEM IN THE PRODUCTION OF GASTRIC EROSIONS IN RATS OF DIFFERING SUSCEPTIBILITY. Menachem Melinek*, Sigurd H. Ackerman*, Nansie S. Sharpless, Myron A. Hofer and Herbert Weiner. Dept. Psych. M.H.M.C. and Albert Einstein College Med., Bx., N.Y. 10467.

Increased gastric sympathetic activity during restraint stress in the rat has been postulated as an important factor in the pathogenesis of restraint-induced gastric erosions. This has been inferred, in part, from the fact that α-adrenergic receptor blockade protects against restraint-induced erosion formation (Djahnguiri, et al., J. Pharm. Exp. Ther., 184, 163 , 1973). We used surgical sympathetic denervation of the stomach prior to restraint, or α-adrenergic blockade with phenoxybenzamine (Pbz) during restraint, in order to disrupt gastric sympathetic innervation. The surgical and pharmacologic interventions were tested for their possible effect on restraint erosion production without predicting the direction of the effect (protection or exacerbation). To detect effects in either direction we used both rats at high risk (prematurely weaned) and low risk (normally weaned) for the development of restraint erosions. Without surgical or pharmacologic intervention, prematurely weaned rats had a 68% incidence of erosions and normally weaned rats had a 7% incidence of erosions when tested on postnatal day 30.

Surgical sympathetic denervation, by removal of the superior mesenteric and coeliac ganglia, had no effect on erosion incidence in either group. Completeness of the gastric sympathetic denervation was indicated by a fall in gastric mucosal norepinephrine content from 369.6$\pm$ 20.5 ng/gm to 11.9$\pm$ 2.9 ng/gm.

α -adrenergic blockade with a high dose of Pbz (30 mg/kg) decreased the incidence of erosions in the prematurely weaned group (from 68.4% to 38.9%). Lower doses (20 mg/kg and 10 mg/kg) had no protective effect. However, lower doses of Pbz completely blocked peripheral α -receptors as indicated by the absence of a norepinephrine pressor response. Pbz reduced O_2 consumption but this was unrelated to its protective effect since Pbz treated rats at all doses had comparable reductions in O_2 consumption. On the other hand, behavioral and electrophysiological studies showed marked increases in sedation with progressive increases in Pbz doses.

We conclude that peripheral surgical or pharmacological disruption of the sympathetic innervation of the stomach had no effect on erosion pathogenesis. Pbz appears to protect against restraint erosions formation though CNS effects which correlate with sedation.

293 CEREBRAL BLOOD SUPPLY IN THE DOLPHIN, TURSIOPS TRUNCATUS. Peter J. Morgane, Willard L. McFarland, and Myron S. Jacobs*. Worcester Foundation for Experimental Biology, Shrewsbury, Mass. 01545; NIH, Bethesda, Md. 20014; NYU, N.Y., N.Y. 10016

The tremendous development of retia mirabilia as the sole cerebral blood supply of Cetacea has attracted considerable interest. The hypertrophy of this system in whales has been thought to be related to diving in these totally aquatic mammals. In our studies on the cerebral blood supply of the dolphin, we have carried out anatomical dissections, vascular casting and angiographic investigations in the anesthetized animal, all of them mutually confirmatory as to the cerebrovascular pattern. The entire cerebral blood supply of the dolphin passes through the massive arterial thoracico-spinal rete mirabile. The internal carotid artery becomes non-patent in the adult extracranially and is reduced to a fibrotic strand in the tympanic cavity. This vessel is patent in early embryogenesis, however (Boenninghaus, '04). Neither vertebral nor basilar arteries are present. The intercostal arteries and posterior thoracic artery supply the huge thoracic rete mirabile which lies retropleurally on the posterior wall of the thorax and is innervated by sympathetic fibers. Extensions of this rete pass through the intervertebral foramina to form the extradural spinal rete mirabile filling the dorsolateral space of the spinal canal. Bilateral spinal meningeal arteries lie on the dorso-medial aspect of the spinal rete, becoming larger as they course rostrad. These extradural vessels enter the foramen magnum, become intradural, and sweep laterally over the cerebellum and temporal poles. They pass ventromedially and form an internal ophthalmic rete investing the pituitary region and optic nerves. Intracerebral arteries then emerge from this retial complex. Vessels possibly homologous in a positional sense to the anterior and middle cerebral arteries were seen. The circle of Willis as such does not exist. Histological examination of the retia revealed presence of collagenous fibers in the walls of the arteries of the spinal rete but relative lack of these in the arteries of the thoracic and ophthalmic rete. While retia mirabilia are not an exclusive feature of aquatic mammals, they have reached their greatest development in the Cetacea. As the retia are innervated, it may be that blood is shunted there to serve as a reservoir of oxygenated blood in pressure-impervious bony channels for the heart and brain during dives. We found that this structure has a marked pulse dampening effect. Presence of retia in other orders of mammals in different ecological niches, makes it important to continue comparative morphological and functional studies of the retia so we can understand how such specializations have evolved in response to environmental conditions and life habits. (Supported by NSF grant BNS 77-08660.)

294 ELECTROPHYSIOLOGICAL EVIDENCE FOR AUDITORY RESPONSIVE AREAS IN THE DIENCEPHALON AND TELENCEPHALON OF THE BULLFROG, RANA CATESBEIANA. Karen M. Mudry* and Robert R. Capranica, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853

In a recent anatomical study Neary (Anat. Rec. 178: 425, 1974) identified an ascending projection from the torus semicircularis to a region of the posterior dorsal thalamus in the bullfrog. Our electrophysiological recordings of evoked potentials verify that this diencephalic center responds to acoustic stimulation. In the bullfrog this center is selectively sensitive to bimodal stimuli containing low-and high-frequency energy which excites the two respective auditory organs (amphibian and basilar papillae) in the inner ear of this species. Such bimodal dependence for excitation of the diencephalon provides support for hierarchial processing of vocal signals and other complex sounds in the central auditory system of anurans.

Evoked potential mapping studies of the telencephalic lobes reveal at least two higher auditory responsive areas (ventral striatum and medial pallium) in the forebrain of the bullfrog. To elicit a response from these regions, like the auditory thalamic area, it is necessary to use stimuli whose spectral and temporal properties resemble biologically significant sounds (such as mating calls); for example, clicks fail to evoke a response from either of these telencephalic centers. Based on our electrophysiological recordings and the previous anatomical studies of afferent projections to the telencephalon by Kicliter and Northcutt (J. Comp. Neurol. 161: 239-254, 1975), it appears that the ventral striatum is the next direct ascending center above the thalamus in the anuran auditory pathway. (Supported by N.I.H. Grant NS-09244).

295 CEREBELLAR EVOLUTION IN CARTILAGINOUS FISHES. R. Glenn Northcutt. Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109.

Cerebellar variation was examined in 58 species representing 43 genera. The ancestral condition was likely a non-convoluted corpus, divided into anterior and posterior lobes of equal size. This condition is retained in chimaerids, in squalomorph and squatinomorph sharks, and in Heterodontus, Scyliorhinus, Gymnura, and most rajiform skates. The corpus has hypertrophied, resulting in a foliated condition, 2 or 3 times independently in other cartilaginous fishes. In each case, the anterior lobe has been selected for and in sphyrnids and most myliobatiforms, it accounts for 50-75% of the total cerebellar volume. In each case, the anterior lobe is divided into rostral and caudal lobules with the greatest increase occurring in the rostral lobule. This condition appears to have independently evolved in advanced myliobatiforms, lamniforms and carcharhiniforms. An allometric analysis of cerebellar foliation indicates that batoids exhibit a 13 fold increase in the volume of the anterior cerebellar lobe, whereas sharks exhibit only a 4 fold increase. The functional significance of cerebellar evolution in these fishes is presently unknown.

This work was supported by a Rackham Faculty Research Grant from the University of Michigan.

296 PROJECTIONS TO EFFERENT VOCAL CONTROL NUCELI OF THE CANARY TELENCEPHALON. F. Nottebohm (Rockefeller Univ., New York, NY 10021) and D.B. Kelley (Dept. Psychol., Princeton Univ., Princeton, NJ 08540).

Canary song is a learned behavior characteristic of adult males during the breeding season. Song is produced by the syrinx, an organ innervated by axons from the hypoglossal nucleus. These motoneurons receive projections from a specialized telencephalic nucleus, hyperstriatum ventrale, pars caudale (HVc) via a synapse in nucleus robustus archistriatalis (RA) (Nottebohm et al., J. Comp. Neurol. 165:457, 1976). Investigation of the efferent connections of the auditory telencephalic nucleus, field L, revealed projections to a thin "shelf" of neostriatum outlining the medial and ventral borders of HVc (Kelley and Nottebohm, Abs. Soc. Neurosci., 1976).

The present study investigated afferent connections of HVc and RA by means of the retrograde tracers, horseradish peroxidase (HRP) and tritiated adenosine. Nucleus HVc receives input from the magnocellular nucleus of the anterior neostriatum (MAN) and from nucleus interface (NIF). Nucleus RA receives projections from HVc and from MAN. A neostriatal area adjacent to the ventrocaudal edge of field L projects to a "cup" of tissue apposed to rostro -ventral RA. Nuclei HVc and RA also receive projections from thalamic nuclei.

Intensified diaminobenzidine staining of retrogradely HRP filled neurons in NIF shows that these cells are multipolar with axons directed dorsally towards the lamina hyperstriatica. Cells in NIF are larger than those in adjacent field L; diameters range from 13 to 20 u. Nucleus MAN has been shown to contain androgen-concentrating cells (Arnold et al., J. Comp. Neurol. 165: 487, 1976), as has nucleus HVc. Hormone concentration by cells in these nuclei may be involved in seasonal modulation of song. However, we still lack direct evidence that connections between NIF, MAN and telencephalic vocal control nuclei function in the control of vocal behavior.

297 TELENCEPHALIC AFFERENTS AND THE VISUAL SYSTEM IN GOLDFISH. S. C. Sharma and B. G. Grover*. Dept. Ophthalmology, N.Y. Medical College, New York, N.Y. 10029.

Knowledge of teleostean forebrain connections is based almost exclusively on data obtained with classical anatomical methods. A recent study of telencephalic efferents in two fish (Vanegas & Ebbesson, J. Comp. Neur., 1976) failed to find any projection to the lateral geniculate nucleus (LGN). These authors suggested that retino-thalamo-striatal system may be lacking in teleosts. However, from recent studies of retinal and tectal efferents, it appears that thalamic nuclei considered to be homologous across teleosts are not homologous with respect to their connections (afferents). Therefore, a retino-thalamo-telencephalic system in teleosts might not involve the nucleus heretofore identified as the LGN.

We have investigated telencephalic afferents and retinal efferents in goldfish using HRP label. Following injection of HRP into the forebrain, labelled cells were found in the contralateral telencephalic lobe, and ipsilaterally in the optic tectum, the nucleus preglomerulosus and in the region of the striolobar bundle in the hypothalamus. Intraocular injection of HRP confirmed the visual projections obtained with degeneration methods. In addition, a few fibers were found entering the telencephalon. Following tectal injection of HRP, labelled cells were found in the central core of the ipsilateral telencephalic lobe.

Since the telencephalon does not appear to receive input from any diencephalic areas which receive direct retinal input, we have no evidence for the existence of any retino-thalamic-telencephalic system in the goldfish.

Supported by N.I.H. EY 01426 and 05137.

298 INFRARED RESPONSES OF NEURONS IN THE LATERAL DESCENDING NUCLEUS OF THE TRIGEMINAL SYSTEM IN THE RATTLESNAKE, CROTALUS VIRIDIS. L. R. Stanford* and P. H. Hartline. Neural and Behavioral Biology Program, University of Illinois, Urbana, IL 61801.

Extracellular single unit responses were recorded in the lateral descending nucleus (DLV) in a snake which has infrared-sensing facial pits. This trigeminal nucleus receives input principally from the afferent fibers that innervate the sensory membrane of the pit organs. Data were collected on the receptive field properties and response characteristics of DLV neurons responsive to infrared stimulation.

Receptive fields of neurons in the DLV are smaller (average horizontal diameter; 25°, range; 9°-41°) than receptive fields reported in either the primary afferents or tectal neurons. Many DLV neurons have receptive fields with excitatory and inhibitory regions. Inhibitory regions do not completely surround the excitatory region but commonly flank it on one or two sides. The units show local adaptation; adaptation of the response to a repeated stimulus in one position in the receptive field does not adapt spatially separated regions of the same field. Small receptive field size, the presence of inhibitory regions and local adaptation indicate convergence of primary sensory afferents onto DLV neurons.

DLV neurons typically displayed a low level of background activity. The response to an infrared stimulus introduced into the excitatory region of the receptive field for three seconds was as follows: 1. a transient increase in discharge (1700 msec.); 2. complete depression of discharge (next 1050 msec.); 3. return of the background discharge. A variable period of post-stimulus depression followed the termination of the stimulus. Stimulation of the inhibitory region of the receptive field produced a depression of the background discharge, followed by a gradual return of activity. No post-inhibitory excitation was noted at the termination of the stimulus, the most common response was a second depression. Simultaneous presentation of stimuli in the excitatory and inhibitory regions of the receptive field resulted in a weaker response than occurred with presentation of the excitatory stimulus alone.

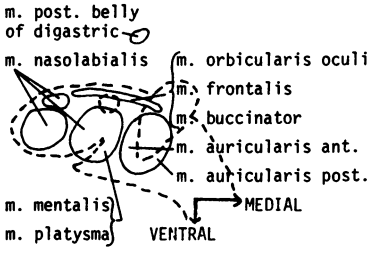
Comparison of these findings with those reported in the primary afferents and tectal neurons indicates that the responses of DLV neurons are more phasic than those of the first order cells but less phasic than the tectal responses. The receptive field data suggests that considerable sharpening of the infrared "image" could occur in the DLV.

299 THE TOPOGRAPHIC ORGANIZATION OF THE RAT FACIAL NUCLEUS. C.R.R. Watson* and S.T. Sakai* (SPON: P.D. MacLean) Depts. of Psychology, Biophysics and Neuroscience Program, Michigan State University, East Lansing, MI 48824.

The topographic organization of facial nucleus motoneurons in the rat was investigated. The horseradish peroxidase retrograde tracing technique with tetramethyl benzidine and hydrogen peroxide as the histochemical indicator was used.

Individual muscles and muscle groups were found to be represented in the nucleus in the same topographic order as is found in the face (see below). Notable findings of this study are: (i) the relatively unremarkable size of the vibrissal muscle representation; there is no increase in size of this part of the nucleus commensurate with the specialized sensory functions of the vibrissal facial area (ii) the posterior belly of the digastric muscle is represented about 1 mm dorsal to the main facial nucleus in a small cell group, the suprafacial nucleus. This cell group may be homologous with the separate dorsal facial nucleus found in non-mammalian vertebrates and monotremes. Since the posterior belly of digastric is involved in swallowing rather than facial expression, its motoneurons may be restrained from further ventral migration by the neurobiotactic influence of the nucleus of the solitary tract.

(Supported by NIMH fellowship MH 05390).



Legend: The subdivisions of the facial nucleus as seen in coronal section are enclosed by solid lines and the muscles represented by them are labelled. The facial nucleus comprises dorsolateral, ventrolateral, intermediate and medial cell columns and a dorsal sheet. The orientation of the rat face as represented in the nucleus is shown in dotted outline.

300 SOMATOTOPIC ORGANIZATION OF THE MOTOR NUCLEUS OF VII BASED ON RETROGRADE AXOPLASMIC TRANSPORT OF HORSERADISH PEROXIDASE FROM SEVERED RAMI TO NEURONAL PERIKARYA. Cyprian V. Weaver. Dept. of Anatomy, University of Minnesota Medical School, Minneapolis, MN. 55455, St. John's University, Collegeville, MN 56321

In an earlier report (Weaver, Anat. Rec., 190:578, 1978), it was suggested that the somatotopic organization of the motor nucleus of VII could be demonstrated on the basis of the uptake, retrograde axonal transport of exogenous protein, and subsequent labeling of cell bodies. The purpose of this suggestion was to introduce an alternate experimental method which could elucidate the discrepancies existing between earlier studies based on degenerative changes effected by retrograde chromatolysis of the perikarya following axotomy of various peripheral branches of the nerve (Papez, J. Comp. Neurology, 43:159, 1927; Courville, Brain Res., 1:338, 1966). In reference to some of the major branches of VII and their cellular groups of origin in the medulla of the cat, this preliminary report indicated both areas of agreement and deviation from the somatotopic organization derived from these chromatolytic studies. Subsequent to this preliminary report, additional rami have been analyzed with further modification of the previous somatotopic divisions and the protocol revised to enhance discrete labeling of cell bodies. In recent experiments, unilateral extirpation of peripheral rami was carried out on eighteen cats. 5 ul of a 25-50% (w/v) suspension of Horseradish peroxidase (Sigma type VI) in buffered saline was applied to the cut end of the nerve for 20 minutes. Survival periods ranged from 16-18 hours. Each cat was then perfused transcardially with isotonic saline and a fixative of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1M Sorensen's phosphate buffer (pH 7.2). This was followed by a perfusate of 0.1M Sorensen's phosphate buffer to which 20% sucrose had been added to remove unbound fixative. No post-fixation was employed in order to reduce the attenuating effects of aldehyde fixation on the enzymatic activity of HRP. Sections were cut 40 u in thickness on a freezing microtome and then processed for the chromagenic oxidation of benzidine dihydrochloride (BDHC) which polymerizes to form the blue reaction product (Mesulam, J. Histochem. Cytochem., 24:1273, 1976). The sections were then counterstained with neutral red to yield optimum contrast between the blue reaction product of the label and the stained cell body under light microscopy. The results have given positive labeling of perikarya localized by histochemical reaction at the sites of HRP activity and have permitted a more satisfactory differentiation between cellular groups within the motor nucleus.

301 TORUS SEMICIRCULARIS AFFERENTS IN THE BULLFROG, RANA CATESBEIANA. Walter Wilczynski. Neurosciences Program, University of Michigan, Ann Arbor, MI 48109.

Horseradish peroxidase (HRP) histochemistry was used to determine afferents to the torus semicircularis in bullfrogs (Rana catesbeiana). Animals survived 4-8 days at 22°C after receiving unilateral toral injections of 75-150 nl of Sigma VI HRP. The animals were then sacrificed and the brains processed for HRP histochemistry by standard techniques.

The ipsilateral superior olive and contralateral dorsal medullary complex of VIII were filled with HRP-positive cells and appeared to be the major sources of toral inputs. The labeled dorsal complex cells were mainly located in the dorsal (acoustic) division. Labeled cells were also seen in the ipsilateral dorsal medullary complex, bilaterally in the reticular formation above the olive, and occasionally in the contralateral superior olive. At obex levels, HRP-positive cells were present in portions of the contralateral perisolitary band adjacent to the spinal tract of V and the dorsal funiculus. Few spinal cord cells were labeled, although autoradiographic experiments have revealed a spinal projection to the torus (Neary and Wilczynski, personal observation). Other afferent populations include the superficial isthmal reticular nucleus and other tegmental fields bilaterally; the contralateral torus; the ventral half of the ipsilateral lateral pretectal nucleus; and possibly the ipsilateral posterior thalamic nucleus. Finally, a few HRP-positive cells were seen in the ipsilateral anterior entopeduncular nucleus and interposed between the lateral and medial amygdala. In addition, autoradiographic experiments have revealed a preoptic projection to the laminar nucleus of the torus overlying the hypothalamic input described by Neary and Wilczynski (Anat. Rec., 187:665, 1977). The cells responsible for the preoptic and hypothalamic inputs have been difficult to visualize with the HRP method. Additional autoradiographic experiments are underway to confirm the inputs seen with the HRP technique and to determine the arrangement of their terminal fields within the torus.

This work was supported by Rackham Dissertation Grant funds, Univ. of Mich., and by PHS Grant 1 R01 EY02485 to R. Glenn Northcutt, Division of Biological Sciences, Univ. of Mich.

303 RETINOFUGAL PROJECTIONS IN THE RED-BACKED SALAMANDER: EVIDENCE FOR AN IPSILATERAL RETINOTECTAL PATHWAY. H. Zakon, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853

The projections of the optic nerve in Plethoden cinereus, the red-backed salamander were studied by the Fink-Heimer technique. Animals were unilaterally enucleated, allowed to survive for 5 to 12 days, perfused, and frozen sections cut at 30μ for staining.

Degeneration was observed bilaterally along the optic tracts, and in diencephalic and mesencephalic neuropil areas. In the contralateral thalamus, a broad continuous sheet of degeneration occurs in the ventrolateral neuropil (VLN) and more dorsally in the neuropil of Bellonci (NB). Ipsilaterally the VLN displays less dense degeneration and extends far less caudally while the NB appears as a distinct compact patch above it. A dense terminal field enters the pretectal neuropil bilaterally.

In the mesencephalon a well developed accessory optic tract and basal optic nucleus are found contralaterally. A broad band of degeneration, with no apparent lamination, appears in the dorsal half of the centralateral tectal neuropil running the entire rostro-caudal extent of the tectum. In addition, a sparse ipsilateral tectal projection terminates just below the level of the contralateral projection, and innervates the rostral third of the tectum.

Recently, direct ipsilateral retino-tectal projections have been described in a number of amphibia using autoradiographic methods. However, these methods may involve transsynaptic transport of label, and since tecto-tectal pathways terminate in the same layer as the presumed ipsilateral retino-tectal pathway, the direct retinal origin of this pathway is unclear. This study using a technique which has no transsynaptic effects at these survival times, verifies the existence of a direct ipsilateral retino-tectal pathway.

302 PATTERNS OF BEHAVIOR IN SOLITARY AND COMMUNAL SPIDERS. Peter N. Witt, N. C. Mental Health Research, Raleigh, N. C. 27611.

When speed and amount of food uptake, web-building activity throughout the day, movement characteristics, and production of offspring in the laboratory and outdoors are compared between the solitary orb web builders like Araneus diadematus Cl. (A. d.) and colonial spiders Mallos gregalis Simon (M. g.) from Mexico, different forms of behavior emerge. While young A. diadematus females (mean weight 55 mg) readily consumed radioactively labeled glucose (injected into a live fly), close to 100% in 120 min, a group of M. gregalis of the same average body weight took more than 1,000 min to ingest the same amount. The latter animals followed a pattern of approaching and leaving the prey repeatedly in 24 hrs. Increasing activity of hungry individuals together with movement to fly-catching surface of the three dimensional web apparently helps M. gregalis to distribute food evenly throughout a 20 member colony. Time-lapse movies and animal counts on different web parts provide data supporting such observations. The relatively greater fly-holding capacity of the Mallos web, measured by Jackson (Beh. Ecol. Sociobiol., 1978), makes it possible for the slower spiders to eventually eat as much of the trapped prey as A. diadematus without attack and wrapping. Slow movements across the web with frequent meetings in M. gregalis seem to have exploratory as well as communication function. In contrast, A. diadematus moves in the laboratory only for web renewal or when prey has hit. No evidence for recycling of silk could be found in M. gregalis using radioactive labeling techniques, while Peakall (J. exp. Zool., 1971) has shown that A. diadematus digests and incorporates 98% of old silk into daily renewed webs. Under controlled, steady laboratory conditions M. gregalis raise offspring all year round; in the changing climate of Mexico, Burgess (dissertation, 1978) found a seasonal rhythm in appearance of spiderlings. A. diadematus maintain circadian and annual rhythms even in a constant environment. — It is hypothesized that in spiders a relatively flexible, environment-dependent behavior is associated with communal living, while predominantly innate patterns of rigid behavior characterize the solitary life style. (Supported by NSF grant No. BNS-75-09915-02A).

DEVELOPMENT AND AGING

304 THE EFFECT OF LEAD ON MYELINATION. S. Aitchison*, A. Goldberg and J. Frangia* (SPON: G. McKhann). Dept. Neurology, Sch. Med. and Sch. of Hygiene, Johns Hopkins U., Baltimore, MD 21205

The developing central nervous system is vulnerable to the toxic effects of lead. Some earlier studies directed at the understanding of the pathogenesis of chronic toxicity have been complicated by the bservation that lead administration also resulted in malnutrition. Under our conditions, malnutrition was not significant.

Mice are exposed to lead immediately after birth by substituting a solution of lead acetate (5 mg/ml) for plain water in the mothers diet, thus exposing the off-spring to lead via the mother's milk. All litters are normalized to 3 pups within 24 hours of parturition. Lead is removed from the drinking water at 18 days and the animals are weaned at 21 days. Body and brain weights obtained were identical in the lead-exposed and control groups of animals.

We have previously shown that the optic nerve is a useful system in which to study myelination. Biochemical and morphological characteristics of optic nerves in lead-exposed and control animals were studied. Light and electron microscopy showed that the axons of lead-exposed animals were smaller than those in control animals. The ratio of myelin to axonal size was found to be constant within each group and between the two groups. This suggests that the effect of lead toxicity is primarily on neurons, rather than oligodendrocytes.

Cerebroside sulfotransferase activity was decreased in the lead-exposed animals, but followed the same developmental profile as the control animals. When measuring 2',3'-cyclic nucleotide phosphohydrolase and myelin basic protein, the onset of myelination appeared to be the same in the two groups of animals. In the lead-exposed animals, however, the total enzyme activity was decreased by 40% and the amount of myelin basic protein decreased by 60%. Thus, the hypomyelination in lead-intoxicated mice may be the result of a primary pathological process involving neurons. The significance of this possibility will be presented.

(Supported by funds from USPHS Grant NS 13402-01A1)

305 SURPLUS ACCUMULATION OF NEUROTRANSMITTER SYNTHESIZING ENZYMES IN CELL BODIES OF DOPAMINERGIC AND SEROTONERGIC NEURONS DURING DEVELOPMENT. H. Baker, L. Iacovitti, D.H. Park, T.H. Joh, D.J. Reis, Laboratory of Neurobiology, Cornell University Medical College, 1300 York Ave, New York, NY 10021

During postnatal development of rat brain there is a substantial increase in the activities of the enzymes subserving the biosynthesis of neurotransmitters. We sought to determine by use of specific antibodies whether the postnatal increase of enzyme activity was the result of changes in the number or catalytic activity of enzyme molecules and to assess the relationship between the increase in enzyme activity in cell bodies and in terminal fields. Tyrosine hydroxylase (TH) was assayed in substantia nigra (SN) and caudate nucleus (CN), and in locus coeruleus (LC) and hippocampus, representing respectively cell bodies and terminals of dopamine (DA) and noradrenergic (NE) neurons. Tryptophan hydroxylase (TrH) was assayed in nuclei raphe dorsalis and centralis and in hypothalamus representing cell bodies and terminals of a serotonin (5HT) system. In cell bodies of DA and 5HT neurons enzyme activity was 10-20% of adult (60 day (d)) values at birth and increased slowly until d 5 in DA neurons, and d 12 in 5HT neurons, at which time enzyme activity began to rise rapidly. TH activity in DA neurons surged to 255% ($p < 0.001$) of adult values by d 18. In 5HT neurons TrH activity increased to 124% ($p < 0.001$) of adult values by d 24. Enzyme activity returned slowly to adult levels by d 30-60. By immunotitration with specific antibodies the overshoot of TH activity in SN and TrH activity in raphe was demonstrated to be attributable to increased accumulation of enzyme protein. The 2.5 fold increase of TH activity on d 18 in SN was not attributable to cell death as the number of SN neurons immunocytochemically stained for TH on days 18 and 60 did not differ (10129 ± 198 vs 9785 ± 830; n.s.). However, on day 18, but not 60, there was marked staining for TH in SN, pars reticulata, perhaps indicating enhanced filling of dendrites with enzyme. In terminal areas of DA and 5HT neurons enzyme activity was 10% of adult values at birth and increased in parallel with enzyme activity in cell bodies, but with a 3 d delay. In contrast, TH activity in LC neurons, which was 20% of adult values at birth, increased slowly from d 6-18 exhibiting no overshoot. TH activity in hippocampus was 70-80% of adult values at birth and increased gradually over time. We conclude that postnatal increases in the activities of brain TH and TrH are due to increased accumulation of enzyme protein, and that in cell bodies of DA and 5HT neurons, but not NE neurons, there is a transient surplus accumulation of enzymes during a period of major postnatal growth of target organs and enlargement of terminal fields. (Supported by NIH grants HL 18974 and NS 03346.)

306 CROSS SECTIONAL ANALYSIS OF HIPPOCAMPAL SYNAPTIC RESPONSE WAVEFORM IN RAT: EVIDENCE FOR CONSTANCY OF CABLE PROPERTIES IN GRANULE CELL DENDRITES. C. A. Barnes and B. L. McNaughton. Department of Psychology, Dalhousie University, Halifax, Nova Scotia, CANADA B3H 4J1.

We previously demonstrated that the waveforms of the extracellularly recorded granule cell response elicited by separate stimulation of the medial and lateral perforant pathways differ in rise time (McNaughton and Barnes, J.Comp.Neur. 1977, 175, 439-454). It was suggested that these differences in rise time may reflect the passive dendritic cable properties of these hippocampal neurons, since the more distally terminating fibers (lateral perforant path) resulted in more slowly rising potentials and the relationship between rise time and width at one half amplitude was similar to that predicted from cable theory for intracellular records.

Observations on both hemispheres of three adolescent (3 months), three middle aged (12 months), and three senescent (28 months) rats show no significant differences in the range or frequency distribution of EPSP rise time when the perforant pathway was systematically explored with multiple stimulating electrode penetrations.

If the assumption that the waveform of the extracellular EPSP reflects the passive dendritic cable properties is correct, then the data indicate that these factors remain constant throughout a large portion of the lifespan of the healthy rat.

307 DEVELOPMENT OF THE ENKEPHALIN AND ENDORPHIN-CONTAINING SYSTEMS IN THE RAT BRAIN. Alejandro Bayon*, Wm. J. Shoemaker, and Floyd E. Bloom. A.V. Davis Ctr., The Salk Institute, San Diego, CA 92112.

Radioimmunoassays (RIA) that use antisera directed either towards β-endorphin or to Leu-enkephalin (see footnote to Table I) were used to determine the regional concentration of brain endorphins and enkephalins during pre- and postnatal development in the rat. Although the absolute amounts of both endorphin and enkephalin, as well as tissue protein, increase with age in all the regions studied (see Table I), the concentrations, expressed on a protein basis, reveal interesting differences. Between embryonic day 20 (ED20) and postnatal day 6 (PN6), the concentration of endorphin decreases in all regions studies; the greatest decrease (about 50%) occurs in the corpus striatum (CS), and continues decreasing to very low levels at adulthood. The CS shows the highest endorphin concentration in the brain before birth, whereas the hypothalamus is the richest in the adult. The concentration of enkephalin does not significantly change from ED20 to PN6 in any of the regions studies; enkephalin concentration remains almost constant after birth except for a marked increase (about 3-fold) in the region containing the preoptic areas and the septum. The CS contains the highest concentration of enkephalin in both the embryonic and adult rat. In contrast to the brain, the pituitary concentrations of both endorphin and enkephalin remain constant from ED20 to PN6; both peptides subsequently increase severalfold by adulthood.

Table I. Total Brain Content of Enkephalin and Endorphin

Age	Enkephalin Units*	Endorphin Units†	Brain Protein (mg)
ED-20	1.3 ± 0.2	6.4 ± 0.8	8.1 ± 0.5
PN-6	7.4 ± 0.5	11.2 ± 1.3	48.5 ± 1.8
PN-25	40.7 ± 2.5	76.7 ± 2.4	263.1 ± 16.5

*Enkephalin immunoreactivity is expressed as the ng of Leu-enkephalin that would give an equivalent trace displacement in the enkephalin RIA.

†Endorphin immunoreactivity is expressed as the ng of β-endorphin that would give an equivalent trace displacement in the endorphin RIA.

The RIA for β-endorphin reads the Leu^{14}-His^{27} segment and cross reacts 100% on a molar basis with both β-LPH and the 31K protein. The Leu-enkephalin RIA shows 3% cross-reactivity for Met-enkephalin. Results are expressed as the mean of 3-4 determinations ± the standard error of the mean.

308 EFFECTS OF SENSORY DEPRIVATION ON THE DEVELOPMENT AND MAINTENANCE OF THE OLFACTORY SYSTEM IN MICE. T.E. Benson* and D.K. Ryugo. (SPON: J. Wells) Depts. Anat., University of Vermont, Burlington, VT 05401, and Harvard Med. School, Boston, MA 02115.

Littermate albino mice have been unilaterally deprived of olfactory sensation by right or left nares closure on postnatal day 1 (PN1) and histologically prepared on PN30. Experimental nasal fossae taken on PN3 and PN6 revealed that no direct damage had occurred to the olfactory receptor sheet (ORS); thus, this study addresses the effects of sensory deprivation without the added complication of transneuronal degeneration.

The olfactory bulbs (OB) of both sides exhibited normal gross morphology, except the deprived OB was reduced in total volume by 30% ± 6 (N = 6). Morphometric volume analyses comparing each of the OB layers between nares-closed OB and nares-open OB were performed by planimetry of traced semi-serial projections and are expressed as mean percent difference (MD% ± SEM). There was always a reduction on the closed side. In untreated littermate controls, the MD% between each component of the two OB's never exceeded 7%.

	Olfactory System Component	N	MD%	SEM
ORS:	a) Receptor packing density (Rpd)	1	20	
	b) Nerve Layer volume (NLv)	4	20	7
Glomerulus number (G#)		9	18	3
Mitral Cell Layer volume (MCLv)		4	19	4
Glomerular layer volume (GLv)		4	29	4
External Plexiform Layer volume (EPLv)		4	46	13
Granule Cell Zone volume (GCZv)		4	36	8

Although our sample is relatively small at this time, we feel that some preliminary conclusions may be drawn. The reduced Rpd and NLv suggest that central changes observed in the OB are related to changes occurring at the periphery. Grouping of these data according to the magnitude of the effect (MD%) further suggests that the deprivation acts differentially on OB components: (1) Commensurate with the ORS changes, there is a direct effect which reduces G# and affects primary postsynaptic cells (MCLv). (2) There is a much larger effect mediated through secondary synapses involving cells and neuropil of predominantly postnatal origin (EPLv and GCZv). Related to this finding are preliminary ^{3}H-thymidine autoradiographic data indicating that the number of postnatally-generated granule cells is reduced on the closed side. (3) Finally, in GLv where both primary and secondary synapses as well as postnatally-generated components are present, there appears to be an intermediate effect. (Supported in part by U.VT. GRS grant PHS 5429-40)

309 METABOLIC REQUIREMENTS FOR GROWTH AND DIFFERENTIATION OF EMBRYONIC SYMPATHETIC NEURONS IN CULTURE. Emanuel M. Bloom and Ira B. Black, Dept. of Neurology, Cornell University Medical College, N.Y., N.Y. 10021.

The embryonic mouse superior cervical ganglion (SCG), which does not require added nerve growth factor for survival in culture, was used to define the metabolic requirements for neurite elaboration and biochemical differentiation of sympathetic neurons in vitro. Ganglia from 14 gestational day mice were cultured in the absence of added nerve growth factor. In the presence of actinomycin-D, which inhibited RNA synthesis by more than 95%, ganglia elaborated neurites for at least 6 hrs. in culture. The activity of tyrosine hydroxylase (T-OH) increased 4-fold by 24 hrs. in control explants, but failed to rise in the presence of actinomycin-D. Ganglion explants also elaborated neurites for at least 6 hrs. in the presence of concentrations of cycloheximide or puromycin which inhibited protein synthesis by more than 95%. However, blockade of protein synthesis prevented the increase in T-OH activity. To define the role of DNA synthesis in differentiation, and to eliminate the influence of non-neuronal support cells, explants were cultured with cytosine-arabinoside (Ara-C), an inhibitor of DNA synthesis. Blockade of DNA synthesis resulted in the virtual absence of support cells. However, neurons elaborated abundant neurites which terminated in growth cones unassociated with support cells. Moreover, T-OH activity increased 3-fold in the presence of Ara-C.

These observations suggest that embryonic sympathetic neurons, cultured in the absence of added nerve growth factor, elaborate neurites in the absence of ongoing protein, RNA or DNA synthesis. Moreover, support cells are not necessary for neurite elaboration at this stage. In contrast, development of T-OH activity requires RNA and protein synthesis, although support cell presence is unnecessary.

(This work was supported by the NIH, the Dysautonomia Foundation Inc., the NSF and the Hirschl Trust Fund.)

310 PHOSPHORYLATION AND AGEING: cGMP DEPENDENT PROTEIN KINASES IN HUMAN STRIATUM. Diethelm H.Boehme* and Neville Marks. The V.A. Hospital East Orange N.J. 07019 and Institute for Neurochemistry and Drug Addiction. Wards Island. N.Y. 10035.

Alterations in synaptic density along with the effects of biogenic amines in vitro on levels of cyclic nucleotides in brain slices point to a decreased synaptic function in senescence. To study this in more detail the changes in protein kinases with age were examined in different brain regions of post mortem tissues from man and compared to those of rat. Synaptosomal membranes were purified from regions known to be subject to senile pathologies such as caudate nucleus and putamen, substantia nigra and compared to those of thalamus and frontal cortex. Membranes from man and rat were incubated according to the conditions of Ueda et al (JBC 248, 8295,1973) followed by analysis on SDS slab-gels and radioautography. Post-mortem tissues within 6 h of death gave a similar pattern of phosphorylated proteins as that observed for rat membranes with a mol.wt range of above 250K to 12K when compared to protein markers. Membranes prepared from human tissues were characterised by a high intensity band of above 250K present only in striatum and largely absent from other brain regions. The intensity of phosphorylation of synaptosomal proteins changed with age in membranes obtained from a 4 month infant to older (senile) patients aged 56-83 years. Human synaptosomal membranes did not respond to cAMP addition but phosphorylation was stimulated in presence of cGMP with the appearance of new band in all anatomical regions in the 80K region. Addition of cGMP gave marked inhibition of the striatal components in the high molecular range and an inhibition of the 12K region dependent on the region studied. These effects were absent in a single case of Alzheimers disease and a case of degeneration of basal ganglia. The availability of an assay system using human post-mortem material capable of responding to cyclic nucleotides such as cGMP provide an experimental system for investigating the diverse effects of agents known to alter cGMP levels or dependent protein kinases in vivo and may be linked to the ageing process.

311 POSTNATAL CEREBELLAR NEUROGENESIS IN RAT IS ALTERED BY HYDROCORTISONE: AN AUTORADIOGRAPHIC AND LIGHT MICROSCOPIC STUDY. Martha Churchill Bohn* and Jean M. Lauder, Dept. Biobehav. Sci., Univ. of Connecticut, Storrs, CT. 06268.

Rat pups injected with hydrocortisone acetate (HCA) on days 1-4 (200ug/day)were sacrificed by ether anesthesia and cardiac puncture on days 2,5,7,10,12,15,18,21 or 24 and brains embedded in paraffin after immersion in Bouin's fixative. Planimetric measurements of the area and width of the external granular layer (EGL) in matched sagittal sections of vermis showed that the growth of the EGL was reduced during the HCA treatment but partially recovered thereafter. Disappearance of the EGL was not significantly retarded. The mitotic index in the EGL (number of mitotic cells/total number of cells) was depressed during the treatment, but exceeded control values during the second week. Nevertheless, the total number of cells produced by the EGL in HCA rats was reduced, presumably due to a severe inhibition of EGL cell proliferation during the HCA treatment.

In treated rats sacrificed by formalin perfusion at 72 days, the foliation pattern in sagittal sections was reproducibly changed. In lobule VIII, the total area and areas of the molecular layer (ML) and internal granular layer were decreased by 14%,17% and12%, respectively. The numbers per unit area of granule cells and small neurons in the inner ML were unchanged whereas the number of stellate cells per unit area in the outer half of the ML was reduced by 23%. Due to areal decreases, however, there were deficits in the total numbers of granule cells and ML interneurons.

A long survival autoradiographic study was undertaken to determine the effects of HCA on the "birthdays" of granule, basket and stellate cells. HCA animals and vehicle injected controls were injected with ^{3}H-thymidine on days 1,2,5,7,10,15,21 or 24 and sacrificed at 72 days. Plots of heavily labeled cells/total cells (HLC/TC) vs age (lobule VIII) indicated that during the treatment greater proportions of both granule cells and ML interneurons completed early final cell divisions. At day 10, HLC/TC was depressed for granule cells, probably as a result of the increase in cell proliferation (mitotic index) following release from HCA treatment. The proportion of neurons formed at later ages (days 15-24) was not significantly changed.

In conclusion, neonatal HCA treatment results in final cell deficits in both granule cells and ML interneurons, apparently as a result of inhibition of EGL cell proliferation. Moreover, a small number of cells stop dividing prematurely and permanently. These cells may be those nearing their last division and thus more sensitive to the HCA treatment. (Supported by NIH Grants 09904, MH05572 and 13481).

312 MORPHOMETRIC STUDIES ON AGING CHANGES IN VISUAL CORTEX AND HIPPOCAMPUS IN THE RHESUS MONKEY. Kenneth R. Brizzee, Neurobiology Department, Delta Regional Primate Research Center, Tulane University, Covington, La. 70433

Brains of three young adult (ages 5-7 years) and three aged (estimated ages 20-24 years) rhesus monkeys were fixed by intracardiac perfusion with glutaraldehyde (1%) and paraformaldehyde (1%) in 0.12 M phosphate buffer, pH 7.3. Tissue blocks from visual cortex and hippocampus were postfixed in OsO_4, embedded in Epon sectioned at 4 μm and stained with toluidine blue.

The mediolateral width of the CA-1 zone of the hippocampus decreased from 1040 μm in young adults to 960 μm in aged animals, but the difference did not attain the level of statistical significance. However, the mean depth of the lamina pyramidale of the hippocampus decreased significantly from 150 um in young adults to about 100 μm in aged monkeys ($p<.05$). The mean number of neurons in a 55 μm segment of the lamina pyramidale in the CA-1 zone decreased from 106 per 55 μm segment to 52 ($p<.01$), while the mean number of glia cells increased from 8 to 32.

The mean depth of visual cortex decreased from 1380 μm in young adults to 1320 μm in aged monkeys but the difference was not statistically significant.

The mean number of neurons per "counting chamber" in the visual cortex (approximately 180,000 μm^3) decreased from 26 to 22, and the mean number of glia increased from 10 to 14 from young adults to aged animals, but the differences were not statistically significant.

These preliminary observations suggest that structural alterations in the hippocampus in nonhuman primates may occur at an earlier age and exhibit greater severity than in cerebral cortex.

313 DELAYED DEVELOPMENT OF THE INPUT-OUTPUT ORGANIZATION FOR KITTEN MOTOR CORTEX. I. C. Bruce* and W. G. Tatton. Fac. Med., Univ. of Calgary, Calgary, Alberta, Canada T2N 1N4 and Playfair Neuroscience Unit, Univ. of Toronto, Toronto, Ontario, Canada M5S 1A8.

The study was undertaken to determine whether 'higher level' reflex circuits, such as those involving motor cortical neurons (MCNs) are organized prenatally or develop postnatally like some neuronal circuits in perceptual systems such as the visual cortex.

This work was carried out in chronically-prepared kittens 9 to 67 days of age. Recordings were made from 411 cortical neurons while the contralateral forelimb was displaced at random intervals by a torque motor so as to rotate the elbow joint and stretch triceps brachii. EMG recordings were made from triceps in response to the displacements and to intracortical and immediately subcortical microstimulation. Average response histograms (ARHs) for the units activity and averaged rectified EMG responses were constructed by computer. 'Synaptic effectiveness' (SE: firing probability/msec/presentation above baseline firing probability) was calculated for the ARH response peaks.

The findings were: 1) SE values for responses of neurons in primary somatosensory cortex (areas 1 and 2) were in the range found for adult cats as early as 9 days postnatally. Response latencies were longer (25-28msec) than those in adults (10-12msec), in keeping with the lowerperipheral and central conduction velocities previously reported. 2) In marked contrast, MCNs (area 4) showed responses with low SE values and long latencies (30-280msec) up to 45-45 days of age. Immediately after this time, the responses attained adult values in both latency and SE values. 3) Microstimulation in the white matter immediately subjacent to area 4 evoked weak triceps EMG responses in kittens less than 20 days old at latencies 5-10 times those of the adult. Adult latencies were attained during the 40-60 day period. 4) Intracortical microstimulation did not evoke triceps activity until approximately 40 days of age. 5) Of the three peaks of the triceps EMG response to displacement seen in adult cats, only one occurs in kittens 9-16 days old. During the following 20-30 days, variable low amplitude EMG activity follows this peak, and at 40-50 days of age the EMG response shows the distinct peaks characteristic of adult cats.

Thus, though mechanoreceptor input to primary somatosensory cortex is effective from 9 days or younger, the immediately adjacent motor cortex does not show adult-like responses until about 45 days. The development of mature mechanoreceptor input to motor cortex immediately lags the development of effective output linkages to alpha motoneurons and occurs over the same interval as the appearance of adult-like EMG responses to forelimb displacements.

ICB is a Dystonia Foundation Fellow.

314 ONTOGENETIC APPEARANCE AND DISAPPEARANCE OF NORADRENERGIC ENZYMES AND CATECHOLAMINES IN RAT EMBRYONIC NEUROBLASTS. Philippe Cochard*, Menek Goldstein and Ira B. Black, Dept. of Neurology, Cornell Univ. Medical College, N.Y., N.Y. 10021 and Dept. of Psychiatry, N.Y.U. Medical Center, N.Y., N.Y. 10016.

The ontogenetic pattern of noradrenergic differentiation in rat embryonic autonomic neuroblasts was defined in vivo. Noradrenergic characters were examined by documenting the appearance of transmitter enzymes and catecholamines (CA) using immunohistochemical and histofluorescent methods. Tyrosine hydroxylase (T-OH), dopamine-β-hydroxylase (DBH) and CA were undetectable in the neural crest cells before or during their ventral migration. T-OH, DBH and CA first appeared at 12 to 12.5 days of gestation (30-35 somite stage) in neuroblasts aggregating at the level of the sympathetic anlage. There was a striking degree of synchrony in the appearance of T-OH, DBH and CA. Fluorescence intensity and the number of fluorescent cells increased dramatically thereafter. In addition, T-OH and CA transiently appeared in scattered presumptive neuroblasts in the gut mesenchyme. The enzyme and transmitter were first detectable at 11.5 days of gestation. During the following day, the number of T-OH and CA-containing neuroblasts in the gut increased rapidly, and thereafter decreased progressively so that by 14.5 days only rare cells were encountered. Once again there was remarkable synchrony in the appearance (and disappearance) of T-OH and CA. These observations suggest that a number of noradrenergic transmitter mechanisms develop in close temporal proximity in the differentiating neuroblast.

(This work was supported by the NIH, the Dysautonomia Foundation Inc. and the DGRST, France and the Hirschl Trust Fund.)

315 REQUIREMENT OF ADDITIONAL FACTOR(S) FOR NORMAL INTERACTION BETWEEN SCHWANN CELLS AND NERVE FIBERS. M. Cochran*, R. Bunge & M. Bunge. Dept. Anat. & Neurobiol., Wash. Univ., St. Louis, MO 63110.

Explants of fetal rat dorsal root ganglia can be cultured under conditions that allow growth and differentiation of neurons and Schwann cells (SCs) in the absence of fibroblasts (Wood, '76). In this type of culture, occasionally there are regions in the outgrowth where nerve fibers (NFs) are not attached to the collagen substrate, appearing as guy ropes. These suspended regions of fascicles are abnormal in that they lack SCs in most areas and those SCs present are in occasional small aggregates along the fascicle. Electron microscopic study has shown that, within these aggregates, SCs are perched on fascicle perimeters and ensheath only a few of the NFs in the fascicle periphery and do not form myelin sheaths. If a plastic strip coated with collagen is placed upon the suspended fascicles, within 1 day the SCs start to divide and migrate along the fascicle and within a few more days the fascicles appear normal and also contain forming myelin sheaths (Bunge and Bunge, '78).

To determine whether this abnormality is restricted to sensory ganglia and to explore the nature of the factors involved in correcting it, cultures of partially dissociated 21 day fetal rat superior cervical ganglia were prepared on collagen (Bornstein, '58) or poly-L-lysine substrates. When the cultures are placed on polylysine, SCs neither increase in number nor ensheath NFs although neuritic outgrowth is comparable to that on collagen. When a collagen clot, polymerized in balanced salt solution, is placed on this type of unensheathed neuritic outgrowth on polylysine, SCs proliferate and align along neurites within 24 hours beneath the clot but not elsewhere. When the culture medium lacks 9 day chick embryo extract (EE), however, SC proliferation and alignment do not occur under the clot. Other cultures grown on collagen in a medium lacking EE exhibit less SC proliferation and ensheathment after 5 days than do cultures on collagen in medium containing 10% EE. When the cultures lacking EE are then given medium with EE, many SCs become aligned along neurites within 24 hours. We conclude that for normal proliferation and differentiation of SCs in relation to NFs in the relative absence of fibroblasts 1) additional factors are needed and that 2) this requirement appears to be satisfied by a collagen substrate modified in some way by a component of EE. (Supp. by N.I.H. Grant NS09923.)

316 EXPOSURE TO ETHANOL IN UTERO MAY DELAY MATURATION OF HYPOTHALAMO-PITUITARY-ADRENAL FUNCTION IN THE RAT. Beatrice Cooley-Matthews* and Anna N. Taylor. Dept. of Anatomy and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Our laboratory has shown that treatment of neonatal rats with corticosterone results in delayed onset of circadian hypothalamo-pituitary-adrenocortical rhythmicity. Treatment with corticosterone or ACTH during the neonatal period leads to persistent alteration of some characteristics of adrenal secretion, suggesting permanent modification of the central neural substrate involved with regulation of adrenal rhythmicity. Since ethanol has been shown to activate the adrenal cortex in man and animals, this investigation was designed to assess the effects of ethanol administration during gestation upon the development of the adrenocortical rhythm in the rat.

Pregnant female Sprague-Dawley rats (Simonsen) were delivered to the laboratory 6-9 days prior to parturition. They were pair-fed until delivery on a liquid diet with 25% of the calories as ethanol or an isocaloric liquid diet with sucrose substituted for the ethanol. A third group was fed Breeder Blox (Wayne) ad libitum. Rats were housed under 14 hours light and 10 hours dark (lights on 0400-1800 h). On day 1 following birth, pups were weighed and culled to 10 (5 males and 5 females where possible) and fostered to untreated lactating mothers which had cast litters in the previous 1 or 2 days and which were fed Breeder Blox throughout pregnancy and lactation. Plasma was collected following decapitation of one-half of each litter at the time of the nadir (0900 h) and one-half at the time of the peak (1700 h) of the adult adrenal rhythm and fluorometrically assayed for corticosterone. Litters were sacrificed on days 15, 18 or 21.

No offspring from any group exhibited the skeletal or central nervous system deformities characteristic of the Fetal Alcohol Syndrome in humans and other species. Neither were there any significant differences in body weights at any time. However, significant elevations of the 1700 h corticosterone value over the 0900 h value were found only in the chow group at day 21. There was no similar rhythm in either the EtOH-fed or sucrose-fed groups on any of the days sampled.

These data suggest a delay in maturation of the hypothalamo-pituitary-adrenocortical axis following this regimen of ethanol and/or liquid diet during gestation. Further investigation of the effect of the diet alone is under way.

Supported by NIH grants AM 05730 and NS 09122 and NSF grant PCM 76-80955.

317 DEVELOPMENT OF THE CONDITIONED ELEVATION OF STEROIDS TO THE FORCED EXTINCTION OF A TASTE AVERSION. S. Coyle*, J. D. Weisberger*, K. M. Salter* and S. Levine. Dept. of Psychiatry & Behavioral Sciences, Stanford Univ. School of Med., Stanford, CA 94305.

In a taste aversion paradigm, the behavioral and physiological responses of an adult animal can be manipulated by varying the amount of deprivation prior to testing. Under "free extinction" procedures of little or no deprivation, adult rats show a behavioral suppression of drinking, and no elevation of glucocorticoids. With acute deprivation (forced extinction), adult animals show a suppression of drinking, and also show an elevation of corticosterone similar to the elevation seen following the injection of LiCl during conditioning. The failure of saline-injected animals to show a steroid response shows that the steroid elevation is due to the reexposure to the conditioned substance, and not to the stress of deprivation.

In our experiments, we examined the development of these behavioral and physiological responses under the conditions of free and forced extinction. Animals aged 22, 26, 32 and 40 days were conditioned to avoid a sucrose solution by pairing the novel taste with LiCl. When tested for the retention of the aversion 4 days later, LiCl injected animals of all ages and under all deprivation schedules suppressed drinking in comparison with their saline controls. The two oldest ages showed the adult pattern of no steroid elevation during free extinction, and a rise in corticosterone levels following forced extinction procedures. The two youngest ages, however, did not show an elevation in glucocorticoids in response to the forced extinction paradigm. This absence of the physiological response is not due to the young animals' inability to show a steroid response, since the injection of LiCl during conditioning caused a long-lasting steroid elevation in all ages.

These data imply that the behavioral responses of an animal may develop independently of the physiological response to the test situation. The dissociation of physiology and behavior suggests that the neural mechanisms which are responsible for the control and coordination of these functions develop at different times.

318 EFFECTS OF ALPHA AND BETA BUNGAROTOXIN ON THE DEVELOPMENT OF TROCHLEAR NUCLEUS AND SUPERIOR OBLIQUE MUSCLE. Tony L. Creazzo* and G. S. Sohal, Dept. Anat., Medical College of Georgia, Augusta, GA 30902

A loss of approximately 50% of the neurons occurs during normal ontogeny of the trochlear nucleus of the white Peking duck embryos. Prior to death all trochlear neurons send their axons to the superior oblique muscle (periphery). The sequence of degenerative changes in the normally occurring and the experimentally induced (by removal of periphery) cell death is identical. These observations suggest that the periphery may be involved in determining whether a neuron survives or dies.

The use of α and β- Bungarotoxin in blocking neuromuscular transmission is well documented. The α- Bungarotoxin binds primarily with nicotinic acetylcholine receptors at the motor endplate whereas the β- Bungarotoxin binds with the presynaptic nerve terminal. The effects of pre and post synaptic blockades on the developing trochlear nucleus and the superior oblique muscles were investigated. α- toxin (50 µg) was directly applied daily to the vascularized chorioallantoic membrane of the white Peking duck embryos from day 8 through 25 of incubation. Embryonic motility was greatly reduced in most embryos and virtually non-existent in others. Brains were fixed in 10% formalin on day 26 and processed routinely for paraffin sectioning. Cell counts were made by counting cells with nucleoli in alternate sections stained with thionin. The extraocular muscles were frozen and processed for esterase and silver stain. Preliminary results indicate an increase in the number of surviving neurons by as much as 50%. The muscle remains largely undifferentiated and the motor endplates are virtually absent despite the presence of numerous nerve terminals.

β- Bungarotoxin with and without phospholipase A_2 activity was directly applied to the chorioallantoic membrane from day 8 through 25 of incubation. Embryonic motility was virtually absent. The gross examination of the brains showed marked reduction in the overall size and particularly that of the tectum and cerebellum. Cell counts and muscle morphology are currently being investigated. (Supported by NIH grant GM 23484)

319 SLOWER RATES OF PROTEIN DEGRADATION IN DEVELOPING RAT BRAIN. June L. Dahl and Victor J. Weibel*, Dept. Pharmacology, Univ. of Wisconsin Medical School, Madison, WI 53706.

The contribution of changes in rates of protein degradation to the increase in protein content of developing rat brain was assessed. Five-day old rats were each given a single intraperitoneal injection of $[^{14}C]NaHCO_3$, and the average rate of protein degradation was estimated from the rate of disappearance of radioactivity in brain over a 12-day time course. In all subcellular fractions examined, decay of protein-bound radioactivity was slower than in the adult animal. Proteins in the soluble and crude mitochondrial (P_2) fractions turned over at 70% of the adult rates, whereas proteins in the microsomal fraction turned over at only 25% of the rate observed in the adult. These results contrast with previous reports that degradation rates in the brains of neonatal animals are faster than in the brains of adult animals. They are similar, however, to observations in bacteria, skeletal muscle, kidney, and cultured mammalian cells which have shown that the rate of disappearance of radioactivity from labeled protein (the degradation rate) is slower under conditions of rapid growth. (This research was supported by NIH Grant DA 00697).

320 ALTERATIONS IN PITUITARY-ADRENAL ACTIVITY OF RATS TREATED WITH $ACTH_{1-24}$ NEONATALLY. Susan M. Dray-Klauss, Berrilyn J. Branch*, and Anna N. Taylor. Depts. of Psychol. and Anat., Brain Research Institute, UCLA, Los Angeles, Ca. 90024.

Early life experiences are known to exert profound effects on adrenocortical reactivity. We were therefore interested in studying whether neonatal hormone levels influence this phenomenon. In a previous study (Lorenz,et al.,Fed.Proc.32:296,1973), we observed that Long-Evans rats treated with $ACTH_{1-39}$ (Armour, 4 IU/10g b wt) on days 7-9 exhibited significantly lower responses to restraint stress than corresponding gel controls. In the present study, Sprague-Dawley rats were injected sc with $ACTH_{1-24}$ in $ZnPO_4$ (Organon, 4 IU/10g b wt) or with an equal volume of vehicle on days 7-9. Animals were housed in 14 hrs light and 10 hrs dark. Eye opening was significantly advanced by ACTH treatment (14.58±.09 [SEM] days vs. 15.82±.15, $p<.0001$).

As adults, animals were subjected to ether stress and jugular tap and stress blood samples were drawn 15 min later for determination of plasma corticosterone (B), just before lights off (peak values) or immediately after lights on (trough values). There was a significant Drug X Sex interaction in both basal and stress levels. ACTH-treated females had significantly elevated basal levels at both peak and trough periods, but did not differ in stress responses. Conversely, there were no differences in basal levels of males, but drug treatment did significantly depress stress levels, especially in the peak period. The percentage change further supported this. Males treated with ACTH showed significantly less elevation of plasma B in response to stress in the peak period than did vehicle males (62%±18% [SEM] vs. 124%±17%). These same animals had smaller adrenals at sacrifice. To rule out a simple adrenal effect, a group of animals, both males and females treated with ACTH on days 3-5 neonatally, was injected with 2 IU of $ACTH_{1-24}$ in $ZnPO_4$, sc, as adults, and bled 2 hrs later, at which time controls have essentially basal levels of B. Neonatal ACTH-treated animals did not differ from vehicle animals in their response to exogenous ACTH. This suggests that the effects on stress responsiveness do not represent hyporesponsiveness of the adrenal to ACTH. Since we have previously shown that exposure of rats at this age to B does not affect adult stress responsiveness (Turner & Taylor,Endocrinol.98:1, 1976), the present data suggest that ACTH produces persistent central effects on pituitary-adrenal reactivity.

Supported by NIH grant NS 09122 and NSF grant PCM 76-80955.

321 NEURONAL RESPONSE TO PYRAMIDOTOMY IN THE ADULT AND 15-DAY-OLD HAMSTER. T.E. Durica* and S.K. Jacob* (SPON: M.L. Klawans). Dept. Anatomy, Rush College Health Sciences, and the U. of Illinois at the Medical Center, Chicago, Il. 60612.

In hamster facial motor neurons the chromatolytic response to axotomy has been shown to vary with the developmental state of the neuron. Pyramidal tract lesions were used to study the chromatolytic response of developing neurons contained entirely within the central nervous system. This study was performed to determine if there is a comparable age dependent response in the developing pyramidal cell after axotomy. Unilateral pyramidal tract lesions were made in the medulla, rostral to the pyramidal decussation, in adult and 15-day-old hamsters. Animals were cardiac perfused with a formol saline-gum acacia solution four days following pyramidotomy. Brain stems and cerebral cortices were then double embedded in Parlodion and paraffin. The brain stems were serially sectioned for examination of the lesion site. The cortices were serially sectioned and every tenth section of the adult and every fourth section of the 15-day-old cortices were collected. Both brain stems and cortices were stained with buffered thionin. The pyramidal cells on the experimental side were pale and rounded in shape due to somal and nuclear swelling. The Nissl substance was dispersed and finely granular. The nucleolus had a predominant dark staining area and small pale staining area unlike that seen in the nucleolus of the normal pyramidal cell which has a small peripheral dark area (the DNA portion) and a larger, central pale area (the RNA portion). Pyramidotomy in the 15-day-old hamster results in a different neuronal response than that seen in the adult pyramidal cells. In the response of the immature neuron there was a more localized chromatolysis, however there was no swelling nor was there any apparent change in the nucleoli. Our findings indicate that for central (pyramidal) neurons there is a differential response to axotomy which is similar to that seen in peripheral (facial motor) neurons in the adult and immature hamster. It has been suggested that these differences in the chromatolytic response may be due to the metabolic state of the developing neurons at the time of the injury.

322 EFFECT OF DORSAL-VENTRAL LIMB ROTATIONS ON THE DEVELOPMENT OF MOTOR CONNECTIONS. Betty Ferguson* (SPON: R. J. Wyman) Dept. Biol., Yale Univ., New Haven, Ct 06520.

Specific motoneuron nuclei innervate particular muscles selectively in the developing chick hindlimb. Each muscle is innervated by a coherent group of neurons in a characteristic position in both the rostro-caudal and medio-lateral planes (Landmesser, L. T. (1978) J. Physiol., in press). However, mechanisms for the establishment of the peripheral connections of motoneurons and of appropriate central connections onto motoneurons are unknown.

To test the possibility that motoneurons are grouped in the spinal cord according to similar function (and therefore connectivity), motor pools for three shank muscles of the chick were localized with the technique of retrograde transport of horseradish peroxidase (HRP): the peroneus, the tibialis, and the medial gastrocnemius. The peroneus overlies the tibialis in the adult limb and both are derived from the dorsal muscle mass (DMM) but are antagonists. The peroneus and medial gastrocnemius are derived from different muscle masses, dorsal and ventral muscle mass (VMM), respectively, yet are synergists (Bekoff, A., Stein, P. and V. Hamburger (1975) Proc. Nat. Acad. Sci., 72:1245-1248).

The position of the shank motoneurons in the spinal cord was found to be related to the embryonic origin of the muscle but not to its function. Thus motoneurons innervating muscles derived from the DMM were situated laterally and those innervating the VMM situated medially regardless of muscle function. From these and similar studies on the thigh (Landmesser, L. T. (1978)) it is evident that central connections onto motoneurons cannot form in a simple topographical fashion according to position.

Supernumerary limb studies in this and other laboratories have shown that motoneurons are capable of innervating muscles that they normally would not have synapsed with. However, in view of the strict correlation found between medio-lateral positions of motoneurons and whether they would innervate the VMM or DMM, dorsal-ventral limb bud rotations (leaving the anterior-posterior axis normal) were done at stage 17 (Hamburger and Hamilton), prior to motoneuron outgrowth.

HRP backfills at stages 35-36 showed that medial motoneurons still innervate the VMM, even though this is now in a different position. Similarly, lateral motoneurons still innervate the DMM.

These results suggest that the limb bud is capable of affecting motoneuron projection patterns to result in selective innervation of DMM and VMM by lateral and medial motoneuron populations by stages 35-36.

Characterization of the actual routes taken by motoneuron axons at earlier stages should clarify the mechanisms involved in the establishment of these specific projection patterns. Supported by NIH Grant NS 10666.

323 ONTOGENY OF VISUOMOTOR BEHAVIOR IN NORMAL AND NEONATALLY COLLICULECTOMIZED HAMSTERS. Barbara L. Finlay, David Cordon (*) and Karen Marder (*). Dept. of Psychology, Cornell University, Ithaca NY., 14853

Neonatal hamsters, after lesions of the superior colliculus, show upon maturity sparing of visuomotor behavior that would be lost after a similar lesion made in an adult. We have analyzed the ontogeny of visuomotor behavior in normal and neonatally colliculectomized hamsters with regard to the mechanism of this sparing of function. Various indices of non-visual sensory and motor development, including general viability, were similar for the two groups, with the exception of general activity, which was depressed in the colliculectomized animals prior to eye opening (75% of normal) and dramatically increased after eye opening (200-400% of normal). Upon eye opening, photophobia, visually-elicited exploration, avoidance of a visual cliff and avoidance of stationary barriers were the same in both groups. Orientation to, and pursuit of small food objects was retarded in initial appearance in the colliculectomized group, but the pattern of deficits observed on first appearance was retained into adulthood. Thus we find little evidence for any role of experience in the sparing of function observed after collicular damage in the hamster. In the neonatal operate, as in the adult operate, deficits in visually-guided behavior after collicular lesions are limited to a restricted class of visuomotor behavior, that of orientation to and pursuit of small moving objects.

(Supported by NSF grant BNS77-07066)

324 RETINAL SYNAPTIC ARRAYS OF LARGE AND SMALL GOLDFISH. Leslie J. Fisher and Stephen S. Easter jr. Dept. Anatomy and Div. Biological Sci. U. Of Mich. Ann Arbor, Mich. 48109.

The retina of goldfish grows, as the goldfish itself grows, continually throughout its life. During growth, the inner nuclear layer (INL), the inner plexiform layer (IPL), the retinal diameter, the number of neurons, and the retinal magnification factor all change. Do these grossly observable changes have concomitant synaptic changes? We have quantitatively compared the IPL synaptic arrays of retinas of small goldfish--less than 1 year old--with those of large goldfish--3 to 4 years old.

Two distinct synaptic types were identified and counted: conventional synapses (made by amacrine cells) and ribbon synapses (made by bipolar cells). The results are expressed as numerical density of synapses (number per unit volume of neuropil). When expressed in synapses per 1000 cubic micra, conventional density was 125±9.1 for small fish and 144±8.6 for large fish while ribbon density went from 29±2.2 for small to 17 ±9.1 for large fish.

A more complete picture of the synaptic organization may be had by accounting for parameters of retinal growth other than numerical density. The synaptic data are analyzed in terms of the INL nuclei (synapses per amacrine or bipolar cell), planimetric density (synapses per unit surface area of the retina), and visual field (synapses per square degree of visual field).

The data so analyzed indicate that bipolar and amacrine synapses differ in their strategies of continuing synapse formation. Conventional synapses are maintained at a constant numerical density regardless of the size of the eye, while bipolar synapses decrease in numerical density as the eye grows. The planimetric density of ribbons remains constant. Since the magnification factor is greater in the larger fish, the number of ribbon and conventional synapses subserving a given area of visual field is increased. The average number of synapses per neuron increases significantly as the retina grows.

Supported in part by NIH grant EY01281 and EY00168

325 AGING IN MOUSE BRAIN: CHANGE IN ASTROCYTE POPULATION. Lynda H. Fleming* and Brian R. Unsworth* (SPON: E. Stein). Dept. of Biology, Marquette Univ., Milwaukee, WI 53233.

Most information on changes in glial cells with age has been provided by electron microscopic and autoradiographic analysis of brain sections. These studies have demonstrated gliogenesis in old brain, but have not enabled biochemical analysis of the glial cell populations. We have applied bulk isolation techniques to the preparation of glial cells from gross dissected regions of mouse brain. Glial cells prepared from young (6 month) and old (24 month) animals were found to band at different sucrose concentrations, when centrifuged on identical discontinuous sucrose gradients. This age-related difference in glial cell population was restricted to the brainstem and telencephalon, and was not observed in cerebellar preparations. Repeated experiments confirmed that the alteration in bouyant density was associated with astrocyte-like cells. The population of astrocytes from young and old animals were readily characterized by their different bouyant densities when separated on continuous diatrizoate gradients. Glial cells have been functionally implicated in the maintenance of the brain permeability barrier, and in the control of the ionic content of the perineuronal intercellular environment. We are presently investigating the possibility that an alteration in the glial cell population, of sufficient magnitude to be reflected in a change in bouyant density, may indicate major age-associated changes at the biochemical level. Supported by NIH Grant NS 12334.

326 ONTOGENY OF [C^{14}]2-DEOXYGLUCOSE UPTAKE IN THE RAT BRAIN WITH SPECIAL REFERENCE TO DAY-NIGHT DIFFERENCES IN THE SUPRACHIASMATIC NUCLEUS. Jannon L. Fuchs* and Robert Y. Moore. Dept. of Neurosciences, Univ. of California at San Diego, La Jolla, CA 92093.

The regional pattern of [C^{14}]2-deoxyglucose uptake (2DG) in the adult rat brain has been shown by Schwartz and Sharp (J. comp. Neurol. 177: 335-360, 1978). In addition, Schwartz and Gainer (Science 197: 1089-1091, 1977) have shown a clear day-night difference in 2DG uptake in the suprachiasmatic hypothalamic nucleus (SCN), in accord with the view (Moore, 1978) that the SCN is a circadian oscillator in the mammalian brain.

In the present study albino rats maintained in a 12:12 light-dark cycle were injected with [C^{14}]2DG at 1, 3, 5, 7, 10, 14 and 21 days postnatally. At each age, one group was injected during the light period and another during the dark. Additional groups of animals were maintained in darkness during the expected light period and in light during the expected dark period.

The 21-day brain exhibits distinct regional differences similar to those observed in the adult. At day 1 there is much less regional differentiation in 2DG distribution. Between days 1 and 21 there is a differential maturation of cerebral metabolism from one brain area to another as demonstrated by the 2DG method. The day-night differences in 2DG uptake in the SCN appear to be present by day 1 and are marked by day 10. This observation suggests that a circadian rhythm in metabolism in the SCN is present early in development, perhaps as early as day 1. No such difference is evident in any other brain area at any age. Consequently, the data obtained here provide further support for the view that the SCN is a circadian oscillator in the mammalian brain. Supported by USPHS Grant NS-12267.

327 EFFECTS OF ENRICHED AND RESTRICTED POST-WEANING REARING ENVIRONMENT ON ACTIVITY, AVOIDANCE, OVERRESPONSIVENESS, EXPLORATION, AND LEARNING. R. E. Gallagher* and Rhawn Joseph* (SPON: Nancy J. Leith). Depts. Psych. & Anat. Vanderbilt U. Nashville TN. 37232

One hundred eighty-five Zivic-Miller rats, reared in either a restricted or enriched environment were removed from their respective rearing conditions at 72 days of age, housed singly in standard laboratory suspended cages, and randomly assigned to one or several experiments. Several aspects of overresponsiveness were studied, including baseline activity, response suppression, locomotor hyperactivity in a complex closed field, the acquisition of repetitive "response habits", and simple passive and escape avoidance. In addition, the influence of exploration in the acquisition of learning errors was assessed in a food seeking task. All subjects were tested between 80 and 105 days of age.

Running wheel and open field testing (Experiments 1 & 2) indicated a sex difference but no environmental influences on activity level. However, when tested in a closed field (Exp. 3) restricted Ss became hyperreactive, and over subsequent testing increasingly responsive, suggesting a failure to habituate. In Exp. 4, hungry Ss were trained to shuttle between 2 goal boxes for a food reward. When complex novel stimuli were added to the testing environment, enriched Ss engaged in more exploration than restricted animals. This observation is similar to an earlier report (Joseph, J. Psychol. In press 1978) and thus disproves the supposition that restricted Ss do poorly on maze learning tasks due to excessive exploration. Restricted Ss may have failed to explore as much as enriched due to their tendency to develop response habits, i.e. a propensity towards repetitive responding in a limited circumscribed manner, in this case shuttling between goal boxes. This interpretation was extended in Exp. 5, in which it was found that restricted animals and females in general have difficulty suppressing a learned repetitious pattern of rewarded response when it is subsequently punished.

In tests of step-down and escape avoidance (Exp. 6), restricted animals were shown to be comparatively deficient in the ability to passively avoid noxious stimulation, or to control and direct their behavior so as to escape electric shock. Moreover, repeated exposure to the testing apparatus several days prior to testing (Exp. 7) improved the passive and escape avoidance performance of enriched animals only.

In all experiments involving learning, enriched animals were superior to restricted, suggesting that the inability of restricted Ss to suppress and inhibit spontaneous behavioral expression, as well as their tendency to overrespond, not only reduces their ability to make behavioral adjustments, but significantly interferes with learning.

328 VASOPRESSIN SYNTHESIS DURING DEVELOPMENT OF HYPOTHALAMIC MAGNOCELLULAR NEURONS IN THE RAT. D.M. Gash, C.D. Sladek, and D.E. Scott. Dept. of Anatomy, Sch. Med., Univ. Rochester, Rochester, N.Y. 14642

Although vasopressin (AVP) from the fetus has been postulated to play an important role in parturition in mammals, little is known about the functional development of the hypothalamo-neurohypophyseal system. We have endeavored to determine the course of appearance of immunoreactive vasopressin in the developing rat fetus and do correlative ultrastructural studies on the magnocellular neurons of the supraoptic (SON) and suprachiasmatic (SCN) nuclei.

The ventral hypothalamus and hypophysis were excised from 16-21 day rat fetuses, homogenized in 0.25% acetic acid, and radioimmunoassayed for vasopressin. Minimum sensitivity of the assay was approximately 10pg AVP/homogenate. Vasopressin was undetectable in 16-day rat fetal homogenates. By 18 days the ventral hypothalamo-hypophysis contained 130 ± 20pg AVP/animal. There was approximately a 3- to 4-fold increase in immunoreactive vasopressin on each subsequent day until shortly after parturition, when neonates contained 6342 ± 545pg AVP/animal.

Preliminary electron microscopic observations are consistent with RIA data. The SON of 21-day neonatal rats (regardless of sex) demonstrated the ultrastructural correlates of heightened synthetic activity. SON neurons exhibited marked nuclear clefting and distinct nucleoli. Their cytoplasmic matrices harbored numerous neurosecretory vesicles in close association and often within extensive Golgi cisterns. Substantial aggregations of rough endoplasmic reticulum, coated vesicles, and free polyribosomes were characteristic at 21 days of intrauterine development. Axosomatic synapses were not frequently observed at this stage of neonatal development.

Supported by USPHS Grants RR-05403, AM-19761, NS-00259.

329 LOSS OF AXO-SOMATIC SYNAPSES WITH ADVANCED AGE. Y. Geinisman, W. Bondareff and J.T. Dodge*. Dept. Anat., Sch. Med., Northwestern Univ., Chicago, IL 60611.

A loss of axo-dendritic synapses was previously shown to occur in the dentate gyrus of 25-month old rats relative to 3-month old rats (Neurosci. Abstr., 1976, 2:194; 1977, 3:106). In this study an attempt has been made to elucidate the question whether axo-somatic synapses are also lost in the rat dentate gyrus with advanced age.

Five young adult (3-month old) and five aged (25-month old) rats were perfused with Karnovsky's fixative. A 1 mm-thick slice, which included the rostral portion of the right dentate gyrus was dissected, postfixed in 1% OsO_4 and embedded in Araldiate-502. The region of the dorsal blade of the dentate gyrus, located opposite to the lateral end of its ventral blade, was trimmed down and cut into 75 nm-thick coronal sections. Three sections, one each from the rostral face, middle, and caudal face of a tissue block, were mounted on formvar coated, slot grids and stained with uranyl acetate and lead citrate. Each section was scanned along the most dorsal row of the granule cell layer to obtain electron micrographs of the first 10 profiles of granule cell somata sectioned through the nucleus. Mean values for each rat were derived from analyses of 30 soma profiles. Mean values for groups of young adult and aged rats were calculated from individual means and treated statistically with the "t" test.

Comparison of data obtained from the two age groups showed no difference in the size of neuronal soma profiles. The length of plasma membrane of neuronal soma profiles was virtually the same in young adult and aged rats. The mean number of synapses per unit length of neuronal soma membrane was significantly lower (by 15%) in the group of aged rats relative to the young adult group. The length of synaptic apposition, as well as the synaptic covering percentage were found to decrease significantly (by 10% and 22%, respectively) in aged rats as compared to young adult rats.

Because the age-related decrease in synaptic numbers per unit length of neuronal soma membrane is not associated with an age-related change in the size of neuronal somata or in the length of their plasma membranes, it suggests an absolute loss of axo-somatic synapses with advanced age. This synaptic loss is also suggested by the substantial age-related decrease in the synaptic covering percentage which cannot be solely explained by the diminution in the length of synaptic apposition. The findings of this study indicate that the loss of axo-somatic synapses contributes to the process of age-related partial deafferentation of neurons in the rat dentate gyrus.

330 MORPHOLOGICAL DEVELOPMENT OF IDENTIFIED NEURONS FROM AN IDENTIFIED NEUROBLAST DURING GRASSHOPPER EMBRYOGENESIS. C.S. Goodman* and N.C. Spitzer. Dept. of Biology, UCSD, La Jolla, CA. 92093.

We are investigating the differentiation of a class of identified neurons from a single identified neuroblast in embryos of the grasshopper Schistocerca nitens, by direct observations with interference contrast optics, intracellular recordings, and intracellular dye injections. We have found that the dorsal unpaired median (DUM) neurons in each segmental ganglion are direct desendents of the dorsal unpaired median neuroblast. Two plates of about 30 ventral neuroblasts, one on either side of the midline, reside at the center of each body segment in a 7-8 day embryo (development takes 21 days at 34°C). Each segmental array of neuroblasts contains a single unpaired neuroblast dorsal to the paired plates of ventral neuroblasts and at the posterior end of the segment. Each neuroblast is a stem cell giving rise to daughter cells by asymmetric mitoses.

In the thoracic ganglia, two chains of pairs of cells are first seen extending anteriorly from the DUM neuroblast, with a single rather than pair of cells at the end of the right hand chain (more chains appear later in development). Specific identified DUM neurons develop from specific DUM daughters along the left and right chains. By injecting Lucifer Yellow into growing neurons, we have been able to identify individually a number of embryonic cells and observe some of the processes of axonal outgrowth, extension of growth cones, and formation of arborizations in vivo. The asymmetric left and right chains contain different types of DUM neurons; the cells on the left extend laterally out peripheral nerves and the cells on the right extend longitudinally in the ventral nerve cords. Intraganglionic DUM neurons are born later than these first DUM neurons. On the left hand chain, the three outside cells, from most anterior, bifurcate and extend bilaterally out peripheral nerves 3, 4, and 5; out nerves 3 and 5; and out only nerve 5 (this third cell is DUMETi). The single cell at the anterior end of the right hand chain is termed the "H" cell since it bifurcates and each lateral branch bifurcates again, running up and down the ventral nerve cords. The next two cells are mirror image cells which extend anteriorly in only a single ventral nerve cord.

The single cell at the end of the right hand chain is the "H" cell in the meso- as well as in the metathoracic ganglia, suggesting that the left and right chains are producing homologous neurons in homologous positions in different thoracic ganglia. Thus, a constant subset of adult neurons are the descendents by a fixed lineage from a single identified neuroblast in each segmental ganglion.

(Supported by NSF, NIH, Sloan and Helen Hay Whitney Foundation.)

331 PROPERTIES OF MONOCLONAL ANTIBODIES DIRECTED AGAINST DEVELOPING BRAIN AND MUSCLE CELL SURFACES. Jeffrey Greve and David Gottlieb. Dept. Anat. and Neurobiology, Sch. Med., Wash. Univ., St. Louis, MO 63110.

An investigation of the cell surface biochemistry of developing brain and muscle cells from the embryonic chick has been undertaken using monoclonal antibodies as molecular probes. Spleen cells obtained from mice immunized with viable suspensions of embryonic chick brain or muscle cells were fused with a drug-marked myeloma cell line. Lymphocyte-myeloma hybrids were selected using the methodology of Galfre et al. (1). Anti-brain or anti-muscle cell surface andibody secreted by hybrid cells (hybridomas) was detected by an indirect radio-immune binding assay. A typical fusion yields a minimum of 24 independently arising clones with activity directed against the muscle or brain cell surface. Antibody secreting cells were cloned in soft agar and clonal lines established. The antibodies from these clones show saturable binding to muscle and brain cell surfaces. Binding activity co-migrates with authentic IgG on Bio-gel A-0.5 chromatography and is abolished by antimouse IgG antibody. Antibodies from different clones differ in banding patterns upon isoelectric focusing, indicating that products from different clones are distinct antibody molecules. Hybridomas produce tumors in syngeneic carrier mice. The serum from tumor-bearing mice is a rich source of monoclonal antibody which can saturate the target cell surface at dilutions of up to 10^5. Antibody from the serum of tumor-bearing animals has been labeled with ^{125}I and direct binding to the cell surface of embryonic brain and muscle cells demonstrated. Competition studies with a battery of unlabeled monoclonal antibodies indicate that different clones recognize distinct antigenic determinants on the cell surface. Therefore, the developing muscle and brain cell surface is not dominated by a single antigenic determinant. At least four such determinants have been demonstrated. The tissue distribution of one antigenic determinant, 59-B5, was studied. This determinant is 10-fold more abundant on brain than muscle, gut, liver or heart cells, and is highly trypsin sensitive. We conclude that monoclonal antibodies are valuable molecular probes for the surface of developing excitable cells.

(1) Galfre et al. Nature 266, 550 (1977)

Supported by NIH Grant NS12867.

332 DEVELOPMENT AND SEXUAL DIFFERENTIATION OF THE SONG SYSTEM IN THE ZEBRA FINCH. M. Gurney* and M. Konishi* (SPON: A. Van Harreveld). 216-76 Div. of Biol., Calif. Inst. of Tech.,Pasadena CA 91125.

Unique to the songbird brain is a robust neural system which controls the frequency patterning of song. In the adult zebra finch, the individual song system nuclei are conspicuously sexually dimorphic in volume [Nottebohm, F. and Arnold, A. (1976) Science 194: 211]. We have examined the ontogeny and sexual differentiation of this system.

The birthdates of nuclei within the song system have been determined using ^{3}H-thymidine autoradiography. The internal and external striata comprise separate proliferative zones. Proliferation in the dorsal ventricular ridge produces a spatiotemporal gradient of time of origin with first born neurons lying deep in the matrix of the external striatum. At hatching, little cytoarchitectonic differentiation has occurred within the telencephalon. In the peri-hatching period rearrangement of neurons through migration, and glial proliferation characterize telencephalic maturation. As the telencephalon matures, the individual song nuclei coalesce and increase in volume, yet as late as 20-25 days post-hatching the sexes are equivalent. During days 25-35, the song system nuclei, RA and HVc, rapidly expand in volume in the male.

The programmed sexual development of the song system occurs independently of either song vocalizations or song crystallization. Injections of estradiol benzoate (1 µg/g body weight) in oil on day 2 post-hatching suppresses song vocalizations and courtship behavior in the male, while the nuclei of the song system achieve normal volumes. Deafening on day 30 post-hatching prevents song crystallization through interruption of auditory feedback yet allows song vocalizations. Again, no effect on song system development is observed.

333 PSYCHOPHARMACOLOGICAL EVIDENCE FOR A SUBNORMAL SENSITIVITY OF SEROTONIN RECEPTORS IN PROTEIN MALNOURISHED RATS. Robert D. Hall and Wendy M. Robertson*. Worcester Foundation for Experimental Biology, Shrewsbury, Mass. 01545.

Stern and coworkers (Exp. Neurol., 1975, 49, 314 - 326) found that rats subjected to chronic protein malnutrition had higher levels of brain serotonin than well-nourished rats at most of the ages at which measurements were made. The model of malnutrition was one in which female rats were placed on an 8% casein diet 5 weeks before mating, and they were maintained on that diet until their pups were weaned at 21 days of age. The pups were then fed the same low protein diet. The present study, using the same model of malnutrition, asked if there might be some adaptation to the chronically high levels of brain serotonin, specifically, whether there might be a reduced sensitivity of serotonin receptors in the malnourished animals.

Thirty malnourished rats of both sexes and 31 well-nourished rats raised on a 25% casein diet were trained to run in a treadmill to avoid electric shock. Following 3 sessions in which only the 1% ascorbic acid vehicle was injected, a 2-mg/kg dose of N,N-Dimethyltryptamine (DMT), a serotonin agonist, was administered i.p. to half of the rats in each diet group 15 min before the treadmill test. The other half of each group received 4 mg/kg DMT. There followed two additional vehicle-only sessions and a second drug test in which each rat was given the 2- or 4-mg/kg dose of DMT it had not received in the first test.

The DMT impaired performance in a dose related way, as measured by the time to the first fall from the treadmill. The malnourished rats were affected significantly less than the well-nourished ones.

Ten of the rats were tested a third time, and methysergide, 10 mg/kg, was administered 30 min before they were given DMT, 4 mg/kg. The methysergide completely blocked the effects of the DMT, suggesting that the effects of the DMT in the first two tests resulted from the stimulation of serotonin receptors. It appears that there is an adaptation to the high brain serotonin levels in the form of a reduced receptor sensitivity in rats subjected to chronic protein malnutrition.

Supported by NIH Grant HD06364.

334 AN INVESTIGATION OF THE OCCURRENCE INTRAMEDULLARY SCHWANN CELLS FOLLOWING X-IRRADIATION OF THE MID-THORACIC AND LUMBOSACRAL LEVELS OF NEONATAL RAT SPINAL CORD. Jeanne K. Heard* and Shirley A. Gilmore. Dept. Anat., Univ. Arkansas Med. Sci., Little Rock, AR 72201.

The presence of intramedullary Schwann cells has been observed following x-irradiation of lumbosacral spinal cords of three-day-old rats (S. A. Gilmore and D. Duncan, Anat. Rec. 160: 675, 1968). In the present investigation intramedullary Schwann cell development was studied in three groups of rats irradiated at three days of age. In one group the irradiated zone was limited to a 5 mm length of mid-thoracic spinal cord (T only), in another group the irradiation was limited to a 5 mm length of lumbosacral spinal cord (L only), and in the third group 5 mm lengths of both mid-thoracic and lumbosacral spinal cord (T/L) were irradiated. All these animals received a single exposure to 4000R of soft x-rays having a half-value layer of 0.16 mm Al. Sham-irradiated littermates served as control animals.

Groups of rats were killed at intervals from 9 through 60 days following irradiation by perfusion of 10% phosphate-buffered formalin through the abdominal aorta. Spinal cords were embedded in paraffin and sectioned transversely at 8 micrometers. Sections were mounted in an interrupted serial fashion and stained by the following methods: hematoxylin and eosin, gallocyanin, Holmes' method, Wilder's reticulum stain, and luxol fast blue-periodic acid Schiff for myelin. Light microscopic observations were made to determine the presence of and area occupied by intramedullary Schwann cells. Schwann cells appeared in the lateral portion of the lumbosacral dorsal funiculus of L only and T/L irradiated spinal cords as early as 9 days post-irradiation. By 17 days post-irradiation Schwann cell development was extensive with the cells occupying the lateral and deep medial portions of the dorsal funiculus. Schwann cells were not observed in the mid-thoracic regions of T only and T/L irradiated rats until 13 days post-irradiation. The accumulation of these cells in the mid-thoracic region was not extensive, and, in general, the cells were confined to the lateral portion of the dorsal funiculus. In animals examined thus far the development of Schwann cells in the thoracic region was never as extensive as in the lumbosacral region. An additional difference between T only and L only irradiated rats is that irradiation of the lumbosacral area results in a decreased number of neuroglia with accompanying alterations in myelin, whereas similar changes were not observed after irradiation of the thoracic region. In general, the T/L irradiated spinal cords showed more necrosis and more Schwann cells when compared to either the T only or L only irradiated spinal cords. (Supported in part by USPHS Grant NS 04761.)

335 DEVELOPMENT OF THE LOCUST FLIGHT SYSTEM: PRECOCIOUS APPEARANCE OF AN IDENTIFIED SYNAPSE. David Heathcote* and David Falk* (SPON: David Bentley). Zoology Department, University of California, Berkeley, CA. 94720.

Insects perform many specific behavior patterns only as adults, for example flight, courtship and copulation. Examination of the neural events underlying the appearance of these behaviors may reveal how patterned neural outputs develop and how the expression of behavior is controlled. Locust flight is a behavior appearing only in the adult. Nymphs lack wings and the coordination necessary for effective flight, even though the flight muscles and their motor neurons are present. The onset of coordination is rapid and it develops during late postembryonic development, in the time immediately around the final molt. Three types of changes could underlie the appearance of coordination in this system. First, previously existing synapses could be strengthened around the critical time; second, new synapses could be formed, and third, previously existing strong synapses could become effective by the removal of an inhibitory block somewhere in the circuit. These hypotheses were tested by examining a critical monosynaptic connection between two identified neurons in the flight system of adults and nymphs.

The Wing Stretch Receptor sensory neuron (SR) has been shown to make a monosynaptic connection with the ipsilateral First Basalar motor neuron (1-BA) and with other wing depressor motor neurons. The SR synapses are essential for flight since their inactivation decreases flight frequency to a nonfunctional level. The forewing SR/1-BA synapse was monitored by intracellular recording from the soma of 1-BA in mature adults and in 6th (last), 5th, and 4th instars. At each stage, the synapse was characterized by measuring (i) voltage amplitude (ii) latency (iii)time course and (iv) decrement with repeated activation. Recordings from 1-BA in the last or 6th instar, show that the synapse is present before the animal can fly. The synapse is also present in 5th and 4th instar animals and it exhibits the physiological properties of the adult synapse.

Therefore, this synapse is present and is effective long before a coordinated flight pattern. It may be that basic connections underlying adult behavior patterns are laid down very early in the development of the nervous system.

336 DEVELOPMENT OF TRIGEMINAL MOTOR NUCLEUS IN CHICK EMBRYO: LIGHT MICROSCOPIC OBSERVATIONS. Marieta B. Heaton and Sally A. Moody. Dept. Neurosci., Univ. Fl. Coll. Med., Gainesville, Fl., 32610.

The development of the trigeminal motor nucleus in the chick embryo was studied, using autoradiographic, cell staining, fiber staining and axonal transport techniques. It was found that this nucleus arises very early in neurogenesis, with the first cells produced at 48 hours of incubation (stage 12), peak cell production at 50-55 hours (stage 16), and neuroblast proliferation completed by 72 hours (stage 18). As has been described in mammalian embryos, the primordial trigeminal cells move from the ventricular layer to accumulate as part of the common medial column, and later migrate in a ventrolateral direction to form the definitive lateral motor nucleus. The first identifiable component of the trigeminal system is the semilunar ganglion, which flanks the neural tube at stage 12, and sends afferents into the metencephalon by stage 13. By stage 14, the medial column is apparent and a few cells have moved to begin formation of a lateral nucleus. At this time, a thin motor root can be seen exiting the brainstem. The temporal sequence of ganglionic afferent ingrowth followed by initiation of medial column migration may be significant, in light of our previous findings demonstrating a profound influence of the ganglionic presence on the development of the lateral nucleus (Moody and Heaton, Neurosci. Abst., 3:114, 1977). During subsequent stages, migratory traffic from medial to lateral column increases, with cells frequently moving in association with fiber processes in the marginal zone. These fibers are presumed to emanate from secondary sensory, reticular, and medial column neuroblasts. By day 5, the medial column is greatly depleted and by day 6-7, the definitive lateral motor nucleus is formed. Beginning at 5 days, the dorsal motor nucleus can be detected, with cells from the lateral nucleus appearing to stream in a dorsomedial direction for its formation. Injections of horseradish peroxidase (HRP) into the mandibular process of the first visceral arch resulted in retrograde labeling of lateral nucleus cells as early as 3 1/2 days of incubation. In addition, migrating cells, intermediate between medial column and lateral nucleus, were similarly labeled. These observations indicate that processes of the lateral nucleus cells and those of migrating cells are well into their peripheral field at this age, but we cannot conclude that neuromuscular affiliations have been established, due to the possibility of HRP diffusion and growth cone uptake. HRP uptake by the migrating medial column cells is the first clear demonstration of the extent of axonal differentiation possible in migrating cells, although such a condition has long been suspected. (Supported in part by NIMH grant MH-27677).

337 TARGET SELECTIVITY OF MOTOR POOLS IN CHICK EMBRYOS. Margaret Hollyday, Dept. Pharmacol. Physiol. Sciences, Univ. of Chicago, Chicago, IL 60637.

A detailed map of the organization of the motor pools supplying muscles of the leg and wing in the hatched chick and in stage 38 embryos has been made using intramuscular injections of horseradish peroxidase (HRP). The adult organization of the motor pools is present before stage 38. The motor pools are grouped according to the embryonic origin of the muscles of the limb which they supply, and not according to the joints on which they act nor to their physiological action during locomotion. Motor pools for muscles derived from the ventral muscle mass are in the medial portion of the lateral motor column; muscles derived from the dorsal muscle mass are supplied by lateral motor pools. The birthdates of motor pools for muscles of ventral mass origin are from stages 17-19 in segments 23-28 and from stages 19-20 in segments 28-30. Motor pools supplying muscles derived from the dorsal mass are born from stages 19-22. In both thigh and calf, the nerves innervating dorsal mass muscles are separate from those innervating ventral mass muscle; in the thigh they are supplied by distinct nerve pathways and in the calf by different fascicles of the sciatic nerve.

Supernumerary legs and wings have been grafted in young embryos so as to be innervated by rostral lumbar segments 23 to 25 or 26. Motor neurons supplying individual muscles of both grafted and host limbs have been labeled using HRP injections in stage 38 embryos. Limb muscles derived from the ventral muscle mass (lat. gastrocnemius and biceps brachii) are supplied by medial motor neurons in clusters whose normal target is muscle of ventral mass origin. Limb muscles derived from the dorsal muscle mass (femorotibialis, post. iliotibialis, peroneus and triceps brachii) are innervated by more lateral motor neuron clusters which normally innervate muscles derived from the dorsal mass. The points of entrance and distribution of the nerves supplying the supernumerary limb are normal even with the reduced number of innervating segments.

These observations suggest that the formation of specific neuro-muscular connections during development may be based on a selectivity for either dorsal or ventral muscle mass tissue. This target selectivity may be further related to motor neuron birthdate and a subsequent expression of pathway selectivity.

Supported by the Spencer Foundation and NS 14066.

338 ABSENCE OF AUDITORY AFFERENTS ALTERS THE GROWTH PATTERN OF AN IDENTIFIED AUDITORY INTERNEURON. Ronald Hoy*, George Casaday*, Sharon Rollins*: (SPON: H. Howland) Cornell Univ., NB&B, Langmuir Lab, Ithaca, NY.

We have studied the effect of the absence of auditory afferent axons during postembryonic development on the adult morphology of a uniquely identified auditory interneuron in the prothoracic ganglion of the cricket Teleogryllus oceanicus. The ear, on the tibia of the foreleg, differentiates wholly during postembryonic development. Thus, unilateral amputation of the tibia immediately after hatching and subsequent amputation of the regenerating tibia prevented the development of any auditory afferent fibers on the operated side. We examined the morphology of the identified interneuron (Interneuron-1)[1] in unilaterally operated and sham-operated animals by retrograde transport of cobalt chloride from the cut end of its axon in the cervical connecture.

In sham-operated animals and on the normal side of unilaterally operated animals, Interneuron-1 has (1) a large lateral dendrite lying in a bundle of incoming auditory afferents and (2) several smaller medial dendrites ramifying throughout the ipsilateral ventral acoustic neuropil (VAN), the region of termination of one class of auditory afferents. In unilaterally operated animals (1) the lateral dendrite on the operated side is usually reduced in diameter and/or length, and (2) some of the radial dendrites originating from Interneuron-1 of the operated side are greater in diameter than normal and, rather than remaining ipsilateral, cross the midline to terminate in the contralateral VAN.

Their results suggest that (1) contact with auditory afferent axons facilitates normal growth of the lateral dendrite, and (2) contact with a normal VAN facilitates termination of the medial dendrites.

1 Casaday, G. B., Hoy, R. R. (1977) J. comp. Physiol. 121, 1-13

339 POSTNATAL DEVELOPMENT OF HAMSTER PREOPTIC AREA: A GOLGI STUDY. Chia-Hung Hsü*, C. Sue Carter, and William T. Greenough. Depts. Psychology and Ecology, Ethology & Evolution, and Program in Neural and Behavioral Biology. Univ. of Illinois, Champaign-Urbana 61820.

We have followed the development of dendritic morphology in Golgi-Cox stained neurons from the medial preoptic area at 0, 5, 10 and 20 days of age in golden hamsters (Mesocricetus auratus). The distance between the anterior commissure and the base of the brain was stable by 10 days of age. Dendritic field area and total dendritic length increased with age through day 20. Soma size increased with age until day 10 and was stable between days 10 and 20. Dendritic spines were infrequent on day 0, more numerous by day 5, and became abundant by days 10 and 20. Only about half of the neurons with first order branches had bifurcations on the day of birth. The probability of bifurcations increased until day 10 and then stabilized, or perhaps actually declined slightly, at 20 days. "Multifurcations" (more than 2 secondary branches), often originating from a varicosity, were most frequently observed on day 5, declined on day 10 and were rare on both days 0 and 20. Prospective branches, from multifurcations and possibly from bifurcations, appear to be lost during development, suggesting that the loss of processes, as well as continuing growth, may be involved in dendritic pattern generation in this region. These findings also indicate that although neuronal growth is probably still continuing to a small extent at 20 days of age, most aspects of development including soma size, and dendritic branching are beginning to stabilize or decline. Supported by PHS RR 07030 and the University Research Board.

340 DEVELOPMENTAL CHANGES IN GANGLIOSIDE COMPOSITION OF HIPPOCAMPUS, RETINA, AND OPTIC TECTUM. Carol Irwin* and Louis Irwin. Dept. Biochem., E. K. Shriver Center, Waltham, MA 02154.

The progressive emergence during development of precise cellular lamination and a topologically organized circuitry in regions such as the hippocampus, retina, and optic tectum of many vertebrates ideally suits these regions for a study of chemical correlates of differentiation and positional coding in the nervous system. We have miniaturized methods for the extraction and analysis of gangliosides in order to correlate changes in the quantity and molecular heterogeneity of these sialoglycosphingolipids with morphological changes during early stages of differentiation in the rat hippocampus and chick retina and optic tectum. Gangliosides from as little as 1 mg of tissue were extracted with chloroform:methanol (2:1, v/v), filtered through Unisil, eluted with chloroform:methanol:water (10:10:3), quantified by sialic acid assay, and resolved into separate molecular species by thin layer chromatography. Ganglioside content varied allometrically (as a log linear function of tissue mass) over a 50 fold increase in tissue dry weight, from very early stages of differentiation to neural maturity. The chromatographic pattern of different ganglioside species developed into the typical adult pattern during the period of maximum differentiation and synaptogenesis, with a disialoganglioside (G_{D1a}) in particular emerging rapidly during this phase. However, even at the earliest stages, prior to a significant degree of differentiation (6 days gestation for chick and tectum, 2 days prenatal for rat area dentata), all the major ganglioside components were present in small amounts. Slight variation in ganglioside content and pattern within subregions of the hippocampus and retinotectal system were detected at early stages, suggesting transient variation in ganglioside distribution within the tissue. These overall results indicate that retinal and brain cells can synthesize all the major gangliosides very early in development, but that shifts in the relative proportion of specific gangliosides occur as the tissue differentiates and becomes topologically specified.
(Supported by NSF Grant BNS 77-20575)

341 DEVELOPMENT OF CEREBRAL CORTEX TRANSPLANTED TO CEREBRAL CORTEX OR TECTUM OF NEWBORN RAT HOSTS. Christine B. Jaeger and Raymond D. Lund. Depts. Biol. Struct. and Neurol. Surgery, Univ. Wash. Sch. Med., Seattle, WA 98195.

We studied the effect of transplantation on the development of the cortex by grafting portions of the dorsal telencephalic vesicle of rat embryos of gestational ages E15-E22 (birth at E22) to the cortex of newborn rats. Embryonic graft tissue was labeled with tritiated thymidine (^{3}HT) prior to transplantation and grown for 1-8 weeks in the host.

Dilution of the ^{3}HT label in neurons of the graft indicates continuation of cell division after transplantation. Furthermore, evidence of ^{3}HT uptake after transplantation by unlabeled graft tissue supports this finding.

Staining of neurofibrils reveals the presence of fiber bundles which course immediately below the surface and within the substance of the transplant. While in cortical implants, fiber fascicles characteristically run between neuron clusters, a markedly different fiber pattern occurs in implants derived from embryonic tectal tissue transplanted to similar host regions. The tectal implants have interwoven fibers not clearly separable into individual fascicles. In cortical implants growing adjacent to the host's midbrain, a prominent fiber bundle can usually be followed running between the implant and the host tectum (or pretectum). This fiber bundle contains, at least in part, efferent axons from the implant.

Golgi impregnation of transplants showed that variations of the two main classes of known cortical neurons, namely pyramidal and nonpyramidal (e.g., stellate) forms, can be recognized in the implant. Pyramidal cells have a single large dendrite which seems to correspond to the apical dendrite except that it has no preferred radial orientation. The basal dendritic field is enlarged. The orientation of neuron dendritic processes relates most obviously to oriented fiber bundles within the implant. In developmentally young implants (up to 4 weeks survival) impregnated neurons have certain characteristics of immaturity, such as spiny somata and hair-like dendritic appendages. These are not seen in older implants. Electron microscopy of cortical implants reveals synaptic patterns characteristic of normal cortex.

These results indicate that embryonic cortical tissue continues differentiation after transplantation and develops connections with the host nervous system. Such connections can persist for at least 8 weeks even though the transplant may be situated in an abnormal site. (Supported by USPHS Grant EY-01950 from the National Institutes of Health.)

342 ULTRASTRUCTURAL STUDY OF MORPHOGENESIS IN THE AUDITORY SYSTEM OF CHICK EMBRYOS: NUCLEUS MAGNOCELLULARIS. Jhaveri*, Sonal, and D. K. Morest, (SPON. J. A. Andrezik). Department of Anatomy, Harvard Medical School, Boston, MA 02115; and University of Connecticut Health Center, Farmington, CT 06032.

The development of the cochlear nerve endings and their target cells was studied with electron microscopy of perfusion-fixed brains from embryonic day 12 (E12) to hatching. E12-13: Somatic processes extend from the perikaryon. The cytoplasm of the soma and processes contains free ribosomes, mitochondria, lysosomes, rough endoplasmic reticulum, golgi apparatus, and an eccentric, heterochromatic nucleus. Small vesiculated profiles of cochlear nerve fibers make specialized contacts, including some synapses on the distal somatic processes but rarely on the proximal processes or soma. The postsynaptic zones contain a flocculent matrix. E15-17: Somatic processes disappear and occasional attachment plaques are seen between cells. The nucleus appears euchromatic. The cytoplasmic organelles form a dense matrix indicative of intense metabolic activity. Somatic spines are evident. The afferent axons form large vesiculated profiles located increasingly on the cell body and somatic spines, with many points of synaptic contact. At each ending a band of amorphous flocculent material fills the postsynaptic cytoplasm. E18-hatching: The somatic cytoplasm becomes less dense; stacks of rough endoplasmic reticulum start to condense. Afferent axon terminals mature, especially the synaptic membrane complex and associated densities. The postsynaptic flocculent material diminishes in extent until it is found associated only with somatic spines.

Conclusions: Primary cochlear fibers initially contact distal parts of the somatic processes of the developing cells. As the somatic processes disappear or withdraw, the axonal endings move to the soma, resulting in large axosomatic end-bulbs. The findings suggest a role of the transiently appearing, flocculent material of the postsynaptic regions in the formation of synapses.

Supported by USPHS grants 7 RO1 NS13463 and 7 RO1 NS 14354, and the Jeffries Wyman Fellowship.

343 ADULT RAT DISSOCIATED SYMPATHETIC NEURONS IN CULTURE: MORPHOLOGICAL AND CYTOCHEMICAL STUDIES. M. Johnson. Dept. of Anat. & Neurobiol., Wash. Univ. Med. Sch., St. Louis, MO 63110.

Although perinatal rat superior cervical ganglion (SCG) neurons (cultured either as explants or as dissociated cells) maintain a number of adrenergic characteristics, they acquire certain cholinergic properties. The neurons accumulate choline acetyltransferase (ChAT) and the vesicles which have dense cores early in vitro become predominantly clear. Synaptic interactions between dissociated neurons are blocked by hexamethonium. Explants taken from adult rats, however, have much reduced levels of ChAT and the vesicles remain dense-cored. Thus, the ability to acquire cholinergic function seemed to be present only during early developmental stages. To further explore this question it was necessary to obtain preparations of dissociated adult neurons. Dissociation was most successful using a 3 hour incubation of small SCG chunks in 0.25% collagenase (Worthington CLS 4194), followed by 1 hour in 0.25% pronase (Calbiochem 53702). After rinsing and gentle trituration the neurons are plated out on air dried collagen in medium containing 10% chick embryo extract, 25% human placental serum and 25 ng/ml NGF. Following dissociation many of the neurons retain multiple branched processes but 24-48 hours later the neurons are rounded and have eccentric nuclei. The growth of new processes is delayed compared to the early neuritic outgrowth from perinatal neurons. Subsequent development is rapid with the neurons increasing in size and the nuclei becoming central. Studies of varicosities or synaptic endings of neurons known to have cholinergic interaction after 28 days in vitro (Wakshull et al., this volume) have shown 64% of the profiles to have a high percent of clear vesicles with a small number of dense core vesicles. The vesicle population is similar to that of the dual function neurons studied by Landis (PNAS 73: 4420-4424, 1976). Because a small number of cholinergic neurons may exist in the SCG and neuronal yield in this culture system is small, studies are in progress to rule out the preferential survival of the cholinergic population. The development of this technique for culture of dissociated adult neurons will allow study of their plasticity, adrenergic properties (such as NGF dependence), and regenerative capacity. (Supported by NIH Grants NS14416 and NS09923).

344 CONCOMITANT AGE RELATED BEHAVIORAL AND NEURONAL CHANGES IN THE NIGROSTRIATUM OF THE RAT. J. A. Joseph, C. Filburn*, S. P. Tzankoff* and B. T. Engel* (SPON: J. B. Appel). NIA, Gerontology Research Center, Baltimore, MD 21224.

Age differences in rotational behavior were examined in two experiments in young (6 mo.) and old (25-29 mo.) Wistar rats. In the first experiment 40 rats were grouped according to age and sex (10/group) and radio frequency lesions were produced in the left substantia nigra. The ratio of left to right turns for each 30 minute session was computed (L/R) following i.p. amphetamine injections so as to eliminate differences in overall turns between young and old unlesioned rats. After lesioning both old and young rats showed dose-related increases in L/R following amphetamine administration; however, age-dependent deficits were observed: young rats showed greater increases in L/R than the old (e.g., 5 mg/kg young rats 80 ± 25; old rats 20 ± 8). No sex differences were observed either prior to or following the lesion. Age related changes were not seen in these animals either when dopaminergic receptors were stimulated directly with apomorphine (.25-5 mg/kg) or when they were blocked with graded doses of haloperidol (.25-2 mg/kg) given prior to a 5 mg/kg dose of apomorphine.

In a second experiment 8 young and 6 old 6-OHDA lesioned males were used in an attempt to potentiate amphetamine-induced turning by pretreatment with 5 mg/kg of l-dopa 1 hour prior to a 1 mg/kg injection of amphetamine. Decided differences in the degree of potentiation were observed between young and senescent animals. Young rats increased their L/R's by 50% while old rats showed a 23% decline in L/R's. These age-related deficits in rotational behavior may be the result of presynaptic changes within the striatum, and subsequent neurochemical analyses have supported this hypothesis, i.e., (a) a 50% reduction in dopamine (DA) (ug/g) in the unlesioned striatum of the old rat making differences in DA levels between lesioned and unlesioned striata less in the old rat (young unlesioned striatum 10.9 ± .9 lesioned 1.8 ± .7; old unlesioned striatum 5.12 ± .7, lesioned 2.03 ± .7); (b) a 12-17% depression in both stimulated and unstimulated tyrosine hydroxylase activity in old unlesioned rats, indicating that basal activity (p mol/min/mg, 15 um tyrosine) measured at saturating (1 mM) and subsaturating (.1 mM) levels of $DMPH_4$ was depressed in the old striata. Addition of heparin (50 ug/ml) to activate the enzyme produced comparable stimulation of activity from both groups at both concentrations of cofactor, indicating a decrease in total enzyme with no significant change in kinetic state. Thus, it would appear that there are deficits in neuronal functioning which can alter rotational behavior in the senescent rat.

345 ASTROCYTIC CELL LINEAGE. AN IN VITRO STUDY. B.H.J. Juurlink*, S. Fedoroff and L. Hertz*. Department of Anatomy, University of Saskatchewan, Saskatoon, Saskatchewan, S7N OWO, Canada.

In order to study a particular cell lineage, one must be able to identify its stem cells and the various progenitor cells as well as the end products of the lineage. Recently developed dissociated brain cell colony cultures (1) facilitate the study of cell lineages in the developing CNS. Dissociated cells of the isocortex of mice of various fetal and postnatal ages, if planted in low numbers, form discrete colonies in culture. The most immature cells in such preparations are believed to be those forming the type A colonies. These cells, in culture, undergo a sequence of morphological changes. In vitro experiments have indicated that the A type colonies develop into the morphologically different C type colonies, which in turn transform into the type B colonies (1). The latter react to addition of the dibutyryl cyclic AMP by intense formation of processes leading to cells which morphologically(1) and biochemically (2) resemble mature astrocytes found in vivo.

In order to determine the precise orgin of the cells which form type A colonies, the subventricular and cortical regions of new born (PO) mouse isocortex were isolated, dissociated and cultured. The number and frequence of various types of colonies that formed were determined. About 80% of all colony-forming cells from the isocortex came from the subventricular zone, and from all astrocytic precursor cells which form type A colonies, about 80% arose from the subventricular zone and 20% from the cortical area of the brain. The colony cultures from P7 mouse subventricular and cortical areas had less than half the number of type A colonies than cultures of PO mice and twice as many type C colonies. These experiments indicate that the cells which form type A colonies in culture come from the subventricular cell population and that some of the cells in newborn mouse brain apparently migrate from the subventricular zone into the cortical area. It also appears that the astrocytic cell lineage progresses from type A colony-forming cells to type C colony-forming cells in vivo as well as in vitro.

(Supported by MRC of Canada, Grant MT 4235).

1. Fedoroff, S. In: Cell, Tissue and Organ Cultures in Neurobiology, ed. S. Fedoroff and L. Hertz, Academic Press, New York, 1977, pp. 215-221

2. Hertz, L., Schousboe, A., Bock, E. and Fedoroff, S. Submitted to J. Comp. Neurol. 1978.

346 THE APPEARANCE OF SYNAPTIC JUNCTIONAL MACROMOLECULES DURING BRAIN MATURATION. Paul T. Kelly and Carl W. Cotman. Dept. Psychobiology, Univ. Calif., Irvine, CA 92717.

Direct biochemical analyses were used to examine the appearance of synaptic junctional (SJ) macromolecules during the formation of asymmetric synaptic contacts in the developing rat CNS. Relatively pure synaptic plasma membrane (SPM) and SJ fractions were isolated from immature rat brain at various stages of postnatal development by subcellular fractionation methods previously developed for mature tissues. Electron microscopy on OsO_4 and E-PTA stained synaptic fractions has revealed that immature SJs are morphologically similar to those isolated from adult brain. In particular, SJs isolated from immature rat brains displayed easily recognizable postsynaptic densities (PSDs). SJs isolated from immature brains (5-10 days of age) were smaller and their PSDs appeared incompletely formed. SJ fractions from immature animals contained a higher proportion of postsynaptic membrane specializations (postsynaptic membrane plus PSD) relative to intact SJs than did adult SJ fractions. The protein yield in SJ fractions increased 4-fold during development, a cnange that paralleled the temporal appearance of asymmetric synaptic complexes in situ.

The protein and [^{125}I] Concanavalin A (Con A)-binding glycoprotein compositions of SPM and SJ fractions were examined at different postnatal ages. A striking degree of similarity was observed in the composition of major proteins in SJ fractions obtained from rats at various postnatal ages (4-60 days of age). The relative levels of tubulin and actin in SJ fractions were constant throughout development. The Con A-binding glycoproteins, which in the adult are localized to the external surface of the postsynaptic membrane overlying the PSD, are present in similar quantities in immature (4-14 days) and adult SJ fractions. However, large differences in the composition of Con A binding-glycoproteins were observed in SPM fractions during postnatal development. An exception to these developmental similarities was the major PSD protein which, when compared to adult, was present in SJ and SPM fractions at greatly reduced quantities during the period of active synaptogenesis (8-18 days postnatal).

These results show that postsynaptic membrane glycoproteins which bind Con A are present in SJ fractions of immature rat brain before and during the stages of active synaptogenesis. The same is true for tubulin and actin. These macromolecules may therefore be involved in the early phase(s) of synaptic adhesion. In contrast, the major PSD protein may be associated with the final stages of structural and functional synaptic maturation. (Supported by NIH grant NS 08597 and post-doctoral fellowship 1F32 NS 05746).

347 FURTHER EXAMINATION OF LEUCINE INCORPORATION BY IMMATURE AND MATURE AXOTOMIZED FACIAL NEURONS. L.A. Kirschen and A. LaVelle. Dept. Anat., Univ. Ill.- Med. Center, Chicago, IL. 60612.

Previous work has shown that immature and mature hamster facial neurons differentially incorporate (^{3}H) leucine at 4 days after axotomy, the height of chromatolysis. This work was extended to correlate changes in isotope uptake with morphological changes known to occur with recovery of facial neurons from injury.

The right facial nerves in hamsters at 15 and 20 days postnatal age and in adults were severed. The opposite sides served as control. On postoperative days 4, 15 and 30 all hamsters were injected subcutaneously with 4 μCi/g body weight and sacrificed one hour postinjection (peak of uptake). Each whole nuclear group was removed by dissection, freeze-dried, weighed, and processed for liquid scintillation counting. Specific activities of the TCA soluble fractions for each nuclear group were taken to represent the free amino acid pool (Br. Res.,111:31,'76). These activities were not significantly different for any age and postoperative time. In addition, there was no increase in capillary endothelial cell (^{3}H) thymidine uptake on the experimental side in adult 4 day postoperatives. This is in contrast to Watson (J. Physiol., 180:'65), who claimed that such an increase in his material indicated local hyperemia. There was, however, a 20-fold increase in the number of labelled glial cells on the experimental side as compared to the normal.

In the 15 day operated series, axotomy results in minimal morphological changes compared to normal neurons. Isotope uptake showed no differences between control and injured neurons at any of the postoperative times. In 20 day operatives, axotomy results for the first time in distinct chromatolysis with somal swelling, features of the adult reaction, although these neurons are still immature morphologically. On postoperative days 4 and 15 the experimental sides showed an increase in isotope uptake over the control sides. By 30 days postoperative the difference in uptake was smaller. In the adult at 4 days postoperatively, the isotope uptake was extremely elevated on the injured side. This difference was diminished by 15 days, and by 30 days uptake was equal on both sides.

The differential degrees of both chromatolysis and isotope incorporation at the various ages and postoperative times are suggestive of stage specific levels of metabolism. The difference in rapidity of recovery between the 20 day and adult operatives reflects the fact that at 20 days, injury occurred at a time when the protein synthetic machinery had not yet fully matured. This correlates with cytological observations that the hamster facial neurons mature only after 20 days of age.

348 EFFECT OF LOW-LEVEL LEAD BURDENS ON CAUDATE NUCLEUS ONTOGENESIS AND SENESCENCE: A MORPHOMETRIC ANALYSIS. Martin R. Krigman, Edward Bertram*, Robert Bagnell*, and Elizabeth G. Bendeich, University of North Carolina, Chapel Hill, N.C. 27514.

Suckling Long-Evans rats were fed a single daily dose of either 0, 25, or 200 mg of lead/kg body weight by gastric gavage from the 3rd through the 30th day of life. Rats were then sacrificed at either 60 or 750 days of age and a predetermined region of the caudate nucleus was prepared for light and electron microscopy. Gross brain development as well as somatic growth and development were unaffected by the lead burdens, and qualitative differences were not discernible in the caudate nucleus of treatment groups evaluated at the two age periods by light or electron microscopy. Morphometric analysis, however, revealed in the 60-day-old group a reduction in the numerical density of neurons, synapses, and synapses per neuron. The degree of these changes at 60 days of age was dose related. However, by 750 days of age, the differences between the treated and control rats were not significant.

Low levels of a lead burden perturb ontogenesis of the caudate nucleus. If the lead exposure is restricted to the period of brain development, this early, but limited, lead exposure does not affect aging of the caudate nucleus. (Supported by USPHS NIH Grant ES 01104)

349 EFFECT OF SPINAL CORD DELETIONS AND REVERSALS ON MOTONEURON PROJECTION PATTERNS IN THE EMBRYONIC CHICK HINDLIMB. Cynthia Lance Jones* and Lynn T. Landmesser. Dept. Biol., Yale Univ., New Haven, CT 06520.

In the chick embryo, the combined use of electrophysiological and retrograde labelling techniques have shown that appropriate neuronal projection patterns, involving a tight correlation between axon termination site and motoneuron soma position, are established prior to muscle cleavage and the peak period of motoneuron cell death (Landmesser, J. Physiol., in press). In order to determine what factors might be involved in setting up these specific peripheral innervation patterns, several experimental manipulations were performed. The first, involving partial spinal cord deletions, was designed to see whether the projection patterns of the remaining spinal cord segments would be altered.

Part or all of chick lumbosacral spinal segments LS1-3 were removed prior to the onset of motoneuron production at Stage 15 or 16 of Hamburger and Hamilton. At Stages ranging from 31 to 37 the innervation pattern of the limb was examined by sequentially stimulating the lumbosacral nerves which were present and recording EMG's from various muscles. The limbs were subsequently fixed for microscopic characterization.

No regeneration of the deleted segments was observed and the distribution pattern of the remaining spinal nerves was electrophysiologically and anatomically normal. Muscles which would normally have been totally innervated by the deleted segments could not be activated by spinal nerve stimulation and showed signs of extensive atrophy. Muscles left partially innervated by the deletion were also atrophic, though qualitatively less, indicating that the remaining motoneurons which projected to these muscles did not entirely compensate for the missing motoneurons. The fact that the projection patterns of remaining segments are unaltered even when examined at stages before most of the motoneuron cell death has occurred, implies that the final innervation pattern observed does not require competitive interactions between different cord segments.

A second series of experiments consisted of the reversal of segments LS1-3 at the same stages. Preliminary results from spinal nerve stimulation at Stage 35 revealed that the displaced motoneurons had established connections with their normal muscles. These observations suggest that, in the case of small positional shifts, motoneurons are capable of forming appropriate connections even though their cranio-caudal position with respect to the limb has been altered. (Supported by NIH Grant NS 10666.)

350 APPARENT ACCELERATION OF BRAIN AGING PATHOLOGY BY PROLONGED ADMINISTRATION OF GLUCOCORTICOIDS. P.M. Landfield, J.D. Lindsey*, G. Lynch, Dept. Psychobiol., Univ. Calif., Irvine, CA 92717.

We previously found a significant correlation in aging Fischer rats between plasma levels of corticosterone (but not aldosterone) and quantitative measures of a sensitive index of age-dependent brain pathology (astrocyte reactivity in hippocampus) (Landfield and Lynch; Neurosci. Abstracts, 1977; Landfield, Waymire, Lynch, submitted). Moreover, it has been hypothesized that endocrine factors, in particular glucocorticoids, may play a major role in the development of age-related brain pathology (Landfield, 2nd Tarbox Parkinson's Dis. Symp.; Plenum; in press). In order to test this hypothesis and to examine the possibility that the above-noted brain-endocrine correlations may in part reflect cause-effect relationships, we have administered various hormonal treatments to groups of Fischer rats for a period of 7 mo., beginning at 16 mo.-of-age. (Groups: deoxycorticosterone acetate (DOCA) 5-8 mg/rat; corticosterone acetate (CTCA) 5-8 mg/rat; ACTH gel: 4-8 I.U. (Organon); and a potent synthetic glucocortcoid: 6-dehydro-16-methylene-hydrocortisone (6 DHMH) 0.4 mg/rat: (E. Merck). 6 DHMH is reported to be relatively more effective in its brain effects (suppressing ACTH secretion) than in peripheral effects. Control groups received vehicle. Injections were s.c., 3 x weekly. Animals were maintained in pathogen-free conditions behind an air-barrier filter system. No symptoms of infection were observed.

Glucocorticoid and ACTH treatments, when combined, significantly increased the degree of astrocyte reactivity in comparison to combined control and DOCA groups. However, some control animals exhibited extensive gliosis, and some glucocorticoid animals exhibited relatively mild pathology. Interestingly, control animals with elevated reactivity possessed the highest adrenal weights and in general, a significant correlation was found across groups between levels of glucocorticoid activity as assessed by adrenal weights and degree of hippocampal astrocyte reactivity. Plasma hormone analyses are in progress.

These data, then, are highly consistent with the hypothesis that glucocorticoids contribute, in part, to the rate of hippocampal age change. Moreover, they agree with our prior report of correlations between corticosterone (and adrenal weights) and astrocyte reactivity in aging animals. However, the possibility of hormone effects on astrocyte staining rather than on reactivity must be considered. Further analyses of our other material will allow for more certain conclusions. Moreover, studies in progress will allow us to determine whether or not we can also retard this syndrome. (NIH grants Ag 00341 (PL) and Ag 00538 (GL). We thank J. Waymire, C. Wurtz and L. Braun for important analyses.)

351 ALTERNATING RETINAL GANGLION CELL TERMINATION BANDS IN DOUBLY INNERVATED FROG OPTIC TECTA. Margaret I. Law* and Martha Constantine-Paton. Dept. Biol., Princeton University, Princeton, NJ 08540.

Two optic tracts were induced to innervate a naive tectal lobe through the implantation of a third eye primordium into the forebrain region of Shumay stage 17 Rana pipiens embryos. Autoradiographic analysis following intraocular injections of ^{3}H proline into either the supernumerary or the double innervating normal eye of animals ranging in age from Taylor and Kollros stage V to 3 mos. postmetamorphic revealed distinct eye-specific bands of labelled superficial neuropil. These bands extended rostral-caudally over the entire dually innervated tectal lobe. Grain counts in the unlabelled eye's bands were at the level of tissue background indicating relatively little overlap between the projections of each eye. These results imply that axons from each of the dually innervating eyes are competetively excluding axons from the alternate eye.

Electrophysiological recordings reveal that both the normal and the supernumery eye maps retinotopically. In many tectal locations the activity from one eye was more pronounced than from the other eye.

A similar banding pattern has also been observed when dually innervated tecta were generated through the removal of one tectal lobe in older animals.

We conclude that ganglion to ganglion cell interactions in addition to pre to postsynaptic neuron interactions are active in establishing the normal retinotectal projection. Moreover, the similarity between this experimentally induced banding and the ocular dominance columns of cat and monkey* suggest that the latter termination pattern may be established through cellular interactions basic to a wide variety of neuronal projections. (Supported by NIH grant EYO 1872 and by NIH service award T32GM 07312.)

*Wiesel et al., Brain Res. 79: 273, 1974; Graybiel, Brain Res. 96: 1, 1975; Hubel et al., Brain Res. 96: 25, 1975; Shatz et al., Brain Res. 131: 103, 1977.

352 DEVELOPMENT OF REACTIVITY TO AUDITORY STIMULI IN NORMAL AND NEONATALLY BRAIN-DAMAGED CATS. M.S. Levine, C.D. Hull, N.A. Buchwald and S.L. Hutchison*. Mental Retardation Res. Ctr.,Sch. Med. UCLA, Los Angeles, CA. 90024.

This is part of a series of experiments designed to assess the effects of early intervention upon behavioral maturation in the kitten. The purpose of this experiment was to determine (1) the development of behavioral reactivity to stimuli in kittens and (2) the effects of neonatal "medial forebrain bundle" ("MFB") lesions on subsequent development of reactivity. Tests were made on 29 normal kittens (varying from 10-150 days of age), 9 adult normal cats and 5 adult cats which had received bilateral lesions interrupting the nigrostriatal pathway ("MFB" lesions) at 10-15 days of age. The "MFB" lesions significantly reduced the concentrations of dopamine and its associated enzymes in the caudate nuclei bilaterally (80% depletion). The test procedure consisted of presenting each animal with a series of taped cat vocalizations and assessing its behavioral responses on a 6 point scale. Each animal received 2 sessions (24 hours apart) of 28 cat vocalizations (each lasting 12-15 sec) presented 1 min apart. During the first few stimulus presentations the amount of behavioral reactivity to the stimuli was directly related to the age of the animals. The youngest kittens reacted least, the adult cats the most. All animals showed a decreasing pattern of reactivity to the repeated vocalizations during session 1. This habituation was most marked and rapid in adult cats, occurring within 7 trials. Ten and 30 day old kittens habituated very slowly; their responses did not stabilize until the last 7 stimuli of the session. Cats with "MFB" lesions were more reactive during the first few presentations and habituated slower than intact adults during session 1. During session 2 the initial reactivity was no longer directly related to age. Adults, and older kittens had similar reactivity scores. These scores were then compared with the scores for the previous session. Only adults showed any savings. Their initial reactivity scores were 60% of session 1 scores. Ten day and 30 day kittens were as reactive on the second day as on the first. Thus, patterns of reactivity to stimuli are markedly different in young kittens than in adults. Kittens habituate more slowly and do not sustain habituation over a 24 hour period. Cats with "MFB" lesions were as reactive during session 2 as during session 1, showing no savings from the previous days experience. Although these animals were adults they displayed an immature pattern of reactivity.

Supported by USPHS grants NS-12324, HD-05958.

353 SEGMENTALLY SELECTIVE INNERVATION OF MAMMALIAN SYMPATHETIC GANGLIA: RELATIVE ROLES OF INTRAGANGLIONIC POSITION AND POST-GANGLIONIC TARGETS. Jeff W. Lichtman, Joseph W. Yip and Dale Purves, Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

The basis of preferential segmental connections in the peripheral sympathetic system was examined in the superior cervical ganglion of the guinea-pig by asking whether the innervation each neuron receives is correlated with its intraganglionic position or some aspect of its postganglionic target.

By analogy with other systems, selectivity might reflect a topographic matching of preganglionic axons from different spinal segments to particular regions of sympathetic ganglia. The influence of intraganglionic cell position in the superior cervical ganglion, however, cannot be very great since neurons located at different positions along the major ganglionic axes receive, on average, the same segmental innervation. Moreover, horseradish peroxidase-labelled ganglion cells innervating a particular target such as the eye are widely distributed. Thus intraganglionic position is probably not the basis for the selective segmental innervation of ganglion cells.

Preferential segmental innervation of ganglion cells might, on the other hand, be determined by postganglionic targets: for example, such innervation could insure appropriate activation of end-organs with similar functions, or proper activation of sympathetic targets within a particular region of the body. To determine whether the pattern of segmental innervation is more closely related to end-organ position or function, we examined the territory and modalities supplied by different spinal segments. Two different end-organs (blood vessels and the dilator pupillae muscle) at the same location (the iris) responded to stimulation of the same ventral roots. Conversely, the territory of a single modality (piloerector muscles) supplied by the neurons of the superior cervical ganglion showed a segmental pattern of innervation: hairs on the anterior face tended to be erected by stimulation of spinal nerves more rostral than those which activate hairs around the ear and back of the head. Thus sympathetic end-organs are activated by similar spinal segments if they are located in the same region, but tend to be innervated by different spinal segments if they occupy different positions.

These results suggest that segmentally selective synaptogenesis is related to the position of a ganglion cell's target rather than the position of its soma within the ganglion.

[Supported by NIH grant NS-11699.]

354 A QUANTITATIVE STUDY OF HIPPOCAMPAL PYRAMIDAL CELL POPULATION IN NORMAL AND SENILE DEMENTED HUMAN BRAINS. M. Linauts* and P.L. McGeer (SPON: W. Gibson). Dept. of Psych., University of B.C., Vancouver, B.C., Canada, V6T 1W5.

The area of the brain most clearly associated with memory is the hippocampus. There is a general clinical impression in humans that memory capabilities decline with age and are grossly deficient in senile dementia. In the latter condition, senile plaques and neurofibrillary tangles are characteristically noted in the hippocampus, particularly in the pyramidal cells of CA1 to CA3. In order to obtain quantitative information regarding the actual number of pyramidal cells in the hippocampus in various normal age groups, and in senile dementia, we counted cells in a series of human cases ranging in age up to 82 years. The hippocampus was dissected out, fixed in 10% buffered formalin, and sectioned at 50 μ on a freezing microtome. Every tenth section was mounted and stained with cresyl violet. There was a consistent decrease in pyramidal cell counts with increasing age in normal individuals, with substantially fewer cells in cases of senile dementia. An average number of cells per defined field is 1778 at age 37, decreasing to 1415 by age 76. In senile dementia cases, the average number was decreased to 1189 for the upper age category.

Supported by Medical Research Council of Canada

355 DEVELOPMENT OF MUSCARINIC AND β-ADRENERGIC RECEPTORS IN RAT PAROTID GLAND; EFFECT OF NEONATAL SYMPATHECTOMY. Jennifer M. Ludford* and Barbara R. Talamo (with the assistance of I. Elashvili). Depts. Neurol. and Physiol. Chem., Sch. Med., Johns Hopkins U., Baltimore, MD 21205.

Rat parotid gland is a favorable tissue for analysis of the development of the secretory process and the influence of autonomic innervation on this development. Maturation of acinar cells is largely a post-natal phenomenon (Redman and Sreebny, Dev. Biol. 25:248, 1971), and sympathetic fibers are actively growing into the gland at birth (Owman et al., Z. Zellforsch. 16:319, 1971). Each cell receives sympathetic and parasympathetic innervation. We have characterized β-adrenergic and muscarinic ligand receptors on membranes of mature and immature glands and determined the density of binding sites in developing glands. [^{3}H]-Quinuclidinyl benzilate (QNB) and [^{125}I]-hydroxybenzylpindolol (HYP) were used as muscarinic and β-adrenergic ligands to measure specific binding. Scatchard analysis of saturation curves gave dissociation constants (K_d) and receptor densities (B_{max}) of 0.10 ± 0.01 nM and 4.8 ± 0.4 pmoles/g tissue respectively for QNB (n=6) and 0.14 ± 0.02 nM and 3.3 ± 0.5 pmoles/g tissue for HYP (n=9) in mature membranes. In young animals (<10 days (d) of age), the K_d for each ligand is essentially the same as for adults. Binding is detectable at birth at 10% and 14% of adult levels (based on tissue weight) for QNB and HYP, respectively. Mature densities of both receptors are achieved by 25 d postnatal. Studies of the development of hormone-sensitive secretion indicated that isoproterenol-sensitive adenylate cyclase activity and amylase secretion are not detectable in 8 d animals, but are present at 15 d (Grand and Schay, Pediat. Res. 12:100, 1978). We find that β-adrenergic receptor levels have reached 22% of adult levels by 7 d and 51% by 14 d, indicating that a complete absence of β-receptors is not the explanation for the lack of sensitivity. Further increases occur in hormone-sensitive adenylate cyclase activity after 25 d, indicating that factors other than receptor number or basal cyclase activity are involved.

Chemical sympathectomy of newborn animals (6-hydroxydopamine, 100 mg/kg, daily, for the first 4 d of life) completely blocks sympathetic innervation of parotid glands in 7-41 d animals (norepinephrine is undetectable, i.e. <0.5% of littermate controls) but does not alter either the K_d or B_{max} for HYP up to 21 d. However, 41 d animals have twice the receptor density of untreated adults, with no change in K_d. Isoproterenol-induced glandular hypertrophy is greater in sympathectomized rats (Gresik and Barka, J.P.E.T. 200:101, 1977); increased β-adrenergic receptor numbers may partially account for the apparent postjunctional supersensitivity of such animals. (Supported by NIH Grant NS12839)

356 THE DEVELOPMENTAL PROFILE OF NEURONAL AND GLIAL ENOLASE IN RAT BRAIN. P.J. Marangos, A.M. Parma, D.E. Schmechel and F.K. Goodwin. (SPON: Henry deF. Webster). Clinical Psychobiology Branch, NIMH, Bethesda, Maryland 20014.

The isoenzymes of the glycolytic enzyme, enolase (E.C.4.2.1.11) from rat brain have been extensively studied in our laboratory. It has been shown that a neuron specific enolase (NSE) and a non-neuronal enolase (NNE) localized in glial cells, accounts for a major proportion of brain enolase activity (Science 199:313, 1978). A specific radioimmunoassay (RIA) has been developed for NSE and NNE which demonstrates that each protein represents about 1.5% of the total soluble protein in brain, making them major constituents of brain tissue.

The developmental profile of each isoenzyme was studied in six brain areas of newborn rat brain up to 25 days of age. In all six regions, brainstem, cerebellum, quadrageminal plate, hypothalamus, forebrain and olfactory bulb, NSE levels are low at birth, (1 ug/NSE/mg soluble protein). The pattern of NSE increase varies between brain regions in a manner that may be correlated with development or acquisition of functional activity. Brainstem levels of NSE increase earliest with the point of inflection occuring at 5-7 days. Other regions are more delayed with cerebellum being the slowest. The late appearance of NSE in development indicates that levels of this neuronal antigen are highly correlated with neuronal differentiation.

In contrast, NNE levels are much higher in the newborn brain (5-8 ug NNE/mg soluble protein) and increase much less dramatically to adult levels (10 ug/mg) at 25 days of age. The NNE/NSE ratio ranges from 5-10 in the various brain areas at birth and decreases to approximately 1 in mature brain. Since NSE is specifically localized in neurons, the low values of NSE in regions where neurons are already generated suggests that NNE is present in immature neurons and that differentiation or acquisition of functional activity correlates with a switch to NSE.

The results are consistent with the hypothesis that NNE is present in immature neurons and is replaced by NSE in a manner coincident with the neuronal differentiation process. Since NSE and NNE have been shown to be products of separate genes, (Brain Research, in press), it appears that the gene responsible for NNE synthesis is repressed, and the gene for NSE derepressed during the course of neuronal differentiation. The appearance of NSE at a late point in development where structural and functional maturation is in its final stages suggests that the neuronal enolase has properties specifically suited to the physiology of the functional neuron.

357 DEVELOPMENT OF GRANULE CELLS IN TWO INBRED STRAINS OF MICE DISPLAYING GENETICALLY-ASSOCIATED VARIATIONS IN THE HIPPOCAMPAL MOSSY FIBER SYSTEM. Dee Ann Matthews and James E. Vaughn. Division of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010.

Certain genetically-associated variations, such as those found in the distribution of mossy fiber synapses upon hippocampal pyramids (JCN 156: 417, 1974), provide an opportunity to study developmental mechanisms involved in establishing specific synaptic connectivities. Previously, we have reported a reversal in the generation pattern of the mossy fiber target pyramidal cells in BALB/cJ mice, but not in SM/J mice. This reversal was limited to the portion of regio inferior that displays genetically-associated variations in the connectivity pattern of the mature mossy fiber system (JCN 173: 41, 1977).

As part of a continuing investigation of the developing mossy fiber system in BALB/cJ and SM/J mice, we have recently examined the genesis of the granule cells and their mossy fiber axons. No differences have been found between these strains either in the development of the granule cells, as examined by ^{3}H-thymidine radioautography, or in the temporal appearance of the mossy fiber system, as revealed by the Timm sulphide silver method. In both strains, the formation of the majority of the granule cells occurred between embryonic day 12 and postnatal day 21 in an "outside-in" generation pattern, and the formation of granule cells in the suprapyramidal limb preceded that of those in the infrapyramidal limb. The peak period of granule cell formation was between embryonic day 19 and postnatal day 7 in both strains. In addition, the percentage of the total labeled granule cell population that was labeled on each injection day was nearly identical for BALB/cJ and SM/J specimens. This finding indicated that granule cells were generated at a similar rate in both strains. In Timm-stained preparations, the mossy fibers were first demonstrated unequivocally on postnatal day 6 and the densely-stained mossy fibers were already visible throughout regio inferior. The prominent appearance of mossy fibers at this time suggests that the mossy fiber system was established prior to its earliest detection by the Timm method. Therefore, the reversed generation pattern observed for regio inferior pyramids of BALB/cJ mice is the only major change thus far identified in a developmental event that might produce the connectivity differences seen in the adult, BALB/cJ mossy fiber system. (Supported by U.S.P.H.S. grants NS10284 and 1 F32 NS05310).

358 NEUROCHEMICAL AND ANATOMICAL STUDIES OF NORMAL AND DEAFFERENTED OPTIC LOBES OF THE MOTH *MANDUCA SEXTA*. Gerald D. Maxwell and John G. Hildebrand, Dept. Neurobiol., Harvard Med. Sch., Boston, MA 02115.

The production of acetylcholine (ACh), γ-aminobutyric acid (GABA), and 5-hydroxytryptamine (5HT) by the optic lobes of the moth *Manduca sexta* is large compared with that in other regions of the CNS (Maxwell and Tait, Soc. Neurosci. Abstr. 3:409, 1977). Using our radiochemical screening procedure (J. Neurobiol. 2:231, 1971), we have compared the production of ACh, GABA, and 5HT in a distal portion of the optic lobe, containing photoreceptor terminals and the lamina (first synaptic neuropil), with that in a more proximal portion containing the medulla and lobula complex (second- and third-order neuropils). Under the conditions of our experiments, the labeling of the pools of neurotransmitter candidates was linear with time. At the conclusion of an incubation, the ratio of radiolabeled product in the distal portion of the optic lobe to that in the proximal portion is about 1.5:1 for ACh, about 1:3 for GABA, and about 3:1 for 5HT.

We have also examined the neurochemical and anatomical consequences of deafferentation of the optic lobe by removing the retinal primordia early in the pupal stage. This surgical intervention results in the development of an animal lacking an eye on the operated side. By light microscopy the lamina is not detectable in the operated lobe, and the medulla and lobula complex are present but slightly smaller than those in the control lobe. The pattern of staining with Luxol-fast blue in the medulla is similar on the operated and control sides. In order to determine the distribution of a likely postsynaptic marker (putative ACh receptors that bind α-bungarotoxin) in normal and operated optic lobes, we examined the binding of [^{125}I]α-bungarotoxin to frozen sections by autoradiography (in collaboration with Dr. L. Hall, MIT). The distribution and density of silver grains over the medulla were similar in operated and control lobes. When the production of ACh, GABA, and 5HT is examined in the deafferented optic lobes, it is found that ACh labeling is reduced about 80% relative to the control lobe; GABA, about 20%; and 5HT, about 50%. Labeling of all three products on the control side is comparable to that observed in an optic lobe from an unoperated animal.

These findings will be discussed with reference to the neurotransmitter chemistry and development of the cellular components of the optic lobe of *Manduca*.

This research was supported by an NIH Postdoctoral Fellowship to GDM and NSF Grant BNS 77-13281 to JGH.

359 GOLGI OBSERVATIONS ON MEDIAL FOREBRAIN BUNDLE (MFB) NEURONS IN NEWBORN RATS. Nathaniel T. McMullen and C. Robert Almli. Dept. Psychol., Ohio Univ., Athens, Ohio 45701.

The MFB is located in the ventrolateral diencephalon and basal forebrain and through its massive reciprocal connections with the limbic forebrain and midbrain forms the major longitudinal conduction tract of the hypothalamus. Interstitial neurons of the MFB (lateral preoptic and hypothalamic neurons) are of the reticular-type and are interspersed within a complex and heterogeneous fiber system. Path neurons of the MFB have fusiform or triangular somas with dimensions of approximately 30 x 18 u and 3-5, long, poorly-ramified dendrites which occasionally bear spines. The stout rectilinear dendrites may exceed 500 u in length and are preferentially splayed across the numerous chemically-coded ascending and descending fiber systems making up the MFB. In the present study, path neurons of the MFB from the LPO posterior to the LH at the level of the VMH were examined in newborn rats less than 12 hr old using a variant of the Golgi method. Serial coronal sections through the MFB, 100 u thick, were cut from the brains of 15 male and female rats, dehydrated, and mounted under a cover slip. Well impregnated neurons were drawn at a magnification of 625 using a Wild drawing tube. In these neonatal rats basal forebrain and hypothalamic neurons were undergoing dramatic development. The somas of these cells had average dimensions of 19 x 11 u and therefore have achieved approximately 62% of their adult dimensions. In contrast to the relatively smooth somas present in adult rats, path neurons in the neonates were frequently covered with thick budding processes and hair-like filopodia. The developing dendrites, similar to that seen in adults, were already aligned in a dorsal-ventral direction across the MFB. A wide spectrum of dendritic development was present within the neonatal MFB with some neurons having short stout processes approximately 20 u in length to long straight dendrites exceeding 250 u in length. The most striking feature of these sparsely-branched dendritic processes was their pronounced beaded appearance. Lumpy enlargements of cytoplasm at the tip and shaft of the growing dendrites-terminal and preterminal growth cones-were present in the majority of neurons. Thin hair-like processes were frequently present on the soma and dendrites of these cells. The developing dendrites appear to lose their varicosities and other contour irregularities as they grow in length. There was no evidence of any gradient in dendritic development within the MFB.

360 MEMORY PERFORMANCE ON A STERNBERG SEARCH PARADIGM IN SENESCENT ORGANIC AND FUNCTIONAL DISORDERS. Nancy E. Miller. National Institute of Mental Health, Bethesda, MD 20857

Anecdotal accounts suggest that primary memory remains intact both in advancing age and in conditions of altered brain function. To examine this hypothesis, a choice-R.T. memory scanning task was administered to 108 persons 50 years of age and up referred for psychiatric evaluation. In this procedure, digit strings ranging in length from 1 to 6 digits were displayed in a horizontal linear array for 3 seconds, followed by a 2-second delay. A single digit was then presented and the S was to decide whether the digit had appeared in the preceding sequence. Each S was evaluated for presence of brain dysfunction and for depression. Regardless of diagnostic classification, all Ss manifested a monotonic decline in accuracy as task difficulty increased. While those with brain dysfunction showed the steepest deterioration in performance with increasing digit set size, depressives also manifested significant impairment in accuracy of information retrieval. Correlations between accuracy of response and number of years of education were substantial and highly significant. These findings suggest that traditional assumptions regarding the imperviousness of primary memory to pathology-linked decline in the aged are not substantiated, and that accuracy of response in memory scanning is strongly related to behavioral measures of cortical intactness and amount of formal education.

361 CHANGES IN NEUROPHYSIOLOGICAL TASTE RESPONSES THROUGHOUT DEVELOPMENT IN FETAL, NEWBORN AND ADULT SHEEP. Charlotte M. Mistretta and Robert M. Bradley. Dept. Oral Biol., Sch. Dent., U. Mich., Ann Arbor, MI 48109.

To study changes in neurophysiological taste response characteristics during development, we recorded from 54 chemosensitive units in the solitary complex (nucleus and tractus solitarius) of fetuses, lambs, and adult sheep while stimulating the tongue with chemicals. Using tungsten microelectrodes, 24 single or few-unit records were made in 18 fetuses aged 84-126 days of gestation (term=147 days); 18 records were made in nine lambs aged 20-82 days after birth; and 12 records were made in six adult sheep aged 2-9 years. Twenty ml each of 0.5 NH_4Cl, 0.5M KCl, 0.5M NaCl, 0.5M LiCl, 0.1M citric acid, and 0.01N HCl were used to stimulate the anterior third of the tongue. The tongue was rinsed with 20 ml of distilled water after each stimulation.

With increasing age, taste units responded to more chemicals: in fetuses $\leq$ 108 days, 67% of units responded to only three (NH_4Cl, KCl, citric acid) of the six stimuli, and 0% responded to all six; in fetuses $\geq$ 114 days of gestation, 11% responded to three stimuli, 33% to six; in lambs and adults, 7% responded to three, 60% to six. Only after 114 days of gestation were responses to NaCl and LiCl recorded.

The adapted response frequency reached higher values in lambs and adults (range: 1-115 impulses/sec) than in fetuses (range: 1-47 impulses/sec). As gestational age increased, the pattern of taste responses during stimulation of the fetal tongue with NH_4Cl and KCl also changed, progressing from a rapidly adapting discharge to the more slowly adapting discharge characteristic of lambs and adults. The response discharge during stimulation of the tongue with citric acid was slowly adapting throughout development. Response latency decreased throughout gestation at a rate of about minus 30 msec/day, but adult values were not reached until after birth.

Developmental changes in response frequency, adaptation pattern, and latency most probably relate to maturation of taste synapses and fibers, whereas the increasing range of chemical responsiveness may reflect taste receptor maturation. Responses to stimulation of the tongue with NaCl and LiCl were only recorded in fetuses $\geq$ 114 days of gestation; receptors for these chemicals may be gradually added, or receptors already present may become more sensitive, as the taste cell matures. These results demonstrate that taste function changes throughout development, concomitant with changes in taste bud structure and with altered feeding experience. (Supported by NSF Grant BNS77-09920, and National Institute of Dental Research, N.I.H., Research Career Dev. Award DE00066 to C.M.M.)

362 DEVELOPMENT OF TRIGEMINAL MOTOR NUCLEUS IN CHICK EMBRYO: ELECTRON MICROSCOPIC OBSERVATIONS. Sally A. Moody and Marieta B. Heaton. Dept. Neurosci., Univ. Fla. Coll. Med.,Gainesville, FL. 32610.

Light microscopic analysis of the migration pattern of V motor neuroblasts has raised questions as to whether the different phases of migration can be identified by distinct ultrastructural characteristics. Initial studies have chosen the day 5 embryo because all three stages are distinct (Phase I=medial column, Phase II= intermediate, Phase III= lateral column; Heaton and Moody, Neurosci.Abst. IV,1978).

Migratory cells of the intermediate phase are elongated and often appear bipolar, aligned parallel to dense bundles of processes. The nuclei are elongated and have dispersed, granular chromatin and one to two nucleoli. The cytoplasm is characterized by abundant ribosomal rosettes, a few endoplasmic reticulum profiles and mitochondria. The processes upon which these cells lie have abundant mitochondria, cisternae and microtubules. Ribosomes are present but sparse. Close membrane appositions are found intermittently not only among fibers in a bundle but also between these fibers and the somas and processes of migratory cells. Such intimate contact mat be indicative of contact guidance and/or some ionic interaction via these junctions. Desmosomes and other adhesive junctions were not observed.

Cells with similar contacts to fiber bundles were also observed in the lateral columns. These cells are often seen aligned in chains to the incoming fascicles. They differ from those of the intermediate phase in that they are often rounded or ellipsoid and also contain Golgi apparati. These neuroblasts are tightly apposed to one another and are connected via direct intercellular bridges identical to those described in the developing cerebellum by Das (Cell,Tiss. Res.176: 475,1977). Up to four cells in thin section have been observed linearly linked via these bridges, which are typically seen as strings of round to ellipsoid vesicles separating two cells. No other membrane structures and no organelles, other than a few small particles are seen between the vesicles. The coupled cells are not undergoing mitosis since they left the mitotic cycle several days earlier (3-3 I/2). Still, it is possible that the joined cells are daughter cells which never totally cleaved during their final telophase and have migrated while remaining in an extended mitotic phase. However, no such bridges have been observed among Phase II cells as yet.

Although the temporal occurence of these junctions during the entire migratory process has not yet been determined, these observations suggest a communicative role important to some phase of migration much like that proposed by Loewenstein (Devel. Biol. Supple. 2: 151,1968).

Supported in part by NIMH grant MH-27677

363 AGE-RELATED CHANGES IN AXONAL AND TERMINAL DEGENERATION AFTER FRONTAL CORTEX (FC) LESIONS IN RATS. Elliott Mufson and Donald Stein. Department of Psychology, Clark University, Worcester, MA 01610

Investigations of FC have shown age-related changes in gross morphology as well as neuronal cell loss. Removal of FC in aged rats produces no significant deficits on spatial learning tasks as compared to younger animals (Stein and Firl, 1976). Differences in neuron density of the aged nervous system has been suggested as an explanation for these behavioral findings. Alternatively, age may also produce changes in the pattern of axonal connections associated with FC. There are, however, no investigations of age-related alterations in axonal pathways associated with brain lesions.

Male Fisher (344) strain rats, 3-4 per group, aged 90, 365 and 720 days sustained unilateral FC lesions intended to remove all cortex 2mm anterior to bregma and 3 mm ventral to the cortical surface, whereas 4 animals served as unoperated controls. Following lesion all rats survived from 1-7 days and were processed for histological analysis using the Fink-Heimer I procedure.

Light microscopic analysis of Fink-Heimer processed tissue revealed no observable age-dependent differences in either the quality or quantity of axonal degeneration. All FC lesions produced a pattern of degeneration similar to that described in young adult rats (Leonard, 1969). In contrast to the findings of Leonard (1969), heavy terminal degeneration was observed in the claustrum independent of age. In addition, only the 720 day old control rats showed consistent fiber debris in the stria medullaris, stria terminalis, anterior commissure, corpus callosum and fornix (see Naranjo and Green, 1977). Axonal debris was also seen in the cochlear, vestibular, medial cerebellar, mesencephalic trigeminal and motor trigeminal nuclei. Degeneration was more extensive in fiber tracts.

The age-related loss of axonal connections seen in control tissue suggests that the central nervous system undergoes atrophy which, over time, may be related to deterioration of sensory, motor or cognitive abilities. Finally, the fact that no age-related differences in axonal degeneration were seen using reduced silver staining does not eliminate the possibility that other more subtle techniques, such as radioautography, may reveal differences in axonal connections across ages.

364 PLACODAL AND NEURAL CREST CONTRIBUTIONS TO THE GLOSSOPHARYNGEO-VAGAL COMPLEX STUDIED BY THE METHOD OF INTERSPECIFIC TRANSPLANTATIONS BETWEEN QUAIL AND CHICK EMBRYOS. C.H. Narayanan and Y. Narayanan*, Dept. of Anat., Sch. Med., New Orleans, LA 70119.

Although cranial neural crest has been implicated in the origin of various cranial ganglia, the function of these crest neurons in the seventh and ninth cranial nerves has not been determined. In this preliminary report we present the results of two experimental procedures aimed at elucidating the relative contributions of placodal and neural crest elements of the glossopharyngeo-vagal complex. In the first series, neural crest and adjacent neural folds of caudal hindbrain levels from quail embryo donors was transplanted orthotopically to replace corresponding hindbrain levels of chick embryo hosts. In the second series, unilateral orthotopic transplantation of placodal material from quail embryo donors was made on to chick embryo hosts. Experimental embryos were recovered at representative stages of development. The heads were embedded in paraffin, sectioned and stained by the Feulgen and Rossenbeck's technique for detailed histologic analysis.

Quail cells were found consistently in the root ganglia of the IXth and Xth cranial nerves, indicating their origin from the neural crest and neural fold graft of quail embryo donors. Chick cells were absent in the root ganglia of IX and X. Placodal material from quail embryo donors transplanted to chick embryo hosts end up in the petrosal and nodose ganglia respectively. Quail cells were not observed in the root ganglion of IX and X in any of the experimental embryos of this series. Confirmatory evidence is provided by these experiments for a purely neural crest origin for the root ganglia, and a purely placodal origin for the trunk ganglia of cranial nerves IX and X.

Supported by USPHS Grant DE04258-03.

365 EFFECT OF ALPHA AND BETA BUNGAROTOXIN ON NATURALLY OCCURRING MOTOR NEURON LOSS IN XENOPUS LARVAE. Anthony J. Olek* and Charles Edwards. Neurobiology Research Ctr., Biol. Dept., State University of New York at Albany, Albany, New York 12222

During normal development there is a decrease in the number of motor neurons in the lumbar spinal cord of Xenopus (Hughes A.J. Embryol. Exp. Morph. 9:269 1961). Recently, in embryonic chick spinal cord, the naturally occurring cell loss has been shown to be reduced by the use of neuromuscular blocking agents (Pittman, R.H., Oppenheim, R.W. Nature 271:364 1978). Similar effects have now been found in Xenopus.

Multiple injections of alpha bungarotoxin (α-BTX), which binds to acetylcholine receptors, into the hind limb bud during the period of normal cell loss, produced a significant increase (up to 50%) in the number of motor neurons compared to neuron counts in saline injected control animals. Although one limb was injected with the toxin, the numbers of motor neurons on both sides of the spinal cord were found to be elevated over the numbers found in untreated animals. These injections slow the rate of development of the animal and produce signs of atrophy in the injected limb. After stage 56, a period when normal rapid cell loss is essentially complete, injections of α-BTX were without effect on motor neuron numbers. Further, single injections of α-BTX at various times during the period of cell death also had no effect on cell numbers. The rapid rate of synthesis of acetylcholine receptors in the developing limb may require multiple injections of α-BTX to block receptors continually.

Beta bungarotoxin (β-BTX) is known to block neuromuscular transmission by actions on the presynaptic nerve terminal. Injections of β-BTX into one hind limb bud produced significantly increased motor neuron loss as shown by a comparison of the motor neuron counts in the injected and contralateral side. However the motor neuron counts on both sides of the injected animal were lower than cell counts in untreated control animals. The effect of β-BTX is maximal in animals injected early during the period of cell death. Injections after stage 56, when normal cell death is largely complete, did not produce a change in motor neuron numbers.

The period of cell death in Xenopus may reflect a time in the developmental history of the animal when motor neurons are particularly sensitive to changes in the peripheral environment.

This work has been supported by grants from the Muscular Dystrophy Association and USPHS (NS-07681).

366 EFFECTS OF ANTIBODIES AGAINST NERVE GROWTH FACTOR ON DEVELOPING AND DIFFERENTIATED ADRENERGIC NEURONS. Uwe Otten*, Martin E.Schwab*, Michel Goedert* and Hans Thoenen,(SPON: A.A.Borbély), Dept. of Pharmacology, Biocenter of the University, Basel, Switzerland

There is good evidence that nerve growth factor (NGF) is an absolute prerequisite for the normal development of the peripheral sympathetic nervous system. Administration of a single dose of purified anti-NGF antibodies to newborn rats leads to an extensive destruction of the superior cervical and stellate ganglion, as manifested by a drastic reduction of all norepinephrine-synthesizing enzymes. These biochemical effects seem to appear first in the neuronal cell body and then to extend to the nerve terminals in the heart, the submaxillary and the pineal glands. In contrast the anti-NGF effects in adults were smaller and reversible. Long-term exposure to antibodies against NGF was achieved by repetitive immunization of adult rats with 2.5 S NGF. Morphological analysis revealed a general reduction in the size of the adrenergic neurons which was also reflected at the biochemical level by a 40% decrease in total protein content and a corresponding reduction in all norepinephrine-synthesizing enzymes. Enzyme levels in target organs however,remained unaffected. All these effects were fully reversible. The recovery of normal enzyme activities followed closely the decrease of the antibody titre after cessation of boosting. The shift from the irreversible to the reversible effect is a gradual one and occured between day 12 and 16 after birth. The time-course of the morphological changes after anti-NGF treatment and the fact that NGF-antibodies are also effective in newborn mice genetically deficient in the C5 complement component suggest that immunosympathectomy does not result from a complement-mediated cytotoxic effect. Thus, adrenergic neurons from newborn animals depend on NGF or a cross-reacting NGF-like material for survival, whereas differentiated adrenergic neurons need this factor for the maintenance of their normal function but not for survival.

This work was supported by the SNF (3.432.74)

367 NEONATAL 6-HYDROXYDOPAMINE: EFFECTS ON THE DEVELOPMENT OF THE ACOUSTIC STARTLE RESPONSE AND MATURATION OF SENSORY SYSTEMS. Thomas Parisi*, James R. Ison, and Carol Kellogg (SPON: V. Laties). Dept. Psychology, Univ. of Rochester, Rochester, NY 14627.

In these experiments we investigated the effects of 6-hydroxydopamine (6-OHDA) administered neonatally on the development of acoustic startle behavior in the rat. In normal rats, the acoustic startle response (to a 10K, 110 db tone) can first be elicited at 12 days of age, and its susceptibility to modification by preliminary stimuli grows over the next seven days, attaining adult levels of facilitation and inhibition by 19 days. At short intervals (10 msec), preliminary stimuli facilitate the response. At longer intervals (50-100 msec), the response is inhibited. In the present experiments, rats were injected with 100 mg/kg 6-OHDA s.c. on days 3, 4, and 5 of life, with litter mates receiving vehicle injections. Behavioral testing began at 13 days. Treated animals lagged behind control animals by one or two days in the first appearance of the response. At day 13, all of the control animals (10) responded to the startle tone, whereas only 2 of 10 of the 6-OHDA treated animals did so. However, once the response was elicited, ontogenetic changes in modification by preliminary stimuli were the same for experimental and control groups.

Over days 14 to 18, the latency of the startle response, measured electromyographically, decreased from 15.0 to 11.8 msec in control animals. Preliminary data suggest that 6-OHDA treated animals respond slightly (1 msec) less rapidly at both ages. The retardation in acoustic/motor development seen in these animals was accompanied by a similar lag in eye opening. In normal animals, this was accomplished by 14 or 15 days, with treated animals again lagging about one day behind. Additionally, there was a difference in body weight, the treated animals running about 10% lower than controls. These data are consistent with hypotheses suggesting a role for norepinephrine in the support of developmental processes. Experiments are now in progress examining the relationship between changes in neuronal uptake of ^{3}H-NE produced by 6-OHDA and the behavioral observations.

(Supported by PHS grant NS-12443.)

368 DEVELOPMENTAL APPEARANCE OF ACETYLCHOLINESTERASE MOLECULAR FORMS IN CHICK MUSCLE CULTURE. Michael R. Patterson*, Hugo L. Fernandez and Barry W. Festoff (SPON: D.K. Ziegler). Dept. Neurology, Univ. of Kansas Med. Ctr. and Neurobiology Research Lab, VA Hospital, Kansas City, MO 64128.

Interest has developed recently regarding the presence and functional roles of several molecular forms of acetylcholinesterase (AChE; Hall, J. Neurobiol. 4:343, 1973) in nervous and skeletal muscle tissue. A large molecular weight form was thought to be a "marker" for nerve-muscle contact (Vigny et al, J. Neurochem. 27: 1347, 1976) and is approximately 18S in eel electroplax, 16S in rat and 19.5S in chicken and endplate-enriched regions of skeletal muscle. A recent report suggested that in rat primary muscle culture, 16S AChE only appears after "induction" by neuronal elements (Koenig and Vigny, Nature 271:75, 1978), while 19.5S was not detected in primary cultures of chick muscle (Rotundo and Fambrough, Neurosci. Abst. 3:527, 1977). Prior to assessing whether "induction" might be an in vitro expression of "trophic" dependence on some neural factor(s), we followed the temporal course of total and separate molecular forms of AChE in chick primaries.

11 day old embryos of specific pathogen free white Leghorn eggs were the source of dissociated muscle cultures (Hartzell and Fambrough, Dev. Biol. 30:153, 1973). Total and individual forms of AChE activity were assayed in the horse serum (HS,10%) and chick embryo extract (CEE) used to feed the cultures, and at 2,4,7,9,11, 14 and 16 days in Lubrol-WX extracts. Forms were separated by sedmentation on linear (5-20%) sucrose gradients in an SW41 rotor and activity estimated by a sensitive radiometric assay using BW284C51 as specific inhibitor. 5 molecular forms of AChE were seen as early as 2 days in culture, with gradual increase in activity until day 14 followed by a sharp decline. 6.5S and 11S forms predominated, but 19.5S, 15.5S and 4S were found. Because all forms of AChE gradually increased over the time course and then sharply decreased with no obvious change in morphological appearance of the cultures, we presently feel that exogenous AChE in HS or CEE may not account for their presence. Alternatively, a form of AChE has been shown associated with membrane surfaces in cultured muscle (Wilson et al Dev. Biol. 33:285, 1973) so we cannot exclude that the increase in minor forms with time represents binding of exogenous AChE to increasing numbers of "receptors" in growing myotubes. "Induction" could not account for their presence since cultures were grown aneurally. Experiments are in progress to determine whether these forms are produced by cells in culture or represents binding of AChE to myotube surface receptors.

(Supported in part by Muscular Dystrophy Assn. and the Medical Research Service of the Veterans Administration).

369 COMPARISON OF TRANSNEURONAL CHANGES IN THE AVIAN ECTOMAMILLARY AND VENTRAL LATERAL GENICULATE NUCLEI. J. D. Peduzzi*, E. S. Baron* and W. J. Crossland. Department of Anatomy, Wayne State University, School of Medicine, Detroit, MI 48201

The avian ectomamillary nucleus (EMN) has been reported to undergo extensive transneuronal degeneration following embryonic eye removal while the ventral lateral geniculate nucleus (GLv) has shown only minor transneuronal changes. Following unilateral enucleation on a day of hatching, we studied the volumetric changes in the EMN and GLv at post-operative survival periods of 2 to 80 days. Since the visual pathway is completely decussated in the chick, the nuclei contralateral to the unoperated eye served as controls. In addition some unoperated animals were also examined.

Initially the relative volume ($\frac{\text{experimental volume}}{\text{control volume}}$) of the experimental EMN decreased rapidly then leveled off at 45% of control volume by the 60th day following enucleation. The experimental GLv also decreased rapidly in volume at first but leveled off at approximately 70% of control volume. However, the experimental EMN changed very little in absolute volume during the post-operative period while the experimental GLv increased in absolute volume paralleling a similar increase in the control side.

Cell measurements based on camera lucida drawings of control and experimental EMN cells on the 48th post-operative day reveal both a lower cell number (20%) and a smaller cross-sectional cell area (25%) on the experimental side. Hence the relative reduction of EMN volume results from both cell size differences and a reduction of cell number.

Our findings confirm and extend earlier observations of the sensitivity of the EMN to deafferentation. Furthermore, our results suggest that the mechanism of change may involve an arrest of development rather than, or in addition to, cell atrophy and death.

(Supported by PHS grant EY-01796.)

370 DEVELOPMENT OF DENDRITIC AND SYNAPTIC STRUCTURE FOLLOWING EARLY LEAD EXPOSURE. Ted L. Petit and Janelle C. LeBoutillier*. Dept. Psychology, Univ. Toronto, West Hill, Ont., Canada, M1C 1A4.

Early lead (Pb) exposure in children and experimental animals leads to a neurological sequel frequently culminating in severe intellectual impairment. To determine what neurobiological factors may underlie this behavioral deficiency, the effect of Pb on dendritic and synaptic development was examined in rats.

All litters were reduced to 6 males on the day following birth and the mothers maintained on an ad-lib diet of either ground chow (control group) or ground chow containing 4% lead carbonate (Pb group) from postnatal day 1 (PN1) to PN25. Brain weights were reduced by 12% and neocortical depth by 14% in Pb treated animals.

For analysis of dendritic development, animals were sacrificed on PN25, their brains processed with the Golgi-Cox method, and Layer V sensorimotor neocortical neurons dorsal to the first hippocampal section were drawn and analyzed according to the Scholl method. Fifty neurons were analyzed from each of 8 Pb and 5 control animals (each from different litters). While there was no difference between the groups in the number of dendritic processes leaving the cell body, the Pb neurons had fewer dendritic branches 80 μm and 100 μm from the cell body. The length of the primary apical dendrite was reduced by 5.6%.

Electron microscopic analysis of EPTA stained synaptic structure was carried out in the molecular layer of the visual cortex of 28 day old animals. For analysis of synaptic density, the number of synapses per 15,000 X field were counted. We did not find any significant differences in the synaptic density between the two groups. However, when one considers the 14% reduction in neocortical depth, there would be fewer total synapses in the neocortex of Pb treated animals.

The following synaptic parameters were measured in 50 synapses per animal, 3 animals per group, at 275,000 X: presynaptic length, postsynaptic length, cleft width, presynaptic thickness, and postsynaptic thickness. Although we did not find any group differences on any of the synaptic parameters, there may be a thinning of the presynaptic terminal in some Pb animals. The brains of more animals are being analyzed to confirm this finding.

Therefore, these findings suggest that early Pb exposure reduces dendritic development and the number of synapses, and may reduce the presynaptic terminal in some Pb animals. These deficiencies in brain development may, in part, account for the behavioral deficiencies observed in Pb exposed animals.

This research was supported by Grant #A0292 from the National Research Council of Canada.

371 DEVELOPMENT OF FLAPPING IN WINGLESS CHICKS. Robert R. Provine. Dept. Psych., Univ. Maryland Baltimore County, Baltimore, MD 21228.

The development of "flapping" was studied in chicks that had their wings amputated at the shoulder on the day after hatching. This is a stage before which the wings are feathered and play a role in flight. The experiment was conducted to evaluate the role played by the periphery in the development of the neuronal circuitry responsible for the generation of the stereotyped, bilaterally synchronized motor pattern of wing-flapping. Flapping of wingless chicks was made visible by mounting sections of soda straws on the stumps of the amputated wings. Flapping was evoked by dropping chicks a distance of 5½ ft. using a tethering device and recorded using stroboscopic photography. The flapping frequencies of wingless and intact control chicks were similar at two weeks of age, a stage at which a mature pattern of wing-flapping is present. These data indicate that after the time of hatching peripheral wing structures and wing-flapping experience are not necessary for the development of the basic motor pattern that is involved in flapping.

372 **SEGMENTALLY SELECTIVE INNERVATION OF MAMMALIAN SYMPATHETIC GANGLIA: FURTHER EVIDENCE FOR THE IMPORTANCE OF POSTGANGLIONIC TARGET POSITION.** Dale Purves, Jeff W. Lichtman, and Joseph W. Yip. Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

The influence of postganglionic target position on the innervation of ganglion cells was explored directly in the guinea-pig superior cervical ganglion by determining the segmental innervation of neurons whose axons run in postganglionic nerves to different regions.

Surprisingly, there is little difference in the average segmental innervation of ganglion cells emerging in the two major postganglionic nerves, the inferior and superior branches. Since the branches of the superior nerve join more rostral craniospinal nerves than those of the inferior postganglionic nerve, the segmental innervation of particular superior cervical ganglion cells is not primarily determined by the position of targets along the rostrocaudal axis. There was, however, a marked difference in the segmental innervation of neurons running in the divisions of the inferior nerve to the second and third cervical nerves: on average, ganglion cells whose axons run to C2 receive stronger innervation from caudal spinal segments than ganglion cells whose axons run to C3. This difference is unlikely to reflect selective innervation based on modality since the postganglionic contributions to adjacent cervical nerves would be expected to supply the same spectrum of sympathetic targets. The only obvious difference between these two cervical nerves is their anatomical distribution. C2 supplies targets occupying medial and dorsal positions, but makes only a small contribution to the cervical plexus which innervates the ventrolateral neck. C3, on the other hand, is distributed about equally to ventrolateral, medial, and dorsal regions. The apparent influence of dorsoventral position on segmental innervation was supported by recording sympathetic compound action potentials from the branches of single spinal nerves in response to stimulation of the thoracic ventral roots. Those branches running to dorsal regions showed a stronger response to stimulation of relatively caudal thoracic ventral roots than branches running ventrolaterally to the cervical plexus.

On the basis of the experiments reported in these abstracts, we conclude that the preferential connections between ganglion cells and preganglionic axons arising from different levels of the spinal cord are formed according to some property of individual ganglion cells correlated with the position of their postganglionic targets. Thus segmentally selective innervation of sympathetic ganglia appears to match positional values of pre- and postsynaptic neurons. [Supported by NIH grant NS-11699.]

373 TEMPORARY SYNAPTIC CONTACTS ON TRANSIENT NEURONS IN THE ALAR PLATE OF THE SPINAL CORD IN RHESUS MONKEY EMBRYOS. P.Rakic, E. Knyihar* and B. Csillik,* Dept. of Neuroscience, Children's Hosptial Med. Center, Boston, MA 02115 and Sect. of Neuroanatomy, Yale Univ., Sch. of Med., New Haven, CT 06510

The development of cellular and synaptic relationships in the posterior quadrant of the cervical spinal cord of rhesus monkey embryos was analyzed by electron microscopic and H^3-thymidine autoradiographic methods. The earliest synapses are found in the alar (sensory) plate on the 31st embryonic day (E31) of the 165 day gestation period in rhesus monkey. These synapses are located in the marginal zone in the region of the prospective posterior columns. The apposed pre-and postsynaptic thickenings are asymmetrical and separated by a 300 A gap. Small, round, clear vessels cluster at presynaptic sites. Electron microscopic montages and reconstructions from serial sections demonstrate that these earliest contacts occur between longitudinally oriented sensory afferent axons and either the borderline cells situated at the interface between the marginal and mantle layers or the leading processes of cells lying deeper within the mantle. H^3-thymidine autoradiographic analysis shows that the permanent neurons, have not yet been generated at this stage and thus are not involved in the initial period of spinal cord synaptogenesis.

The borderline cells undergo changes in position and morphology over the next three weeks of embryonic spinal cord development. By E40, the cells lie fully within the dorsal columns and become surrounded by fascicles of newly arrived afferent axons. Around E50, many borderline cells show cytological evidence of degeneration; some disintegrate and begin to disappear. Some dying cells retain synaptic contacts with apparently healthy axons. No borderline cells can be detected in the posterior columns at later developmental stages or in the adult. Thus, borderline cells seem to represent a transient cell population that forms temporary synaptic contacts during early embryonic stages. The fate of the axons that terminated on these cells is uncertain. At least some appear to enter the posterior horn following death of their primary target cells and terminate on the permanent substantia gelatinosa neurons that begin to be generated around E38, a few days before borderline cells start to degenerate. Thus, the complex synaptic architecture of the substantia gelatinosa in primates may be developed through a considerable rearrangement of cellular and synaptic relationships involving the death of an entire population of neurons and the translocation of its synaptic connections.

Supported by NIH grants NS14841 and NS12200.

374 BLOOD-BRAIN BARRIER IN AGED RATS. Stanley I. Rapoport, Kikuo Ohno* and Karen D. Pettigrew. Lab. Neurosciences, Natl. Inst. of Aging, Balt., Md. 21224 and Theoret. Stat. Branch, Natl. Inst. o Mental Hlth. Bethesda, Md. 20014.

Cerebrovascular permeability to ^{14}C-sucrose and other water-soluble nonelectrolytes normally is extremely low and equal to nonelectrolyte permeability in aporous, bimolecular lipid membranes (Ohno et al., Am. J. Physiol., in press). It has been suggested that cerebrovascular permeability increases with age, so that brain-reactive protein antibodies can enter the brain through the vasculature and damage brain cells. In order to test this hypothesis, we used the method of Ohno et al. (op. cit.) to measure cerebrovascular permeability to ^{14}C-sucrose in 3-month and 28-month old restrained, conscious Fisher 344 male rats. 5 μC of tracer was injected intravenously. Tracer concentration in arterial plasma was followed until the rat was decapitated 50, 100 or 240 min after injection. Parenchymal tracer concentration, C_{brain}, was obtained by subtracting intravascular tracer (product of blood concentration and brain blood volume) from net regional brain radioactivity. It was assumed that tracer entered brain as follows (1): $dC_{brain}/dt = PA\ (C_{plasma} - C_{brain}/V_e)$, where PA = capillary permeability-area product (sec^{-1}), V_e = cerebral distribution space of ^{14}C-sucrose, t=time, C_{brain} = brain concentration (dpm/g) and C_{plasma} = plasma concentration(dpm/ml). Plasma concentration was represented by the following equation (2),

$$C_{plasma} = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} \ldots\ldots,$$

which when inserted in Eq. gives C_{brain} (for i = 1,2,...)

$$C_{brain} = PA\left[\frac{A_i}{PA/V_e - k_i}\left(e^{-k_i t} - e^{-PAt/V_e}\right) + \ldots\ldots\right]$$

Eq. 3 was fit by computer to concentration data to obtain PA and V_e. PA in 3 month-old rats averaged 7.53 x 10^{-6} sec^{-1} in 14 brain regions and was not elevated in brains of 28-month old rats, except possibly at white matter and then by not more than 50%. Furthermore, Ve fell from an average of 0.126 in young animals to 0.070 in 28-month old rats. This 44% reduction probably reflected a reduced brain extracellular space in the older animals, although other interpretations are possible. The findings indicate that cerebrovascular permeability is normal in old rats, but that the brain extracellular space may be reduced.

375 FUNCTIONAL UNCOUPLING OF AN IDENTIFIED ELECTRICAL SYNAPSE: DEVELOPMENTAL MODULATION OF SYNAPTIC TRANSMISSION IN *APLYSIA*. Stephen Rayport* and Eric R. Kandel (SPON: V. Castellucci). Div. of Neurobiology & Behavior, Depts. Physiology & Psychiatry, Columbia University, P & S, New York, N.Y. 10032.

Recent studies of neuronal plasticity and of the cellular concomitants of simple forms of learning have indicated that chemical synapses differ from electrical synapses in being able to undergo profound and prolonged changes in effectiveness. For example certain types of chemical synapses become functionally inactivated for a week or more following long term habituation training (Castellucci *et al.*, in press). In contrast, electrical synapses between neurons are thought to be relatively non-plastic in post-embryonic life and to undergo only brief modulation consequent to conductance changes in the adjacent electrically excitable membrane (Spira & Bennett, Brain Res. 37:294, 1972).

We here describe a profound change in the functional effectiveness of an identified electrical synapse in *Aplysia* between the two giant cells, R2 in the abdominal ganglion and P1 in the left pleural ganglion, during a specific stage of development. We find that these two cells are tightly coupled electrically until a late stage (stage 11) in post-embryonic life and then become functionally uncoupled during a period of about one week.

Until stage 11, action potentials in either cell lead to one-for-one firing of the other cell. Depending on slight variations in thresholds, either cell may lead in response to common input. DC current flow is symmetrical from one cell to the other with a coupling factor of about 0.3. Loss of one-for-one firing occurs at the transition between stages 11 and 12. In parallel, the coupling factor decreases to about 0.1 and the input impedance of the cells drops by an order of magnitude. In the young adult, stage 13, the coupling factor becomes unmeasurable, and all that remains is a less than one mv biphasic PSP in P1 following an R2 spike, characteristic of the adult (Meunier & Tauc, Arch. Ital. Biol., 111:305, 1973).

This phenomenon is unlike the generalized coupling and uncoupling that occurs between all cells during embryonic development (Potter, Furshpan & Lennox, PNAS 55:328, 1966). Since R2 and P1 develop in separate ganglia, the two axons are presumably not initially coupled but rather seek each other out to form this very effective connection. The R2-P1 junction persists throughout the animal's life but becomes functionally uncoupled in adulthood. This finding provides a functional parallel in an electrical synapse to the synaptic uncoupling of a chemical synapse considered in the companion abstract, and suggests that certain types of synaptic contacts may have ontogenic or behavioral roles restricted to specific periods of development.

376 INFLUENCE OF UNDERNUTRITION ON THE DEVELOPMENT OF THE SLEEP - WAKE CYCLE OF RATS. José A. Rojas-Ramírez* and Ana Ma. Martínez-Rivas* (SPON: R. R. Drucker-Colín). Depto. Farmacología, Fac. Medicina, UNAM, México 20, D.F. México.

Since relationships between protein synthesis and sleep mechanisms seems to exist, it was decided to explore the effects of undernutrition on the sleep-wake cycle of rats. Fourteen pregnant rats at term were separated in two groups. The method of different numbers of litters was followed to induce undernutrition in the pups. One group of seven mothers (normally nourished, N) were allowed to rear 6-8 pups. The other group of seven mothers (undernourished, U) were allowed to rear 16-18 pups. These conditions were kept up until weaning (21-22 days after birth). From each litter a pup was retired at 6-9 (1st week), 14-16 (2nd week), and 21-23 (3rd week) days after birth for recording. After weaning, all pups were individually caged and those from the U group were provided with half amount of nutriments than those of the N group. On days 28-32 (4th week) and 60-64 (8th week) after birth another rat from each group was recorded. Needle electrodes on fronto-parietal areas and wire electrodes into the neck muscles were permanently implanted to record EEG and EMG respectively. Records were made in a 7B Grass polygraph keeping the animals within individual cages. Seven rats were studied for each group and age. In all cases visual observations were made along recordings during eight diurnal hours. Wake (W), slow wave sleep (SWS), and REM sleep (REM) states were measured and compared among groups. Results showed that sleep and wake times practically remained unaffected on the 1st and 2nd weeks. W time was significantly higher and SWS time lower in U group compared with N group on the 3rd week; REM time did not change. Similar changes for W and SWS times appeared at the 4th week, but in addition, U animals lasted less time in REM than N rats. At the 8th week the only parameter affected was REM time which was lower in U group. This study shows that undernutrition from birth is able to alter normal development of sleep-wake cycle, being REM the state that apparently endures permanent changes, and support the hypothesis that relates sleep with nutritional factors.

377 MATURATION OF COCHLEAR POTENTIALS IN THE RAT, AND THE EFFECT OF KANAMYCIN OF THE ONSET OF THE AUDITORY FUNCTION.

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Maturation of cochlear potentials has been studied in 40 rat pups. Two potentials have been recorded on the round window, such as the cochlear microphonic (CM) and the compound action potential (AP). The CM has been obtained with tone bursts stimulation at various frequencies and the AP after clics and filtered clics stimulation of 0.5, 1, 2, 4 and 8 kHz. For each frequency, the input-output curves for amplitudes and latencies have been analysed. The CM has been recorded on the 8th postnatal day and the AP 5 days later. Adults values have been obtained at the end of the second week for the CM, and during the fourth postnatal week for the AP. Compared with others mammals such as the cat, rats show a faster cochlear potentials maturation.

On this model of maturation, the effect of kanamycin has been tested in relation with the period of administration after birth. Results show a clear ototoxic effect on the cochlear potentials when the treatment is performed during the second postnatal week. In contrast, if the treatment is achieved during the first postnatal week, none effect can be demonstrated. This result suggests a relationship of the ototoxic effect of the drug with the onset of the auditory function.

378 LIFESPAN CHANGES IN THE DIURNAL SLEEP PATTERN OF RATS. Richard S. Rosenberg and Allan Rechtschaffen* Sleep Laboratory, University of Chicago, Chicago Ill. 60637.

Awakenings during the night and naps during the day increase dramatically with advanced age in humans. These changes describe a decrease of the diurnal sleep rhythm amplitude. In a previous study (Sleep Res. 5:88, 1976) decreases in sleep rhythm amplitude with age were found in rats, but each was recorded for only a single day. The present study reexamines age-related changes in rat sleep in more detail with much longer recordings.

Eight male Sprague Dawley rats were recorded in a 12:12 L:D schedule for 10 days each. Three groups were distinguished: 4-8 months (Young), 15-17 months (Middle), and 25-28 months (Old). Measures of rhythm amplitude similar to the "A" statistic of Borbely (Brain Res. 95:89-101, 1975) were obtained using a moving 12 hour window which detected the maximal 24 hour rhythm amplitude in individual animals. In general, these rhythms corresponded to the L:D cycle. Rhythms of waking (W), NREM sleep (S), absolute amount of paradoxical sleep (P), and delta activity (Δ) were measured. Length of sleep bouts and latencies from S onset to P were scored for the last five recording days.

TABLE: Changes in rhythm amplitude measures with age.

	Young	Middle	Old
W	35.54	31.80	25.22
S	22.18	24.01	11.51
P	25.72	21.72	24.80
Δ	59.24	57.36	48.27

Diurnal rhythm amplitudes W, S, and remained fairly stable up to about 18 months of age and then dropped sharply (see Table). Paradoxical sleep rhythms, however, were stable in amplitude across the lifespan. The day-to-day variability of all rhythm amplitudes increased with age. Bout lengths and P latencies tended to decrease with age, but individual differences were large.

Six animals were recorded for 20 additional days in constant dark, and the S rhythms were analyzed with a modification of the Enright periodogram. Young rats showed expected free-running rhythms with period length near 24 hours. Rhythm amplitudes of the old rats were so low that reliable estimates of circadian period length could not be obtained. An ultradian rhythm with period length of three to six hours was prominent in both the cycling and constant environments in old rats.

Decreases in diurnal sleep and waking rhythm amplitudes and increases in day-to-day variability suggest that the rat may provide a model of some of the changes in human sleep patterns with age. Preliminary results in three animals indicate that reentrainment following 180° phase shifts of the light:dark cycle was more rapid in old rats, suggesting that the rhythms seen in the cycling environment are more driven than entrained.

379 EFFECTS OF AGING AND HYPOXIA ON LEVELS OF CATECHOLAMINES IN RAT BRAIN REGIONS. Isaac F. Roubein and Larry J. Embree, Veterans Administration Hospital and Department of Neurology, Louisiana State University Medical Center, Shreveport, Louisiana 71130.

Central catecholamine (CA) neurons through modulation of synaptic transmission and the brain's vasculature may play a key role in the regulation of cerebral metabolism, activity and reactivity. Age-related changes in catecholamines have been reported in the brains of different species. The brain however does not respond uniformly to aging, hypoxia or other insults, hence this study was undertaken to investigate the effects of aging and of aging and hypoxia on the level of catecholamines in seven discrete brain regions which are known to have varying vulnerability and functions.

Male Sprague-Dawley rats (15 months and 20 months old) were sacrificed by decapitation, the brains were removed and immediately dissected into the following regions: cerebral cortex, striatum, midbrain, cerebellum, pons and medulla, hypothalamus and hippocampus.

Spectrofluorometric determinations of norepinephrine (NE) in cerebral cortex, midbrain, cerebellum, pons and medulla, hypothalamus and hippocampus and of dopamine (DA) in striatum indicated that the level of NE was reduced in the cerebellum, midbrain, pons and medulla, and hypothalamus of aged animals when compared with young adult rats (3 months old). Further, our findings suggest that the changes in catecholamine levels in rat brain may begin at mid-age. If similar changes in catecholamine levels are confirmed in man the possibility exists for a pharmacological approach to the amelioration of some of the signs and symptoms of senile brain disease.

The effect of hypoxia on the level of CA in the seven brain regions from aged rat is currently under investigation and will be reported.

Supported by the Medical Research Service of the Veterans Administration.

380 LOCATION OF MOTOR AND SENSORY NEURONS SUPPLYING IDENTIFIED MUSCLES IN NORMAL AND SUPERNUMERARY HINDLIMBS IN THE FROG (XENOPUS LAEVIS). David I. Rubin and Lorne Mendell. Depts. Anatomy and Physiology, Duke Univ. Med. Ctr., Durham, NC 27710.

Transplanted supernumerary hindlimbs in the frog (Xenopus) show movement coordinated with normal limbs, but are innervated by a separate set of motoneurons. The purpose of this study was to investigate the location of motoneurons innervating muscles in the normal and extra limbs. The location of motoneurons supplying identified muscles was determined by retrograde transport of horseradish peroxidase (HRP) after injection into the appropriate muscle.

Peroxidase activity was demonstrated by an enzyme method described by Hanker et al. (Histochem J. 9, 1977) in which p-phenylenediamine (PPD), a benzene derivative, and pyrocatechol (PC), a phenol, are used as substrates. This mixture yielded brown reaction granules which were more abundant and more readily visible in brightfield than when diaminobenzidene was used as substrate.

The location of motoneurons innervating extensor and flexor muscles in the leg and thigh was investigated in normal and extra leg animals. The position of each labeled motoneuron within the lateral motor column was plotted along the mediolateral and rostrocaudal axes. In normal hindlimbs, motoneurons supplying gastrocnemius, an ankle extensor, are located at the medial end of the motor column; those innervating tibialis anterior, an ankle flexor, lie at the lateral end. In each extra leg animal, HRP was injected into an identified muscle in the extra leg, and the corresponding muscle in the contralateral normal leg. Motoneurons supplying gastrocnemius in extra limbs lie in the same mediolateral position as those for normal gastrocnemius. Extra leg tibialis anterior motoneurons also lie in the same region as for the normal muscle.

Along the rostrocaudal axis, groups of motoneurons supplying different muscles are normally overlapping, so detection of changes in extra leg animals is difficult. Motoneurons supplying extra leg muscles, however, did appear to be slightly shifted toward the segment of innervation.

Counts of dorsal root ganglion cells and motoneurons revealed increases on the experimental side in most cases. For motoneurons, the increases were distributed differently in different animals (cf. Hollyday and Hamburger, J. Comp. Neurol. 170, 1976). For dorsal root ganglion cells, those ganglia supplying the normal limb on the experimental side always showed an increase in cell number; cell numbers in ganglia supplying the extra limb were more variable, and frequently showed a decrease. (Supported by NIH.)

381 UNCOUPLING OF AN IDENTIFIED CHEMICAL SYNAPSE: TRANSIENT APPEARANCE OF MORPHOLOGICALLY IDENTIFIABLE CONTACTS ON THE SOMA OF DEVELOPING NEURONS IN *APLYSIA*. Samuel Schacher* and Eric R. Kandel (SPON: K. Pfenninger). Div. of Neurobiology & Behavior, Depts. of Physiology & Anatomy, Columbia Univ., P & S, New York N.Y. 10032.

We previously described axo-somatic synaptic contacts which are unique to the early developing nervous system in *Aplysia* (Schacher & Kandel, Neuroscience Abstracts, 1977). Since these synapses are absent in adult animals, they may represent initial contacts that later "move" to more appropriate sites on the postsynaptic cell, or they may represent contacts that are later retracted. We here report that 1) these contacts retract, and 2) perhaps trigger profound morphological changes in the postsynaptic neuronal cell bodies.

Prior to contact, the postsynaptic cells contain few of the characteristic morphological features of mature neurons. The cells often have irregularly shaped nuclei containing large amounts of heterochromatin, numerous glycogen particles, few mitochondria, free ribosomes, and a poorly developed or no Golgi apparatus. In addition, the cells do not as yet have axons. Following the appearance of the contact, the neuronal nuclei round up, rough ER and Golgi sacs become evident, and axon formation begins.

In order to link the transient appearance of a soma contact with the changes in the postsynaptic cell, we have followed the time course of formation and retraction of a specific axo-somatic contact on an abdominal ganglion cell that can be reliably identified from animal to animal in the same and succeeding developmental stages. We found that 1) the axo-somatic contact invariably occurs on the same topographic region of the cell body; 2) the contact is always present at a particular time in development; 3) the contact is not present on any portion of the cell after the initiation of axon process formation.

These findings suggest that transient chemical synaptic contacts can be formed in development to mediate specific inductive signals critical for aspects of neuronal differentiation. As will be considered in the accompanying abstract, electrical synapses can also be formed and functionally uncoupled during development.

382 DECREASE IN ADRENERGIC AXON SPROUTING IN THE SENESCENT RAT. Stephen W. Scheff, Larry S. Benardo* and Carl W. Cotman (SPON: James L. McGaugh). Dept. Psychobiology, Univ. Calif., Irvine, CA 92717.

In the present study we investigated the capacity of adrenergic neurons to sprout in the septum and hippocampus of aged (26-31 month old) and young (3-6 month old)Sprague-Dawley rats. Axon sprouting of adrenergic neurons innervating the rat septal and hippocampal area was studied following a unilateral transection of the fimbria using a modified glyoxylic acid histofluorescence method for the cellular localization of monoamines. Both young adult and aged animals were subjected to a unilateral lesion of the fimbria-fornix which denervates portions of the septal area and hippocampus. In the septal area of both age groups we observed morphological changes in the catecholaminergic innervation in response to denervation. Thirty to sixty days after fimbria transection the most marked increase in noradrenergic innervation was found in those areas of the septum which receive the heaviest projections from the hippocampus. However, while aged animals with equivalent lesions showed a reinnervation pattern qualitatively similar to that of the younger animals, it was much less extensive.

The response of sympathetic catecholaminergic fibers in the hippocampus following transection of the fimbria was also examined. Previously it has been reported that fimbrial transections induce the growth of sympathetic catecholamine neurons to innervate the dentate gyrus. In four aged animals we found no indication of the anomalous innervation by the superior cervical ganglion. In one aged animal we observed a very minor reaction. These results indicate that axon sprouting of central catecholaminergic systems does occur in aged animals but to a much lesser extent than that in young adult animals. This may indicate that as neurons die with age reactive synaptic growth may provide an ongoing mechanism to replace those connections lost and thereby maintain synaptic populations in the wake of neuronal loss. If the new connections will functionally substitute for the original ones axon sprouting would serve as a homeostatic mechanism against neuronal loss. On the other hand, if the connections are inappropriate such processes could slowly and progressively disorder circuitry further hindering the processing of information in the aged brain. This might contribute to the deterioration of function with age. (Supported by grant AG00538)

383 GRANULE CELL NEURONS IN DEVELOPING RAT AND MONKEY CEREBELLUM CHANGE FROM GLIAL TO NEURONAL ENOLASE. D.E. Schmechel*, M.W. Brightman*, P.J. Marangos*, and I.J. Kopin. NIMH and NINCDS, Bethesda, Maryland 20014.

Two distinct isoenzymes of the glycolytic enzyme enolase (E.C. 4.2.1.11) exist in mammalian brain: neuron-specific enolase (NSE) found only in neurons and non-neuronal enolase (NNE) present in glial cells (Schmechel et al., Science 199: 313, 1978). During early development of rat brain, NNE levels are higher than NSE whereas in the adult the two isoenzymes are present in roughly equivalent amounts. In rat cerebellum, NSE only attains near-adult levels at one month of age.

In the present study, immunocytochemical localization of NSE and NNE in developing cerebellum was studied in a series of early postnatal rat brains and a rhesus monkey fetus (Macacca mulatta) at 100 days of gestation (E100) using the unlabeled antibody enzyme method of Sternberger. Antisera directed against purified rat NSE and NNE were used for rat and antisera against purified human NSE and NNE for monkey. In both rat and monkey cerebellum, cells of the external granule cell layer, a subventricular zone derivative which generates granule cell neurons, contain NNE but not NSE. Migrating granule cells located in the molecular layer also did not contain NSE despite the presence of immunoreactive NSE in the adjacent parallel fiber axons of the molecular layer and granule cells already present in the internal granule layer. In postnatal rat brain as well as the E100 monkey fetus, Purkinje cells and deep cerebellar neurons are uniformly NSE-positive. Likewise, NNE stains Bergmann glial cells and other glial elements as well as the external granule layer.

These results indicate that granule cell neurons of both rat and monkey are derived from cells which contain NNE--the glial isoenzyme. Significant amounts of NSE are still absent in the granule cells during elaboration of their parallel fiber axons and migration inward and only appear when the granule cell reaches its final destination in the underlying internal granule layer. The switch in production of glial enolase (NNE) to the separate gene product neuron-specific enolase (NSE) apparently occurs during migration or soon after arrival in the molecular layer.

The appearance of neuron-specific enolase (NSE), is therefore, a relatively late event in the differentiation of granule cells of the mammalian cerebellum.

384 DEVELOPMENT OF NEOSTRIATAL ACETYLCHOLINESTERASE (AChE) AND DOPAMINE (DA) CORRELATED WITH THE ONTOGENY OF MOTOR BEHAVIORS IN THE RAT. Cheryll A. Smith (1) and Larry L. Butcher (1,2). Dept. of Psychology (1) and Brain Res. Inst. (2), U.C.L.A., Los Angeles, California 90024.

Developmental studies are particularly useful in elucidating the neurochemical bases of motor behaviors since the ontogeny of such behaviors can be readily correlated with the maturation of neurobiological systems. We have focused our attention in this regard on the neostriatum (caudate-putamen complex) because that structure is known to play an important role in modulating movement and is rich in neurochemicals (e.g., AChE and DA) for which sensitive histochemical techniques are available.

The development of various motor behaviors (e.g., locomotion, exploration, and grooming) in albino rats aged 1-24 days were observed and compared to the ontogeny of these same behaviors in rats of the same ages given 20mg/kg of the muscarinic blocker atropine every 8 hours for 24 days. The forebrains of animals of different ages from both normal and atropinized groups were then processed histochemically for AChE and DA.

Many of the motor behaviors exhibited by the normal animals progressively increased in frequency until approximately 15 days postnatally, after which there was a decline to the lower, adult frequencies. In the animals treated with atropine, however, many of the same behaviors reached a higher incidence of occurrence than in normal rats and displayed no decline in frequency throughout the 24 day testing period. Our histochemical data suggest that in the neostriatum the DA system attains an adult distribution pattern earlier than does the AChE system: the former appears to reach the adult pattern between 12 and 15 days of age, whereas the latter achieves such a pattern between 15 and 17 days of age. The final maturation of the AChE system is concurrent with both a prominent upsurge in rat striatal acetylcholine (S.H. Butcher et al., submitted) and the decline in motor behavior frequencies noted in normal animals. These findings support the idea that central DA sytems facilitate, while central cholinergic systems inhibit, motor behaviors and suggest an interaction between these systems in behavioral development.

(This research supported by USPHS grant NS 10928 to L.L.B.)

385 POSTNATAL DEVELOPMENT OF RETINOGENICULATE PROJECTIONS IN SYRIAN HAMSTERS: AN ANTEROGRADE HRP STUDY. Kwok-fai So* and Gerald E. Schneider. Department of Psychology, M.I.T., Cambridge, MA 02139.

In a study of the early development of the retinal projections (Brain Res.'78,142:343), our ability to detect the early appearance of terminating axons was limited by the relatively higher background radioactivity in younger compared to older animals. In order to overcome this problem, we have used horseradish peroxidase (HRP) histochemistry as an anterograde tracing technique. One eye in each of 29 animals was injected with 0.4 mg of HRP dissolved in 0.5 µl of Tris buffer (pH 8.6) at various postnatal ages. After 20 hr survival, each brain was processed using a sensitive HRP method with tetramethyl benzidene as chromogen (Mesulam and Rosene, Neurosci. Lett.,'77,5:7). In every case but 2, the optic tract and its terminals were clearly labeled with HRP reaction products, with an almost total absence of background labeling.

On day 0, axons have already begun to penetrate the nucleus from the superficial optic tract on both sides of the brain, but they have penetrated much more extensively in the mid-portion of the nucleus than at the dorsal and ventral edges. Thus, if one assumes that at this early age the mid-portion of LGBd is innervated by the central retina as in the adult, then the leading fibers appear to originate in the central retina. This finding is similar to that reported for the contralateral projection to the optic tectum of the chick by Crossland, Cowan and Rogers (Brain Res.,'75,91:1). The ipsilateral fibers, which had not been detected at this age with the autoradiographic technique, appear to lag slightly behind the most advanced contralateral ones, since they have reached only halfway into the nucleus, while the contralateral axons are seen at the medial margins.

Retinogeniculate axons distribute throughout the entire contralateral nucleus by day 1, but they remain sparser at the medial edge until day 4. The dorsal part of the ipsilateral nucleus has become completely penetrated by labeled fibers on day 3; beginning at this age, the labeling is denser at the medial edge, indicating the onset of an adult-like pattern of terminal arborization.

By day 7-8, the density and distribution of ipsilateral fibers in LGBd are similar to that seen in the adult animal. Confirming the autoradiographic study,the contra- and ipsilateral fibers overlap in LGBd until day 7. On day 7, partial segregation of the fibers from the two eyes is observed. By day 8, the nearly complete segregation of the contra- and ipsilateral fibers in LGBd closely resembles that of the adult.

Supported by NIH grant # EY00126 and NASA

386 ULTRASTRUCTURE OF THE NIGROSTRIATAL SYSTEM IN EARLY PRENATAL RAT BRAIN BY IMMUNOCYTOCHEMICAL LOCALIZATION OF TYROSINE HYDROXYLASE. L.A.Specht, V.M.Pickel, T.H.Joh, and D.J.Reis Lab. of Neurobiol., Cornell Univ. Med. Coll., New York, NY 10021.

By the immunocytochemical localization of the catecholamine synthesizing enzyme tyrosine hydroxylase (TH) for light microscopy, we have described the anlage of the nigrostriatal system at the end of the second week of gestation in the fetal rat (Neurosci. Abs. 3:369, 1977). In the present study, we sought to determine (a) the ultrastructure of the earliest appearing catecholaminergic perikarya of the substantia nigra (SN) and their processes which project to the caudate nucleus (CN), and (b) the relationship of the primitive TH containing distal proceses to cells of the CN. Sections of fixed brain from rat fetuses of embryonic day 13, 14, and 15 were incubated with specific antiserum to TH and immunocytochemically labeled for electron microscopy. Labeled perikarya of the SN had a lobulated nucleus surrounded by a scant rim of cytoplasm containing numerous free ribosomes, an immature Golgi apparatus, scattered mitochondria, a few saccules of granular endoplasmic reticulum, and occasional centrioles and multivesicular bodies. Labeled proximal processes of the nigrostriatal bundle contained ribosomes, saccules of smooth membrane, occasional mitochondria and microtubules, and small (300-500Å) round particles. Two populations of distal processes containing TH were examined in the rudimentary CN. One group was located in the ventral lateral CN and could be traced to the neurons of the SN. These processes were adjacent to unlabeled neuronal perikarya and processes, and did not form any specialized contacts. The second group of labeled distal processes was located along the ventricular lumen of the CN and could not be followed for more than 4µ; thus, their origin was uncertain. These processes were adjacent to mitotic ventricular cells and apical processes of the non-mitotic ventricular cells. Gap junctions of 70Å or less were often observed with the apical processes, and less frequently with the mitotic cells. Both groups of labeled distal processes contained organelles similar to those observed in the proximal processes. The application of the immunocytochemical technique for the localization of the catecholamine synthesizing enzyme TH to electron microscopic analysis in the fetal brain, has permitted the fine structural characterization of the chemically homogeneous group of neuroblasts of the SN, including the relationship of their earliest appearing distal processes to neuronal elements of the CN. The presence of TH within perikarya of the SN and their processes in the CN prior to the functional maturation of this system, and the association of TH containing processes with mitotic ventricular cells, are consistent with a possible regulatory role of catecholamines in early central nervous system development. (Supp: MH05651, NS06911, HL18974, RCDA MH00078).

387 PHYSIOLOGICAL DEVELOPMENT OF IDENTIFIED NEURONS FROM AN IDENTIFIED NEUROBLAST DURING GRASSHOPPER EMBRYOGENESIS. N.C. Spitzer and C.S. Goodman* (SPON: S. Barondes). Dept. of Biology, UCSD, La Jolla Ca. 92093.

We are investigating the cessation of electrical coupling and the onset of electrical excitability during the differentiation of a class of identified neurons in embryos of the grasshopper Schistocerca nitens, by intracellular recordings and dye injections of cells visualized with interference contrast optics.

In the thoracic ganglia, the dorsal unpaired median (DUM) neuroblast initially gives rise to two chains of DUM mitotic daughter cells. These chains extend anteriorly, and the DUM daughters divide again to produce specific identified DUM neurons. The process of temporal differentiation for a single DUM descendent cell is represented spatially in the chain of cells extending anteriorly from the DUM neuroblast at any one time. Neuronal differentiation of cells increases anteriorly with distance from the DUM neuroblast. In a 12 day embryo, the DUM neuroblast is still dividing and is surrounded by a string of daughter cells. These cells are electrically coupled, dye coupled (Lucifer Yellow, 450 MW), and electrically inexcitable; they have resting potentials of -70 to -80 mV and are not depolarized by 10^{-5} gm/ml veratridine, which specifically depolarizes cells possessing voltage dependent Na^+ channels. The cells more anterior along the chains become electrically uncoupled, dye uncoupled, and the input resistance increases. The next anterior cells begin to grow axons, and soon thereafter the axons become capable of carrying action potentials in which the inward current is carried predominantly by Na^+, since they are rapidly abolished by replacement of this ion or addition of 10^{-9} gm/ml TTX. Furthermore, these cells, like those still further anterior along the chains, are rapidly depolarized by more than 25 mV by exposure to veratridine; prior removal of Na^+ or addition of TTX prevents this depolarization.

Cells at the ends of the chains have -40 to -55 mV resting potentials and produce overshooting action potentials, 2-4 msec in duration. The inward current of the soma spike is carried by both Na^+ and Ca^{2+}; it persists in either the absence of Na^+ or the presence of Co^{2+}, but is blocked by both treatments applied simultaneously. Action potentials in a mature DUM neuron require the presence of both Na^+ and Ca^{2+} (Goodman & Heitler, Soc. Neurosci. 3, 426, 1977).

The onset of axonal outgrowth and electrical excitability occur after the disappearance of electrical coupling, but it is not known whether or not uncoupling is causally related to the other aspects of differentiation.

(Supported by NSF, NIH, The Sloan and Helen Hay Whitney Foundations.)

388 TIME OF ORIGIN OF NEURONS IN NUCLEUS CUNEATUS LATERALIS IN THE MOUSE. Elizabeth Taber Pierce, Seri Slastad* and Inger J. Onshus*. Dept. Anat., Harvard Med. Sch., Boston, MA 02115

Female mice Balb c/Gn were mated to SJL males. The pregnant mice were injected with tritiated thymidine, 5 uC/gm body weight. A series of off-spring was obtained pulse labeled with triated thymidine at a known hour during gestation. The brain of each off-spring was taken at two months after birth and processed by the autoradiograph technique to obtain data on the time of origin of neurons in the brain stem. The position of labeled neurons within specific nuclei was plotted by camera lucida from Nissl stained sections cut at 10 u in the transverse plane. For each day of gestation studied, every 5th section was plotted at a magnification of 200x. It has been determined that neurons within the nucleus cuneatus lateralis are born within the hours 255-294. Both large and small neurons arise at the same time. The peak time of neuron formation was observed at 281 hours. No definitive gradient, rostral to caudal, or medial to lateral, was observed.

389 EXPRESSION OF CATECHOLAMINE BIOSYNTHETIC ENZYMES DURING DEVELOPMENT OF THE AUTONOMIC NERVOUS SYSTEM

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After its detachment from the neural axis, the prospective sympathetic neurons migrate ventrally and eventually give rise to the sympathetic chain, paraganglia and adrenal medulla. We sought to determine, applying immunohistochemical techniques for visualization of the catecholamine (CA) biosynthetic enzymes tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT): (a) at what point in their ventral migration these different enzymes appear, (b) if they do so in a sequential or in a simultaneous fashion, and (c) whether PNMT, which is principally, but not exclusively, found in the adrenal medulla, is detected only in those sympathoblasts that reach the adrenal anlage or if it has a more general distribution. Rat embryos at different stages of development were fixed in formalin, sectioned on a cryostat and processed for staining, by the PAP technique, either with rat anti-rabbit TH, DBH, or PNMT or with bovine anti-rabbit PNMT. The CA synthesizing enzymes were not detected prior to the 11th day (d) of development; by d 11 TH and DBH appeared simultaneously and were detected in cells localized in a) the future site of the primary sympathetic chain and b) the mesoderm of the gut wall. Since these latter noradrenergic cells were not observed after d 14 or in adult animals, they may eventually die or be transformed into enteric ganglioblasts expressing other neurotransmitter functions. By d 15 cells containing TH and DBH started to migrate from the sympathetic (suprarenal) ganglia towards the adrenal anlage. However, PNMT was detected 3 days later only in those sympathoblasts that reached the adrenal cortical tissue. We conclude that sympathetic neuroblasts express TH and DBH simultaneously during development, that contact of these cells with the adrenal cortex is required for the expression of PNMT and that a transient population of noradrenergic cells is present in the developing gut. We propose that migrating sympathoblasts express a labile noradrenergic phenotype. The microenvironment of their final destination will determine if they will synthesize only norepinephrine, or, provided they populate the adrenal gland, also epinephrine. If, on the other hand, the sympathoblasts colonize the gut wall, they may switch to the synthesis of other neurotransmitters. Supported by grants HL 18974, MH 24285 & NSG-2259.

390 PRENATAL DIAZEPAM EXPOSURE IN RATS: EFFECTS ON GROWTH AND BEHAVIORAL DEVELOPMENT OF THE OFFSPRING. Donna Tervo*, Carol Kellogg, Richard Miller*, and James Ison. Depts. Psych. and Obs-Gyn, Univ. of Rochester, Rochester, NY 14627.

Pregnant female rats of the Long-Evans Hooded strain were injected on days 13-20 of gestation (sperm positive vaginal smear= day 0) with diazepam (2.5, 5, or 10 mg/kg) or the vehicle. Marked sedation of the dam was noticeable only at the highest dose of diazepam (dz). Other rats were left undisturbed. All pups were fostered to uninjected dams within 24 hours after birth. All uninjected and vehicle-injected rats (total of 18) delivered on day 22 of gestation whereas all dz-injected rats (7) delivered on day 21. Hence, prenatal dz exposure shortened the gestation period by 5%. Birth weights did not differ among the groups, nor was there any apparent difference between the rate of growth over the first three weeks of life while all pups were nursed by uninjected dams. However following weaning on conceptual days 43-44 (postnatal day 21) differences in weight between the control and dz-exposed pups became increasingly apparent as the dz-exposed pups did not sustain the same growth rate as the control groups. By 104 days conceptual age the weights of the prenatally exposed rats were 84, 87% (male, female) of the uninjected controls.

The development of spontaneous locomotor activity was evaluated in isolated animals. The usual developmental pattern was observed in both control groups with activity increasing markedly to reach a peak at 38 days conceptual (16 postnatal) age and declining sharply thereafter. Animals exposed to the lowest dose of dz exhibited a similar developmental pattern, however peak activity was reached at 36 days conceptual (15 days postnatal) age. Animals exposed to the two highest doses of dz, however, showed only a gradual increase and decrease in activity over this time period, with no precise delineation of a peak. The development of the acoustic startle response was also evaluated from postnatal days 12-22. Animals from both control groups showed an increase over this period in the startle amplitude to a 110 db, 10 K tone against a background of 28-30 db whereas there was no apparent increase in any of the dz-exposed animals. Evaluation of the startle response against increasing background intensities demonstrated further effects of prenatal dz exposure. All control groups showed potentiation at a background intensity of 75 db whereas no potentiation was observed in any of the dz-exposed pups.

These studies have demonstrated that exposure of the fetus to dz during the period of marked neuronal differentiation can have pronounced and long lasting consequences.

391 EFFECTS OF POSTGANGLIONIC AXOTOMY ON THE REGENERATION OF SELECTIVE SYNAPTIC CONNECTIONS IN THE GUINEA-PIG SUPERIOR CERVICAL GANGLION. Wesley Thompson, Arild Njå, and Dale Purves. Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

Neurons of the guinea-pig superior cervical ganglion are selectively innervated. Thus each neuron receives innervation from a contiguous subset of the thoracic spinal segments, one of which is dominant; these rules of segmental contiguity and dominance are presumably the neuronal basis for the characteristic pattern of end-organ responses elicited by stimulation of each thoracic ventral root in vivo (Njå and Purves, J. Physiol. 264: 565, 1977). The experiments we report were designed to test whether altering a ganglion cell's peripheral connections changes the specificity of the synapses it receives.

The major postganglionic nerves of the cervical ganglion were crushed in adult animals; at the same time, the preganglionic nerve was cut. We then examined the segmental innervation 1, 3 and 12 months postoperatively by recording intracellularly from ganglion cells while stimulating the thoracic spinal nerves in vitro. At all intervals axotomized neurons, as neurons after denervation alone, showed a strong tendency to be reinnervated by a contiguous subset of spinal segments, and to be dominated by one of them. Moreover, the final proportion of reinnervated neurons dominated by different spinal segments was about the same whether or not the postganglionic axons had been interrupted.

In contrast, the responses of end-organs to ventral root stimulation in vivo were decidedly abnormal following reinnervation of ganglion cells which had themselves reinnervated peripheral targets. Normal animals, or animals with reinnervated superior cervical ganglia, show marked pupillary dilation and widening of the palpebral fissure in response to stimulation of the first and second thoracic ventral roots, a weaker response upon stimulating T3, and little or no response to T4 activation. Three months after postganglionic axotomy, however, reinnervated ganglia no longer showed this rostrocaudal gradient. Stimulation of thoracic ventral roots now caused end-organ responses proportional to the number of ganglion cells innervated by each segment so that stimulation of T1 and T4 affected the eye nearly equally.

These results are consistent with the view that postganglionic axons reinnervate peripheral targets in an indiscriminate way, and that connections with peripheral targets different from (or in addition to) normal fail to elicit compensatory changes in ganglionic innervation. [Supported by NIH grant NS-11699.]

392 AUTORADIOGRAPHIC LOCALIZATION OF ^{3}H-ESTRADIOL IN RELATION TO STEROID RESPONSIVENESS IN CULTURES OF THE HYPOTHALAMUS/PREOPTIC AREA. C. Dominique Toran-Allerand, John L. Gerlach*, and Bruce S. McEwen. Columbia Univ., Coll. P&S, New York, N.Y. 10032, and Rockefeller Univ., New York, N.Y., 10021.

Earlier studies (Toran-Allerand, Brain Research, 106, 407, 1976) have shown that estradiol (E_2) and testosterone elicit a selective acceleration and enhancement of neuritic proliferation in specific regions of the newborn mouse hypothalamus/preoptic area (POA) in vitro. In order to relate this response to the presence of the specific steroid receptor, the distribution of E_2-concentrating cells in 7-day-old organotypic cultures was determined autoradiographically. Coronal explants of the newborn female mouse were maintained in Maximow assemblies. After 72 hrs exposure to E_2-deficient medium, cultures received $2x10^{-9}$ M ^{3}H-E_2 for 1 hr. 2 µm frozen sections were processed for thaw-mount autoradiography and, after 150 or 622 days exposure, were developed and analyzed. Radioactive label was observed in specific explant regions. Despite some in vitro architectonic rearrangements, the topographic distribution of the labelled cells was similar to that of the neonatal rat (Sheridan et al., Endocrinology, 94, 1386, 1974) and of the adult mouse (Warembourg, C.R. Acad. Sci., 250, 152, 1970; Stumpf and Sar, Anatomical Neuroendocrinology (Karger) 82, 1975). The largest number of labelled cells were found in the following general regions: medial POA; suprachiasmatic; interstitial nucleus of the stria terminalis; arcuate and ventral premamillary nuclei. A few cells were also seen in the anterior hypothalamus, ventromedial and periventricular nuclei and lateral septal region. Small clusters of cells, labelled solely cytoplasmically, were also observed. There is a strong correlation between the presence and topography of the E_2-concentrating neurons and the pattern and regional localization of the steroid response. In the dorsal (strial) POA and premamillary regions, for example, silver impregnation shows that the localized areas of steroid responsive neuritic proliferation appear to emanate from regions containing the labelled cells. The purely cytoplasmic labelling may be related to the immunocytochemical demonstration of intraneuronal α-fetoprotein (Benno and Williams, Brain Research, 142, 182, 1978), an E_2-binding protein. (Supported in part by NIH grant HD-08364; NSF grant BNS77-09859; National Foundation-March of Dimes grant 1-564, NIMH Research Scientist Development Award MH-00192 to DT-A and an Institutional grant from The Mellon Foundation; and by NIH grant NS-07080 to BMc and an Institutional grant RF-70095 from the Rockefeller Foundation).

393 CYCLIC AMP-DEPENDENT PROTEIN KINASE (PK) AND PROTEIN PHOSPHORYLATION IN HUMAN BRAIN DURING AGING. L. Truex*, R. Conway*, A. Routtenberg, and M. Schmidt. (SPON: J. Clemens). The Lilly Res. Labs., Indianapolis, IN and Northwestern Univ., Evanston, IL.

The activity of soluble cAMP-dependent PK and the phosphorylation of synaptosomal proteins were studied in autopsy samples of the cerebral cortex of humans. Subjects were 2 days - 82 yrs of age. PK activity ± cAMP was measured in the 27,000 g supernatant. Phosphorylation of synaptosomal membrane fragments was measured in vitro ± cAMP (5 µM). To assess the effects of postmortem autolysis on PK and phosphorylation, similar determinations were carried out on rat cortex 10 min - 16 hr after death.

No change occurred in the cAMP-dependent PK activity in rat cortex postmortem. Basal PK activity was higher 10 min after death than 6 or 16 hr postmortem. These changes parallel the postmortem rise and fall in endogenous levels of cAMP in the brain. Postmortem autolysis also did not alter cAMP-dependent or -independent phosphorylation of synaptosomal membrane fragments. These studies using the rat cortex established the validity of conducting similar experiments on human brain tissue postmortem.

We were unable to demonstrate age-related differences in phosphorylation of synaptosomal proteins from human cortex. The number and staining densities of the protein bands were also similar across age. PK activity was also not significantly different between ages. Maximal stimulation of PK occurred at 10^{-6} M cAMP (426 pmoles Pi incorporated/mg prot./min) with a basal activity of 54 pmoles Pi incorporated/mg prot./min. Subjects over 60 yrs of age tended to have lower PK activity but based on the limited sample size, these differences were not significant.

A comparison of 3 and 24 mo. old Wistar rats also failed to reveal significant differences in PK activity, cAMP stimulation of PK activity, or phosphorylation of synaptosomal proteins in the cerebral cortex.

These studies show the phosphorylation system in the cerebral cortex to be stable in the rat and human for periods as long as 16 hr postmortem. cAMP-dependent PK activity and the number, type, and phosphorylation of endogenous synaptosomal proteins in the human appear similar to analogous systems in the rat cortex. No changes in the system with advanced age were detected in either rats or humans. Additional tissue is being collected for study and findings with other components of the phosphorylation system (e.g., phosphoprotein phosphatase) will be discussed.

394 EFFECTS OF NEURONAL ACTIVITY AND CYCLIC NUCLEOTIDE DERIVATIVES ON THE DEVELOPMENT OF TRANSMITTER FUNCTION IN CULTURED SYMPATHETIC NEURONS. Patricia A. Walicke* and Paul H. Patterson. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Recent evidence strongly suggests that individual sympathetic neurons taken from superior cervical ganglia of neonatal rats can either secrete catecholamines (CA) and form adrenergic synapses or, if grown in medium previously conditioned by incubation on appropriate non-neuronal cells (CM), secrete acetylcholine (ACh) and form cholinergic synapses. The effects of neuronal activity and exogeneous cyclic nucleotide derivatives on this choice of transmitter were studied. Adrenergic and cholinergic development were evaluated by determination of the rate of synthesis and accumulation of ACh and CA from radioactive precursors. Neurons treated with depolarizing agents, elevated KCl or veratridine, or stimulated directly with electrical current, either before or during exposure to CM, displayed up to 300-fold lower ACh/CA ratios than without depolarization, and thus remained primarily adrenergic. Since depolarization increases Ca^{++} entry into the neurons, the effects of several Ca^{++} agonists and antagonists were studied. The former (Ba^{++}) augmented the K^+ effect, while the latter (Mg^{++}, EGTA, or D600) largely blocked the effect of K^+ on cholinergic development.

Because cyclic nucleotides are known to be involved in the control of a number of cellular processes, often in conjunction with Ca^{++}, the ability of exogeneously added nucleotides to mimic the effects of depolarization were studied. Neurons exposed to 1 mM mono-or dibutyrl cAMP or cGMP (+phosphodiesterase inhibitors) for periods of 10 days or more, either before or during exposure to CM, displayed up to 10-fold lower ACh/CA ratios than controls. 1 mM butyrate did not mimic these effects. Agents which elevate cAMP levels (adenosine and PGE_1, +phosphodiesterase inhibitors) also lowered the ACh/CA ratio up to 10-fold. Since addition or withdrawal of the nucleotides for 2 days prior to assay did not significantly alter the ACh/CA ratio, these effects are interpreted as changes in developmental fate rather than acute alterations of transmitter metabolism. Thus the neurons can be influenced to remain adrenergic either by agents which increase cAMP and/or cGMP levels or by depolarizing agents whose effects may be mediated by Ca^{++} entry. Assay of the effects of depolarization and CM on neuronal cyclic nucleotide levels are in progress in an attempt to further elucidate the intracellular mechanisms controlling this developmental transmitter choice. (Supported by the American and Massachusetts Heart Association and NINCDS.)

395 ATTENTION AS AN ALTERNATIVE TO SELF-INDUCED MOTION FOR THE PERCEPTUAL BEHAVIOR OF KITTENS. Richard D. Walk, Jane D. Shepherd*, and David R. Miller*. Dept. Psychology, Geo. Wash. Univ., Washington, D.C. 20052.

In a classical study Held and Hein (J. Comp. Physiol. Psychol. 1963) reared kittens in the dark for 8 wks and then allowed them 3 hr/day light exposure under two conditions. One group actively locomoted in the light while the second group was passively exposed to the light in holders that prevented sight of the limbs. Only the first group developed depth discrimination. The authors concluded: "self-produced movement with its concurrent visual feedback is necessary for the development of visually-guided behavior."

In a prior study (Miller and Walk, Eastern Psychol. Assoc., 1975) we showed some depth discrimination in kittens reared in the dark for 4 wks. We have also replicated the Held and Hein study with 8 wk old kittens and found much the same results as theirs. While depth discrimination of 4 wk old passive kittens was essentially normal the depth discrimination of passive kittens reared in the dark for 8 wks prior to the period of light exposure was delayed until after active locomotion in the light; it did not appear with passive exposure to the light.

Depth discrimination may be innate but what maintains it? Is it self-produced movement or attention?

We raised kittens in the dark for 40 days and then divided them into 5 groups: (1) active locomotion, (2) passive exposure, (3) passive exposure to an attention provoking display, (4) passive exposure in a "go-cart" on wheels which the kitten could move forward by closing a microswitch above its head, (5) raised in the dark until tested. Groups 1-4 had 3 hr/day exposure to the light in a patterned environment for 10 days starting on day 40. All kittens were tested on the 49th day and thereafter until depth discrimination appeared. All passive kittens had the same type of holder; none could see the limbs or locomote.

Preliminary results indicate that passive exposure to an attention provoking display is sufficient for depth discrimination on the visual cliff. (Supported by NIH Grant MH-25864).

396 AGE-RELATED CHANGES IN SPINE NUMBERS ON CEREBELLAR BASKET CELLS IN POST-WEANLING RATS. Christopher D. West* (SPON: Michael J. Malone). Harvard Neurol. Unit, Beth Israel Hosp., and GRECC Unit, Bedford V. A. Hosp., Bedford, MA 01730.

Age-related changes in the numbers of dendritic spines were studied in two types of neurons of the cerebellar cortex which receive synaptic input from granule cells. Basket cells and Purkinje cells were examined in Rapid Golgi preparations of the rat cerebellar vermis. Albino rats, 1 to 6 months of age, were perfused through the aorta with either Karnovsky's fixative or 10% buffered formalin. Cerebella were fixed in classical Rapid Golgi solution, silvered, embedded in low-viscosity-nitrocellulous and sectioned in the sagittal plane at 150 micra. Only well impregnated neurons were studied. Basket cells with cell bodies located in the lower portions of the molecular layer and with at least one prominent pial-directed dendrite were photographed and drawn with a camera lucida. Purkinje cell terminal branch dendrites located in lower levels of the molecular layer near the cell body were photographed and drawn. Dendritic spine profiles were counted on terminal portions of the dendrite located in a single focal plane. Drawings and photographs of basket cells revealed marked decreases in dendritic spine numbers over the age range examined. Photographs, drawings, and counts of Purkinje cell dendritic spines revealed no corresponding decrease. (Supported by USPHS Grant No. 1 R23 AG00607-01 from the NIA and by the Veterans Administration).

397 MORPHOGENESIS OF SYNAPTIC ENDINGS OF COCHLEAR FIBERS IN THE CHICK BASILAR PAPILLA. M. C. Whitehead and D. K. Morest. Department of Anatomy, University of Connecticut Health Center, Farmington, Connecticut 06032.

Developing acoustic ganglion cells provide an opportunity to study the morphology of identified populations of fibers and their target cells. These cells have peripheral processes contacting hair cells and centrally directed axons ending on neurons in N. magnocellularis and N. angularis. This study, using rapid Golgi and Golgi- aldehyde methods, details the growth of the peripheral processes of acoustic ganglion cells and the formation of their endings in the basilar papilla (organ of Corti).

At 4½- 5 days of incubation (equivalent Hamburger- Hamilton stage), acoustic ganglion cells lie just beneath the receptor epithelium: their peripheral fibers, sprouting from the soma, bear growth cones with filopodia. A few longer, tapered fibers, have already entered the epithelium where they emit 1- 2 short, fine branches. At 6- 6½ days,most fibers run lengthwise below the basal lamina where they branch and end as thin, tapered terminals or growth cones. Some end- branches take an abrupt radial course into the epithelium. By 7½- 9 days,the acoustic fibers have many end-branches in a restricted zone of the receptor epithelium and small varicosities, growth cones, filopodia, and tapered endings extending between the young hair cells often as far as the luminal surface. Between days 10- 13,the fibers develop large, bulbous, terminal swellings from which irregularly shaped processes extend into the hair cell region. Thin, beaded, efferent fibers are first seen in the epithelium during this stage. By late embryogenesis (days 14- 17), the swellings of the acoustic endings are smaller and have thickened, foot- shapes with a few claw- like appendages (end- bulbs) around the bases and sides of hair cells.

The sequence of structural events defined here compares to that of the acoustic axons in N. magnocellularis (Jhaveri and Morest, Neurosci. Abstr., 1977). Between days 11- 13, while peripheral endings have terminal swellings, the central axons expand and ramify. After day 15, while the peripheral endings become smaller, there is a corresponding condensation of the central terminals to form, ultimately, the end- bulbs of Held.

(Supported by PHS grants 1 F32 NS 05910- 01 and 5 R01 NS14354.)

398 SEGMENTALLY SELECTIVE INNERVATION OF MAMMALIAN SYMPATHETIC GANGLIA: COMPARATIVE INNERVATION OF CERVICAL AND THORACIC GANGLIA. Joseph W. Yip, Dale Purves, and Jeff W. Lichtman. Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

The guinea-pig superior cervical ganglion can be dissected in continuity with the cervical trunk and the thoracic portion of the peripheral sympathetic system. The ventral roots of the spinal segments which contribute innervation to the ganglion can then be stimulated in vitro while recording synaptic responses in individual ganglion cells. Such recordings show a segmentally selective pattern of innervation. Although each neuron in the mature ganglion typically receives synaptic contacts from about a dozen different preganglionic axons arising from an average of 4 of the 8 ventral roots which contribute innervation to the ganglion, the segments of origin of the preganglionic axons to individual neurons are nearly always contiguous. Typically, one of the ventral roots supplying preganglionic axons to a neuron provides the dominant innervation to that cell (as measured by either the amplitude of the postsynaptic potential or the number of innervating axons), while adjacent ventral roots contribute a synaptic influence which diminishes as a function of distance from the dominant segement.

These rules of contiguity and segmental dominance are also evident when similar recordings are made from the stellate and the fifth thoracic ganglia. These three ganglia differ from one another, however, in the spinal levels from which their innervation arises. Most of the axons innervating the superior cervical ganglion arise from thoracic spinal segments T2 and T3, while the stellate receives most of its innervation from T3-T5, and the fifth thoracic ganglion from T5. Thus, although the innervation to these ganglia arises from the same set of thoracic segments, there is a net caudal shift in the origin of the majority of axons innervating progressively more caudal ganglia. In addition, these several ganglia differ in the relative homogeneity of their spinal inputs: while individual cervical or stellate ganglion cells may be dominated by any one of several innervating segments, most neurons in the fifth thoracic ganglion are dominated by T5. A possible explanation for this difference in segmental innervation is that the fifth thoracic ganglion innervates a more restricted region than the superior cervical or the stellate ganglion.

These findings show that the segmentally selective innervation of superior cervical ganglion cells is characteristic of other sympathetic ganglia, and suggest that differences in the segmental innervation of ganglia may be a function of the territory they innervate. [Supported by NIH grant NS-11699.]

DRUGS OF ABUSE

399 INFLUENCE OF BRAIN CATECHOLAMINES ON BARBITURATE WITHDRAWAL CONVULSIONS. William M. Bourn. School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209.

Rats were addicted to sodium barbital by daily administration of the drug with weekly incremental increases in the daily dosage for a four week period. Interference with brain catecholamine function by intracerebroventricular administration of 250 ug of 6-hydroxydopamine one week prior to withdrawal resulted in no increase in audiogenic seizure susceptibility, but a marked increase in severity of the sound-induced convulsions at 48 hours post-withdrawal. Ro 4-1284, an agent which depletes brain amines (catecholamines and 5-hydroxytryptamine), produced an increase in severity of- and susceptibility to sound-induced seizures at 48 hours post-withdrawal from barbital. Desipramine, an agent which enhances noradrenergic function by blocking uptake at the noradrenergic nerve terminal, provided significant protection from audiogenic seizures during withdrawal. These data support the hypothesis that brain norepinephrine acts as a modulator of sound-induced convulsive seizures during barbiturate withdrawal.

400 COMPARISON OF NARCOTIC AGONISTS AND ANTAGONISTS IN THE TAIL-SHOCK VOCALIZATION TEST IN RATS. P. J. K. Dobry. The Upjohn Company, CNS Research, Kalamazoo, MI 49001.

Narcotic agonist ("analgesic") and antagonist properties were compared in an up-down shock titration procedure, which measured the threshold for vocalization during an electric shock. All compounds were administered subcutaneously in 0.9% saline.

Methadone hydrochloride, morphine sulfate, profadol hydrochloride, meperidine hydrochloride, and codeine phosphate were good-to-excellent agonists (elevated vocalization threshold above 750 uA). None antagonized morphine (16 mg/kg) analgesia. All except 64 mg/kg profadol were completely antagonized by naloxone hydrochloride (5 mg/kg).

At the other extreme, naloxone hydrochloride, nalorphine hydrochloride and cyclazocine completely antagonized morphine, and levallorphan tartrate almost completely antagonized morphine. None of these elevated vocalization threshold above 750 uA, and none was antagonized by naloxone. Only naloxone was completely devoid of agonist activity at all doses tested (up to 64 mg/kg).

Dezocine, pentazocine lactate (Talwin^{R}, injectable), buprenorphine hydrochloride, and butorphanol tartrate antagonized morphine to the level of analgesia shown by the antagonist alone. These four intermediate compounds varied in their amount of agonism and in their response to naloxone. Dezocine and pentazocine caused the most analgesia of these four compounds, but naloxone almost completely antagonized 64 mg/kg dezocine and did not antagonize 32 or 64 mg/kg pentazocine. Buprenorphine and butorphanol caused weak analgesia which was completely antagonized by naloxone. Buprenorphine is the only compound tested whose dose-response curve showed a ceiling effect.

In conclusion, the vocalization threshold test for analgesia is a stringent assay which demonstrates the greatest efficacy for strong narcotic agonists and weak analgesic efficacy for strong antagonists. In general, test results are consistent with the results of other laboratories demonstrating that naloxone is a better antagonist against relatively pure agonists than against mixed agonist/antagonists with strong antagonist properties.

401 CYTOLOGICAL EVIDENCE OF A CHANGE IN RNA/PROTEIN SYNTHESIS IN HAMSTER NEURONS FOLLOWING INGESTION OF ETHANOL. C.R. Dunmire-Graff* and F.W. LaVelle* (SPON: C.H. Anderson). Dept. Anat., Loyola Univ. Stritch Sch. Med., Maywood, IL 60153.

The neuronal intranucleolar body (INB) of the hamster, first reported by LaVelle and LaVelle (Exp. Neur., 49:569, 1975), is regarded by us as a reserve of nucleolar RNA-rich material destined to take part in the synthesis of cytoplasmic protein in neurons. In this study chronic ingestion of ethanol was found to alter the size and frequency of INBs in the hamster brain. Adult golden hamsters were fed either (1) water and food pellets or (2) a 10-15% ethanol solution and food pellets for periods of 23 or 46 days, after which they were perfusion fixed. Brains were double-embedded and prepared for light microscopy. Transverse sections (4 μm) were cut at all brain levels and stained with buffered thionin. The diameter and frequency of INBs were then determined in: (1) facial motor neurons, (2) cells of the medullary and pontine reticular formation, and (3) cerebellar Purkinje cells. Each neuron containing a nucleolus was judged to possess either no INB, a punctate-sized INB, or a prominent INB. In all three brain areas the cells of the alcohol treated animals exhibited a higher percentage of prominent INBs than the controls ($p < 0.005$). The greatest change occurred in Purkinje cells: ±40% of control cells contained a prominent INB vs ± 70% of cells exposed to alcohol. Measurements of the prominent INBs in both control and alcohol treated animals showed for all brain areas studied a statistically significant ($p < 0.05$ - $p < 0.005$) increase in diameter following chronic ingestion of alcohol. Furthermore, the longer the exposure to alcohol, the greater the INB size. For example, in facial neurons, which contained the largest INBs, the mean diameter increased from 1.67 μm (controls) to 1.88 μm after 23 days and 2.07 μm after 46 days of alcohol ingestion. Within the cerebellum an additional study was made of a specific regional difference found in the nodule; here the Purkinje cells were normally less advanced than their counterparts elsewhere, as evidenced by a high percentage of nuclei with multiple nucleoli and nucleoli still attached to the nuclear membrane. After injection of ethanol, there was a significant shift ($p < 0.005$) to single nucleoli in this region, with many of them containing a prominent INB. Since our evidence consistently indicates a build-up of nucleolar RNA reserves (larger INBs) after alcohol ingestion, we suggest a decreased utilization of this material, which agrees with biochemical evidence of a decreased synthesis of cytoplasmic brain proteins following chronic exposure to dietary alcohol. We suggest that the nucleolar portion of this chain of synthetic events remains intact.

402 ALTERATIONS IN BEHAVIORAL RESPONSIVENESS AMONG OFFSPRING OF MALE MICE INJECTED WITH MORPHINE PRIOR TO MATING. G. Friedler* (SPON: C. Kornetsky). Boston University Medical School, Boston, MA. 02118.

We have previously reported a decrease in response to morphine sulfate (morphine) challenge among offspring of rodent parent(s) which were injected prior to mating (Pr) with morphine (In Perinatal Pharmacol. (Raven), 207-216, 1974; Pharmacol., 16: 203, 1974). Two studies were designed to explore whether alterations in responsiveness among offspring of opioid parentage were restricted to response to morphine challenge or would be extended to other behaviors. In the first study, female Swiss albino mouse offspring-- derived from male parents injected Pr with morphine or saline-- were tested at 12 weeks of age on a step-through avoidance procedure (.16mA scrambled footshock, 2 sec duration). At 18 weeks, offspring were retested in a straight alley swimming maze (water temp 18°C) for 20 trials with 30 sec cutoff and 20 sec intertrial interval. Swimming time (as a measure of performance) and errorless trials (as a measure of maze learning) were recorded. Offspring of Pr morphine-injected fathers showed an increase in swim time and decrease in errorless trials (<.001) when compared with saline-derived controls. In the second study, both male and female offspring -- derived from male parents injected Pr with morphine, methadone or saline -- were tested on the avoidance procedure at 6 weeks and on the swimming maze at 8 weeks. Significant deficits in both swim time and error-free trials (<.01) were again observed among morphine offspring. Methadone offspring did not differ from saline-derived controls. In both studies, the morphine and saline groups did not differ on the swimming maze alone, i.e., if not preceded by the avoidance task. The findings suggest that an altered responsiveness to stress may be responsible for the behavioral changes observed in offspring derived from rodent parents injected Pr with morphine.
(Supported in part by grant DA 01204 from NIDA).

403 ENHANCED PLACENTAL TRANSFER OF MORPHINE WITH INCREASING GESTATIONAL AGE. Margaret L. Kirby. Department of Anatomy, School of Medicine, Medical College of Georgia, Augusta, GA 30902

Placental transfer stdies of dihydromorphine and etorphine on the 21st day of gestation have shown that both drugs reach higher concentrations and are present longer in the fetuses than in maternal brain (Sanner and Woods, JPET 148:176, 1965; Blane and Dobbs, Br., J. Pharmacol. 30:166, 1967). The present study was undertaken to compare the amount of placental transfer of morphine on different days throughout gestation. On days 11, 13, 15, 17 and 19 of gestation pregnant Wistar rats were injected with 180 μCi of ^{3}H-morphine containing 4mg/kg cold morphine. At 1/2, 1, 2, 6 and 12 hours after injection, the fetuses were delivered by Caesarian section and all but 11 day fetuses cut into head and body pieces, which were analyzed individually. Placentas, maternal liver, brainstem and diencephalon-caudate were also analyzed. One group of animals was injected chronically with 10mg/kg of morphine twice daily between days 7 and 15 of gestation. On day 15, these animals were injected with ^{3}H-morphine and treated as described above. The tissues were homogenized in 0.5 to 4 ml ethanol and centrifuged. Free ^{3}H-morphine in the supernatant was isolated by thin-layer chromatography. Less than 100 ng/g of free morphine was found in individual whole fetuses on day 11. The amount of morphine found in fetuses grows progressively higher between days 11 and 17 of gestation. The highest concentrations of morphine occur at broad peaks between 1 and 2 hours on days 11, 13 and 15. On days 17 and 19 there is a sharp peak at 1 hour. The peak concentration on day 19 is less than on day 17. This decrease may represent metabolism by the placenta and fetal liver. On days 15, 17 and 19 measurable amounts of morphine are still present in the fetuses 12 hours after injection but not in the maternal brains. On day 11 the fetuses have less free morphine than the maternal brains. However on all the other days measured fetal tissues have higher concentrations than maternal brains on a per gram basis. On day 15 of gestation fetuses chronically exposed to morphine show a peak concentration at 1/2 hour after injection. This concentration is higher than the 1 hour peak in naive fetuses. In conclusion, these results imply that morphine passes through the placental barrier more easily as gestation progresses and that the concentration of morphine in the fetus can be altered by prior morphine exposure.

Supported by NIH Grant RR-05365

404 MORPHINE AND NALOXONE EFFECTS ON SPONTANEOUS AND EVOKED EEG AND UNIT IMPULSE ACTIVITY - TESTS FOR TOPOGRAPHIC AND DIURNAL DIFFERENCES. W. R. Klemm and C. G. Mallari Dept. Biology, Texas A&M Univ., College Sta., TX 77843.

Studies were conducted on 27 adult rats, locally anesthetized, paralyzed with Flaxedil, and artificially respired. Recording semi-microelectrodes were implanted in the caudate, amygdala, and at two levels of the central grey (CG); stimulating electrodes were put in the olfactory bulb and substantia nigra (SN). Stimuli of 4 intensities were delivered as 5-sec trains of 10/sec pulses. Unit activity was filtered, stimulus-artifact suppressed, differentiated, discriminated, and tallied every 5 secs. After baseline activity was determined, the 1st drug (morphine, 15 mg/kg, or saline) was injected, and activity re-determined 30-min later; then a 2nd drug (naloxone, 1 mg/kg) was given and activity re-determined. Diurnal effects of morphine were not evident in either spontaneous or evoked field potential (EEG) or unit activity. Morphine's main effect on spontaneous EEG was a naloxone-reversible slowing of frequency in all areas. With olfactory stimuli, morphine caused a naloxone-reversible increase in response in the amygdala of many rats and a decrease in others. Responses to SN stimuli in all areas were usually decreased by morphine (naloxone reversible). Morphine's main effect on spontaneous unit activity was a naloxone-reversible depression, especially evident in the caudate and CG. Both CG loci had depressed activity, but neurons of a few rats discharged faster; naloxone usually reversed the morphine depression, but not the excitation. In saline controls, naloxone had no clear effects in any brain area. Olfactory stimuli caused mostly excitatory responses, most evident in the amygdala; morphine not only failed to depress responses well, but often enhanced or reversed them; naloxone usually reduced the effect. Morphine usually caused a naloxone-reversible depression of evoked activity in the other areas, when they were responsive. SN stimuli generally caused excitatory responses, but were least effective in the amygdala. Morphine caused a naloxone-reversible depression of responses, especially in the CG. In saline-injected controls, naloxone commonly depressed or even reversed responses to both kinds of stimuli, suggesting involvement of enkephalinergic pathways. (Supported by NIDA grant, DA 00784).

405 OPIATE RECEPTORS IN AGGREGATING CELL CULTURES OF EMBRYONIC RAT BRAIN. E.L. Knodel* and E. Richelson. Depts. of Psychiatry and Pharmacology, Mayo Fdn., Rochester, MN 55901.

Mechanically dissociated fetal rat brains in rotation-mediated aggregating cell culture have been extensively characterized in our laboratory for their neurochemical properties(1). To study opiate drug interactions with these cells, we cultured fetal rat brains of 15-16d gestation, and characterized opiate receptor binding in homogenates of these cultures using [^{3}H]dihydromorphine ([^{3}H]DHM). [^{3}H]DHM bound to homogenates of aggregates in the absence of sodium ions in a saturable and stereospecific manner. Scatchard plots of binding data from studies of aggregates cultured for 10, 20 and 28d *in vitro* showed a single binding site for [^{3}H]DHM, with an average K_D = 1.2nM (range 0.9-1.3 nM) and B_{max} = 8-16 fmol/mg protein. After 4 weeks of treatment with 50 nM dextrorphan or 50 nM levorphanol, opiate receptor binding was unchanged compared to controls with respect to K_D and B_{max}.

In preliminary experiments, morphine sulfate (1-10μM) decreased endogenous levels of adenosine 3',5'-monophosphate in cultures incubated with this drug for up to 30 min. However, the effects of morphine were not reversed by equimolar amounts of naloxone. These data suggest that aggregating cell cultures of fetal rat brain may be a useful system for studying biochemical correlates of opiate binding and their possible effects on brain developmental processes.

(1) P. Honegger and E. Richelson, Brain Research: 109, 335-354, 1976; 133, 329-339, 1977; 138, 580-584, 1977.

(Supported by Mayo Fdn. and USPHS Grant DA 1490.)

406 HIPPOCAMPAL UNIT RESPONSES TO LOW DOSES OF MORPHINE AND NALOXONE IN DRUG-NAIVE AND MORPHINE-DEPENDENT RATS. M.A. Linseman. Addiction Research Foundation, Toronto, Canada, M5S 2S1.

The effects of opiates on single unit responses of the hippocampus were studied and compared to those previously obtained and reported for units in medial thalamus. The hippocampus, in comparison to medial thalamus, is characterized by a low density of opiate receptors, and there is no or negative evidence of its involvement in opiate tolerance and dependence. The comparison was of interest to determine whether the two areas might be distinguishable also on the basis of neurophysiological responses to opiates.

The spontaneous activity of hippocampal units was recorded prior to and following an i.v. injection of 0.625 mg/kg morphine (M) in drug-naive, chronically-prepared, paralyzed rats. Previous experiments established this dose to be generally just above threshold for producing a change in the simultaneously recorded fronto-cortical EEG. Responses of hippocampal units did not differ in terms of absolute latency, or latencies relative to cortical EEG changes from those previously recorded in medial thalamus. However, there was a marked difference in the pattern of responses in the two areas. Whereas medial thalamic units had shown consistent sustained decreases in rate in response to the drug, the hippocampal responses were more heterogeneous and variable. The majority of hippocampal responses were increases in rate, but there were also several decreases, as well as changes in the direction of increased or decreased variability of rate compared to baseline. Even when the overall rate had increased or decreased, it was often accompanied by greater variability than had been evident prior to M.

In contrast to the effects of M, the majority of responses of hippocampal units following an injection of 0.0125 mg/kg naloxone in morphine-dependent animals (1-75 mg pellet implanted s.c. for 3 days) were sustained decreases in rate. Hippocampal units were no less responsive to this low dose of naloxone (also just above threshold for producing a change in the cortical EEG) than were medial thalamic units, and like medial thalamic units, the latency of hippocampal responses tended to coincide with or follow the change in cortical EEG.

These results constitute evidence that medial thalamus rather than hippocampus might be a primary site of action of morphine only if one assumes that an internally consistent pattern of response to morphine might be characteristic of such an area. Neither area may be a primary site of action of naloxone as both hippocampal and medial thalamic changes following naloxone in dependent animals may have been secondary to previously occurring changes in the cortex.

407 OPIATE RECEPTORS MEDIATE THE EXCITATORY (EUPHORIGENIC?) EFFECT OF ETHANOL, CHLORDIAZEPOXIDE AND MORPHINE ON BRAIN STIMULATION REWARD. S. A. Lorens and S. M. Sainati*. Dept. Pharmacology, Stritch School of Medicine, Loyola University, Maywood, Illinois 60153.

Rats were trained to press a lever to deliver a 0.2 sec train of bidirectional 0.1 msec pulses (100 pairs/sec) through bipolar electrodes implanted in the lateral hypothalamus (LH) and medial frontal cortex (MF). The animals were run in 10 min sessions at least once daily. A 5 min period separated testing at each electrode site. Drug tests were initiated after responding for a threshold current intensity had stabilized (see Psychopharmacol. 48(1976)217). Different drugs and doses (expressed as the base) were administered i.p. following a 10 min control session once every 4-7 days according to a randomized design. Naloxone (NOX) was injected 5 min before or 1 hr after saline (1.0 - 2.2 ml/kg) or the other compounds. The animals then were tested hourly for 5 hr post-injection.

Ethanol (ETOH, 0.2 - 0.8 g/kg, 30% v/v) and chlordiazepoxide (CDP, 2.0 - 8.0 mg/kg) enhanced the response output for LH but not MF self-stimulation (SS). In contrast, morphine (MOR, 1.0 mg/kg) elevated responding for both LH and MF SS. NOX (0.5 - 16.0 mg/kg) did not affect SS rates at either site. However, NOX (5 mg/kg) both prevented and reversed the increase in LH SS response output produced by ETOH (0.4 g/kg) and CDP (4.0 mg/kg). Likewise, NOX (1.0 mg/kg) blocked the facilitatory effect of MOR (1.0 mg/kg) on both LH and MF SS. Dose-response relationships indicate a competitive antagonism.

That the excitatory effect of ETOH, CDP and MOR on LH SS is mediated by opiate receptors is strongly supported by preliminary findings indicating that the (-) isomer (M2266), but not the (+)isomer (M2267), of the narcotic antagonist, 5,9α-diethyl-2-(3-furylmethyl)-2'-hydroxy-6,7-benzomorphan (5.0 mg/kg), blocks the facilitatory effect of ETOH (0.4 g/kg) and CDP (4.0 mg/kg) on LH SS. M2266 (1.0 - 5.0 mg/kg) alone does not affect SS responding, but prevents the excitatory effect of MOR (1.0 mg/kg). CDP and ETOH thus appear to release an endogenous opioid which acts on opiate receptors associated with LH SS, but not MF SS, resulting in enhanced rates of response. This mechanism of action, furthermore, may mediate the positively reinforcing or euphorigenic property of ETOH and CDP.

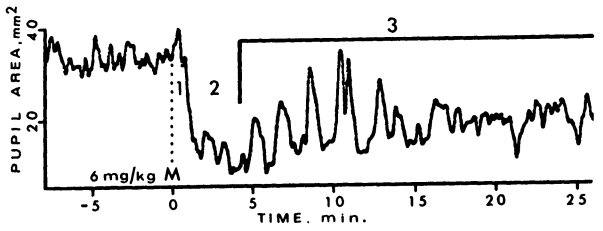
408 THE TRIPHASIC RESPONSE OF THE RABBIT PUPIL TO MORPHINE. R. B. Murray* and R. J. Tallarida* (SPON: E. B. Geller) Dept. Pharmacol., Temple U. Sch. Med., Philadelphia, Pa. 19140.

Change in pupil size upon narcotic administration is commonly determined by direct observation or still photography. A study using serial photographs taken at 30-sec intervals reported the surprising finding that morphine (M) administered i.v. to albino rabbits produces only a transient miosis which is followed by large amplitude fluctuations in pupil size (Tallarida et al., JPET 201:587,1977). The present study was undertaken to describe in detail the action of M on the rabbit pupil using continuous pupillography.

The pupillary responses of 36 albino rabbits to i.v. doses of M (1 to 12 mg/kg) were recorded with a specially designed infrared video pupillometer which measures pupil area in real time (Murray and Loughnane, Fed.Proc.37:275,1978). All experiments were carried out under constant low ambient light (luminous flux at eye = 0.87 $mW \cdot cm^{-2}$). The typical response (see figure) consists of 3 phases: 1) an immediate mydriasis lasting up to 20 sec; 2) a brief miosis which peaks within 4 min; and 3) large amplitude fluctuations in pupil area lasting approx. 2 hrs. This triphasic response is blocked by prior i.v. administration of 0.2 mg/kg of naloxone. The magnitude of phase 2 miosis is dose related. The amplitude of phase 3 fluctuation may reach 100% of the control pupil area. Time series analysis reveals that the power of pupil area fluctuations increases by 2 to 6 times and that the peak frequencies have periods in the range of 1.5 to 3 min after M administration.

Local blockade of the iris sphincter with scopolamine abolishes all phases of the M-induced pupil effects. Based on evidence from other investigators that M's pupillary action is of central origin, this finding indicates that M-induced transient miosis and pupil fluctuation are produced by perturbation of the pupil servo-control (parasympathetic) system.

Supported by grant #DA00376 and fellowship #1F31DA-05119-01 from NIDA.



409 **INTERACTION BETWEEN MORPHINE AND REWARDING OR AVERSIVE BRAIN STIMULATION IN THE SHUTTLEBOX.** **Alex E. Popov*, Carol Palmer* and Robert A. Levitt. Dept. Psych., So. Ill. Univ., Carbondale, Ill. 62901 and Dept. Psych., Univ. Ala. Birmingham, Birmingham, Ala. 35294**

Systemic injections of morphine increase the total time rats leave on hypothalamic electrical stimulation in the shuttlebox paradigm, as well as increasing the average ON time per crossing. Further, in the lever-press paradigm, rats given naloxone, a narcotic antagonist, during intracranial self-stimulation (ICSS) of the central grey, show rate decreases. In the current study, using the shuttlebox, the effect of morphine on hypothalamic (LHA) ICSS was replicated and compared with the ICSS effects with and without morphine in the septal area (LSA)., periaquaductal gray (PAG) and the mesencephalic reticular formation (MRF). The results indicate marked stimulation site dependent differential morphine effects, as well as inter-subject consistency. All experimental groups differed markedly from non-stimulated controls. The LSA subjects, compared to the LHA subjects, showed moderate rates of shuttling and a similar increase in total ON time, decrease in shuttling and increase in average ON time when given morphine. The PAG subjects showed extremely high baseline rates of shuttling and reliable increases in ON time, decreased shuttling and greatly increased average ON time under morphine. The MRF subjects, who showed marked aversion to the ICSS, had baselines characterized by low rates of shuttling and very low ON time. These MRF subjects under morphine generally further decreased ON time and rates of shuttling. Thus the general findings indicate morphine increases the amount and duration of reinforcing ICSS tolerated and does not facilitate tolerance of aversive ICSS. These results demonstrate a possible interaction between morphine and putative transmitters and add evidence to an aversion suppression hypothesis of morphine action.

410 ENHANCED SECRETORY PROTEIN SYNTHESIS RATES IN DISCRETE BRAIN REGIONS ASSOCIATED WITH DEPENDENCE DEVELOPMENT ON MORPHINE. K.C. Retz* and W.J. Steele. Dept. of Pharmacology, College of Med., Univ. of Iowa, Iowa City, IA 52242.

Ramsey and Steele (in Opiates and Endogenous Opioid Peptides, H.W. Kosterlitz, ed., Elsevier, 1976) have shown that the degree of dependence on morphine (1 pellet over 3 days), as assessed by wet dog shakes, correlates well with increases in secretory protein synthesis rates in the pons-medulla. However, because it has been reported (Psychopharmacology 52:55, 1977) that such treatment produces a low degree of dependence we decided to re-examine this question.

Rats were rendered dependent on morphine as follows: 1 pellet over 2 days (S1), 3 over 4 days, i.e., 1 on day 0, 2 on day 2 (S2) and 6 over 7 days, i.e., 1 on day 0, 2 on day 2, 3 on day 4 (S3); controls received placebo pellets. Protein synthesis rates were measured in vivo by pulse-labeling for 7 min with ^3H leucine, 2.5 mCi/0.5 mmol/kg. Brains were dissected into regions and free (non-secretory) and membrane-bound (secretory and membrane) polysome compartments were isolated therefrom (J. Neurochem. 28: 517, 1977). Separate groups were used for assaying dependence following pellet removal and naloxone, 10 mg/kg, s.c. Total drug dose was estimated as the product of daily dose times days, assuming delivery of a constant proportion of the pellet's morphine content over 3 days (Psychopharmacologia 28:35, 1973).

A linear relationship between increases in protein synthesis rates and log dose was exhibited in the secretory protein compartment of the pons-medulla (PM) and striatum-septum (SS). No changes were observed in either compartment of the cerebrum, cerebellum, mesencephalon, hippocampus-amygdala, or thalamus-hypothalamus. There was a linear relationship between maximal body weight loss during withdrawal and log dose, indicating that the former correlates well with the degree of dependence. In contrast, hypothermia was observed only with S2 and S3, suggesting that it is a less sensitive measure of dependence than weight loss. Finally, the magnitude of the increases in secretory protein synthesis rates in the PM and SS (up to 20-30% with S3) correlated closely with that of dependence assessed by maximal weight loss (17-20% with S3). Our findings not only confirm that morphine enhances secretory protein synthesis in the PM, but also extend earlier work by demonstrating that morphine induces similar changes in the SS, regions important as sites of opiate action and binding, in association with dependence development measured by an objectively quantifiable change. Supported by USPHS DA-00710.

411 THE CHRONOPHARMACOKINETICS OF SECOBARBITAL IN FASTED AND NON-FASTED RATS. Alvin L. Sermons,* Fred H. Ross* and Charles A Walker. Florida A and M University, School of Pharmacy, Tallahassee, FL 32307.

The toxicity of secobarbital in rodents have been shown to vary significantly with the time of day. Male Sprague-Dawley rats weighing approximately 200 gm each were used in this study. All animals was anesthetized with ether and the aorta was permanently cannulated by the method of Popovic (1963). Following cannulation, the animals were adapted to a 12-hour light, 12-hour dark illumination cycle for three weeks at 23 ± 1° C. The control (non-fasted) and 24-hour fasted groups were injected with sodium secobarbital (80 mg/kg) intraperitoneally every four hours. Blood samples were collected at 30, 60, 120, 180 and 240 minutes following treatment. The blood levels of the drug were determined by the method of Baer (1965). Secobarbital concentrations peaked in both the fasted and non-fasted animals at 0600 hours and troughed at 1200 hours respectively. Blood levels differed significantly between groups. The fasted were lower than the non-fasted. Changes observed for the blood concentrations of secobarbital in both groups correlates positively with the chronotoxicity of secobarbital. The results of this study suggest that fasting can influence the blood levels and chronotoxicity of specific drugs. Fluctuation in the levels of the drug in the blood and daily variations in toxicity may suggest a biological rhythm in barbiturate drug metabolism. (Supported by a grant from National Aeronautics and Space Administration).

412 ROLE OF n.ACCUMBENS IN MORPHINE INDUCED LOCOMOTOR HYPERACTIVITY IN THE C57/bl MOUSE. Herman Teitelbaum, Paul Giammatteo* and G. Andrew Mickley. Physiol. Psychol. Div., Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Mice of the C57/bl strain display locomotor hyperactivity which increases with increasing dose of morphine. A dose of 30 mg/kg of morphine produces a response that is the behavioral equivalent of the optimal dose of amphetamine (4mg/kg). Since it is known that 6-hydroxydopamine lesions of n.Accumbens block the response of amphetamine in the rat (Kelly et al., 1976) we compared the effects of lesions of posterior n.Accumbens and the bed nuclei of the stria terminalis (Group I) to the effects of lesions of the anterior olfactory nucleus and the anterior tip of n.Accumbens (Group II) on responses to both drugs.

The posterior n.Accumbens lesion was effective in completely blocking locomotor hyperactivity to amphetamine; it reduced the morphine response to half preoperative levels. The anterior lesion reduced the amphetamine response to half preoperative levels but had no effect on the response to morphine. The effects of posterior lesions were mimicked with bilateral microinjections of haloperidol into n.Accumbens. These results confirm the findings of Kelly et.al., 1976 showing that dopaminergic synapses in the n.Accumbens are necessary for amphetamine induced hyperactivity. The neural mechanism for morphine induced locomotor hyperactivity is not identical to that for amphetamine. Dopaminergic neurons of the n.Accumbens are involved in this response, but other neural elements are involved in the morphine response as well.

413 β-ENDORPHIN-INDUCED ALTERATIONS IN DOPAMINE (DA) AND SEROTONIN (5HT) METABOLISM IN DISCRETE REGIONS OF RAT BRAIN. G.R. Van Loon,* E.B. De Souza* and C. Kim* (SPON: Y. Israel). Depts. of Medicine and Physiology, University of Toronto, Toronto, Canada, M5S 1A8.

Interactions between brain monoamines and the effects of opiates have been demonstrated, although specific mechanisms remain to be defined. Endorphins have been characterized as peptides with opioid activity synthesized in brain and pituitary. It was clearly important to examine possible interactions between the endorphins and their behavioural effects, and brain monoamine metabolism.

Effects of acute intracisternal administration of synthetic human β-endorphin (15 µg) on DA and 5HT metabolism in discrete brain regions were investigated in adult male Sprague-Dawley rats. β-endorphin increased striatal concentrations of the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) as well as producing catalepsy. All of these effects were inhibited by naloxone. Rates of pargyline-induced decline in striatal concentration of DOPAC and HVA were greater in endorphin-treated than in saline-treated animals, supporting the concept that β-endorphin increases striatal dopamine turnover. β-endorphin also increased the rate of decline in striatal DA following synthesis inhibition with α-methyl-tyrosine, further suggesting that endorphin increases striatal DA turnover. It is probable that this apparent increase in striatal DA turnover is compensatory since β-endorphin appears to inhibit neuronal release of DA. β-endorphin and probenecid interacted competitively to decrease the effects of each other to increase striatal HVA, and naloxone prevented this effect of β-endorphin.

In addition to the effects on striatal DA metabolism, β-endorphin increased concentrations of 5HT and its metabolites, 5-hydroxyindoleacetic acid (5-HIAA) in brain stem and hypothalamus and decreased 5-HIAA in hippocampus. β-endorphin increased in brain stem and hypothalamus and decreased in hippocampus the rate of pargyline-induced decline in 5-HIAA, and decreased the rate of pargyline-induced accumulation of 5HT in all brain regions. Thus, β-endorphin appears to increase 5HT turnover and release in brain stem and hypothalamus and decrease 5HT turnover and release in hippocampus, while also decreasing 5HT reuptake in these brain regions or increasing 5-HIAA egress from brain.

Acute administration of β-endorphin clearly alters brain DA and 5HT metabolism. The relationship of these changes in brain monoamine metabolism with β-endorphin-induced alterations in behaviour, pain threshold, thermoregulation, etc., remain to be defined.

Supported by MRC DA-48 and MRC MA-5183.

414 THE EFFECT OF INTRAVENOUS ETHANOL ON VOLUNTARY ALCOHOL CONSUMPTION BY ALCOHOL-PREFERRING RATS. Marshall B. Waller, William J. McBride, Lawrence Lumeng* and Ting-Kai Li*. Institute of Psychiatric Research and Departments of Psychiatry, Biochemistry and Medicine, Indiana University School of Medicine, Indianapolis, IN 46202.

Sensory cues, a CNS pharmacologic effect or a combination of these factors appear to be the major determinants of ethanol (ETOH) self-selection in the free-fed unstressed rat. The present study examined the effect of intravenously administered ETOH solutions (w/v in saline) on the voluntary consumption of ethanol (10% v/v in water) by a line of rats genetically selected for alcohol preference (P-line). After a pre-infusion control period, a polyethylene cannula was implanted in the external jugular vein in male rats following the procedure of Pickens and Dougherty (1972). The animals were then divided into two groups. One group received 24 hourly infusions of ETOH daily to total 20-130% of their voluntary oral ETOH intake expressed as g of ethanol/kg of body weight/day. The other test group received 12 infusions each day to total 75-150% of the daily ETOH consumption, delivered only during the rat's dark cycle. Throughout the experiment, 10% ETOH and water were available ad libitum. When 75% or more of the ETOH usually ingested was infused intravenously, the amount of ethanol consumed orally changed dramatically. Voluntary consumption of ETOH in both groups decreased approximately 60% from 6.2 to 2.6 g/kg/d. Furthermore, the amount of ETOH infused correlated negatively with the amount of alcohol consumed orally. The r values are -0.55 for the 24 infusions/day group and -0.71 for the 12 infusions/day rats. The corresponding t values are 5.494 and 8.511 both significant beyond the 0.005 level. As alcohol intake decreased, the amount of water consumed orally increased from 5 to 30 times the volume (0.5-1.5 ml) ingested during the pre-infusion period. Thus, the total daily fluid intake was not compromised. Moreover, the total daily caloric intake remained stable throughout the experiment even though the amount of food eaten progressively declined. This suggests a voluntary compensation by the animals rather than an adverse effect of the alcohol. Finally, during a post-alcohol infusion period (saline infused), the amount of ETOH consumed orally returned to 80-100% of the pre-infusion level within 3-7 days; water intake, however, remained elevated. These results suggest that, although orosensory cues are present, the CNS pharmacologic effect provided by the infused ETOH was an important determinant of ethanol drinking behavior in these genetically selected alcohol-preferring rats. (Supported by Grant No. AA03243).

EPILEPSY

415 LESIONS OF THE INTERPEDUNCULAR NUCLEUS RETARD DEVELOPMENT OF AMYGDALOID-KINDLED SEIZURES IN RATS. Robert F. Ackermann , and Jerome Engel, Jr., Reed Neurological Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

Effects of ventral tegmental lesions on electrical amygdaloid kindling were assessed in Sprague-Dawley rats chronically implanted with bipolar electrodes aimed at the central amygdaloid nucleus. Animals were divided into 3 groups and treated as follows:

(1) lesioned: radiofrequency lesions were made in the ventral tegmental area at the time of amygdala implantation.

(2) lesion controls: the lesioning electrode was only lowered and then withdrawn with no current passed.

(3) normal controls: no ventral tegmental treatment.

Following 2 weeks of daily handling, each animal was stimulated once a day with 60 cps, 400 uA current for 1 sec. until 3 consecutive stage 5 seizures were induced. Subsequently, the locus of the stimulating electrodes, and the locus and extent of the ventral tegmental lesions were determined with routine neurohistological procedures.

The mean number of stimulations to stage 5 seizures did not differ between the normal controls (x=4.0) and the lesion controls (x=5.0); therefore they were combined into a single control group. Animals lesioned in the interpeduncular nucleus (IPN) required significantly more stimulations to achieve stage 5 seizures (x=12.4); this effect was proportional to the extent of IPN damage. By contrast, animals lesioned elsewhere in the ventral tegmentum (medial substantia nigra, ventral tegmental area of Tsai, ventral raphe) did not differ from controls (x=4.5). The fact that lesions immediately rostral to the IPN were among those having no effect suggests that these results were due to disruption of intrinsic IPN structures rather than longitudinal fibers of passage.

These data indicate that, in rats, the development of amygdaloid kindled seizures is retarded by IPN ablation, suggesting that the IPN normally facilitates kindled epileptogenesis. At present, a mechanism for this facilitation is not obvious, but it is interesting to note that the IPN is rich in acetylcholine and enkephalin, and both substances are known to readily induce seizures.

416 EXCITATORY EFFECTS OF SODIUM VALPROATE ON SINGLE NEURONS IN RAT BRAIN. H.Blume, Y.Lamour*, E.Arnauld*, L.Renaud. Division of Neurology, Montreal General Hospital and McGill University, Montreal, Quebec, Canada.

Sodium valproate (N-Dipropylacetate) is now widely recognized as an anticonvulsant drug. It has also been associated with behavioral effects in the reduction of signs of morphine and alcohol withdrawal in human and animal studies. These pharmacological effects of sodium valproate have been ascribed to the increases in levels of GABA seen in many areas of the brain including the cerebral cortex. However, the precise mode of action of sodium valproate at the single neuron level has not yet been fully investigated. In order to observe the direct effects of this drug on neurons, extracellular recordings were carried out in rat cerebral cortex and hippocampus during application of sodium valproate by microiontophoresis.

Extracellular recordings were obtained from 50 neurons in the sensory-motor and parietal cortex and dorsal hippocampus in male Sprague-Dawley rats under pentobarbital anaesthesia. Recording electrodes of saline filled micropipettes were fixed to 7-barrel microiontophoresis electrodes filled with L-glutamate, GABA, acetylcholine (ACh), bicuculline, and sodium valproate (.5M, pH 8.0). 40 neurons displayed enhanced excitability of rapid onset and termination during ejection of sodium valproate at thresholds ranging from 0-15 nA negative current. Application of L-glutamate also showed enhanced excitability of rapid onset with all 40 of these neurons. The effects of sodium valproate were rarely seen without the simultaneous ejection of L-glutamate although only a small "priming" current was necessary. The sodium valproate and L-glutamate enhanced neuronal excitability in additive fashion. ACh also increased the excitability of over half the neurons tested. Sodium valproate increased the excitability of both cells sensitive and non-sensitive to ACh. However, the simultaneous ejection of sodium valproate and ACh did not give additive effects. GABA decreased the excitability of all neurons tested and antagonized the effects of L-glutamate, ACh and sodium valproate. An understanding of the significance of the reported findings, relative to previously observed behavioral and anticonvulsant effects of sodium valproate, awaits further in-vivo studies of single cell recording in conjunction with behavioral and epileptic animal models.

(Supported by MRC)

417 CHRONIC IRON-INDUCED EPILEPTOGENIC FOCI: AN ELECTROPHYSIOLOGICAL STUDY IN RATS. W.M. Boggs*, S.A. Reid*, G.W. Sypert, J.B. Munson, and L. J. Willmore (SPON: K.M. Heilman) VA Hospital and Dept. of Surgery and Neuroscience Univ. of Fla. Coll. of Med., Gainesville, FL 32610

Subpial injections of 5 ul of 100 mM $FeCl_2$, $FeCl_3$ or 0.9 NaCl were instilled into the sensorimotor cortex of Sprague-Dawley rats. Regular cortical electroencephalographic recordings through extradurally implanted screw electrodes revealed the following: 1) Both ionic salts of iron caused focal spiking activity within 48 hours, with spread of this epileptiform activity contralaterally; 2) Frequent and sustained bursts of epileptiform activity developed within 10 days; 3) Behavioral convulsions and electrocorticographic discharges continued to persist beyond 12 weeks in 94% of the iron-injected rats, and for as long as 31 weeks in one animal before being sacrificed; and 4) Four of the 14 animals injected with NaCl exhibited transient focal spike activity lasting less than 14 days. Preliminary electrophysiological data from continuing microelectrode recording of single cortical neurons reveal that most neurons within and immediately surrounding the site of iron injection ("focus") burst in synchrony with spiking activity. Neurons more distant from the focus appear to be unaffected by such spike activity and few neurons in any location seem to be inhibited during spiking. The similarities of these findings to pathophysiological changes in man suggest the applicability of this experimental model of focal epilepsy to the study of the disease in humans. (Supported by VA Hospital Medical Research Service.)

418 DECREASED SEIZURE SUSCEPTIBILITY IN RATS FOLLOWING LESIONS OF THE LATERAL MIDBRAIN TEGMENTUM. R. A. Browning, R. L. Simonton* and M. L. Smith*. Southern Illinois University School of Medicine, Carbondale, Ill. 62901.

In an attempt to localize the seizure antagonizing effects of norepinephrine (NE), seizure susceptibility was examined in rats after surgical interruption of the ascending noradrenergic (NA) pathways. Mechanical lesions designed to interrupt the dorsal noradrenergic (NA) bundle were placed bilaterally in the midbrain tegmentum of male Sprague-Dawley rats (300-400g). The lesions were produced by lowering a piece of stainless steel tubing (1mm diameter) into the brain at the level of the inferior colliculus according to the procedure described by Erinoff et al. (Proc. Soc. Exp. Biol. Med. 150, 748, 1975). Biochemical assessment of the lesion 28-35 days post-operatively revealed a 50-60% reduction in forebrain NE, with no significant alterations in forebrain dopamine or serotonin and no effect on spinal cord NE. Histological evaluation revealed a 0.5-1.0mm wide lesion tract, which passed through the midbrain at the level of the inferior colliculus damaging the following structures: lateral aspects of periaquaductal gray, dorsal NA bundle, lateral portion of decussation of the brachium conjunctivum, reticular formation, and medial edge of ventral NA bundle. All seizure testing was conducted at least 30 days after lesion placement. In contrast to our expectations, we found a significant ($p<0.05$) reduction in the percentage of lesioned rats exhibiting hindleg extension in response to maximal electroshock (150mA, 60Hz, 200mSec) as compared to sham operated controls. Moreover, the incidence of tonic extension in the pentylenetetrazol seizure test was found to be significantly decreased ($p<0.01$) by the lesion. However, no difference in the threshold for minimal electrochock seizures was detected between sham-operated and lesioned animals. Inasmuch as seizure facilitation has been consistently observed following widespread destruction of NE neurons with 6-hydroxydopamine, the present results cannot be attributed to the lesion-induced reduction in forebrain NE. It seems more likely that damage to structure(s) other than the NA neurons are responsbile for the seizure antagonizing effects of the lateral tegmental lesion. However, the precise anatomic and biochemical changes responsible for this effect remain to be elucidated.

419 KINDLING STIMULATION OF THE RAT HIPPOCAMPUS: ALTERED NEURONAL SENSITIVITY TO MICROIONTOPHORESIS OF ACETYLCHOLINE. James L. Burchfiel*, Michael S. Duchowny*, and Frank H. Duffy (SPON: M. BIBER). Dept. Neurol. and Seizure Unit, Children's Hospital Medical Center, Harvard Med. Sch., Boston, MA 02115.

The term "kindling" describes the phenomenon whereby repeated focal electrical stimulation induces electrophysiological and behavioral changes consisting of increasing duration and spread of afterdischarge (AD) activity and increasingly more widespread motor seizures. We have been exploring the hypothesis that the electrophysiological changes of kindling reflect altered neuronal responsiveness to neurotransmitter agents. Neuronal responsiveness to microiontophoresis of glutamic acid (GA):0.02M pH4.5, gamma-amino butyric acid (GABA): 0.1M pH4.0, and acetylcholine (ACH): 0.01M pH5.2 was assessed for CA1 pyramidal cells in adult male Sprague-Dawley rats anesthetized with chloral hydrate (400mg/kg IP). Kindling stimuli (0.1 msec bipolar squarewave pulses, 100 Hz frequency, 1 sec train) were delivered to the ipsilateral fornix. The major change seen after the induction of a hippocampal AD was a prolonged period of neuronal supersensitivity to ACH. ACH supersensitivity usually occurred at 40-60 minutes post-stimulus, and could still be observed at four hours. The period of ACH supersensitivity was often preceded by a period of ACH subsensitivity. A subsequent kindling stimulus delivered during the period of ACH supersensitivity resulted in a substantially longer AD. In contrast, re-stimulation before the onset of ACH supersensitivity resulted in no growth of the AD or an actual decrease in its duration. These effects were not observed for GA or GABA. These results suggest that kindling in the rat hippocampus may be a function of altered synaptic sensitivity to ACH.

420 KINDLING IN SENSORY THALAMUS AND NEOCORTEX. A PRELIMINARY STUDY. Donald Peter Cain and Marilyn Kilbreath Dept. of Psychology, U. of Western Ontario, London, Ontario N5A 5C2 Canada

The kindling response of a number of sensory and nonsensory areas of thalamus (lateral geniculate, medial geniculate, lateral posterior, and posterior nuclei), neocortex (areas 17, 18, 41), and midbrain superior colliculus, reticular formation) was studied. A total of 110 male hooded rats were implanted bilaterally with bipolar twisted nichrome electrodes and stimulated between 100 and 200 times through each electrode at the individually determined afterdischarge (AD) or aversive response threshold. Stimulation was delivered once daily and consisted of 1 sec of constant current sine waves at 60 Hz or 4 min of high frequency bursts of biphasic square waves. Currents were in the range of 2 to 200 μA. EEGs were recorded through both electrodes before and immediately after each stimulation. In no case did animals with electrodes in the superior colliculus or reticular formation show sustained AD or behavioral convulsions. A number of animals in each of the other groups showed either AD alone or behavioral convulsions that eventually generalized with associated AD. The proportion of animals showing AD or convulsions in each group was 20 to 60 per cent. The AD and convulsion manifestations differed markedly from those obtained from limbic areas in that AD was spikier and less rhythmic, convulsions required many stimulations to develop and were of abrupt onset and atypical in form, and often did not occur in response to each stimulation after they had appeared once or a few times. However, convulsion frequency increased gradually over time, and the resulting state of seizure susceptibility was permanent. These results suggest that much of adult mammalian brain, including primary sensory areas, is plastic and subject to seizure production if directly activated with electric current.

421 REDUCED POTASSIUM CURRENTS IN THE PRESENCE OF A PERSISTENT INWARD CURRENT LEADS TO BURSTING OF CAT SPINAL MOTONEURONS. Wayne E. Crill and Peter C. Schwindt*. VA Hospital and Depts. Physiol. & Biophys. and Med., Univ. Wash. Sch. Med., Seattle, WA 98195.

Voltage clamp studies of cat spinal motoneurons reveal a steady negative resistance 10-30 mV positive to resting potential. This negative resistance is caused by a persistent inward current (I_i) presumably carried by Ca^{++}. We have postulated that I_i is an important factor in pencillin-induced bursting in motoneurons since the underlying synaptic currents measured during voltage clamp are shorter than the prolonged bursts. This is in marked contrast to the large synaptic currents seen to underly strychnine-induced motoneuron bursting. In addition, I_i is necessary for penicillin-induced bursting since no bursting is observed when I_i disappears due to injury produced by electrode impalement. Thus, we have hypothesized that I_i can dominate neuron firing behavior if the repolarizing potassium and leak membrane currents (I_K and I_L, respectively) are decreased or if I_i is increased. Specifically, a relatively small decrease in potassium equilibrium potential (E_K) caused by the raised extracellular potassium accompanying intense neuronal activity may reduce I_K sufficiently to allow I_i to produce bursting. To test this hypothesis we have adopted the expedient of decreasing E_K (and, thus, I_K) by intracellular injection of the relatively impermeant cation tetramethylammonium (TMA). This agent appears simply to displace intracellular K^+ rather than to block K^+ channels in frog node of Ranvier or spinal motoneurons. After TMA injection sustained bursts of repetitive firing could be evoked by brief stimulation only after a decrease in I_K and a small decrease in I_L allowed I_i to become net inward over a certain range of membrane potential. The interspike plateau potential during bursting was well correlated with the steady current-voltage relation of the bursting cell. Both slow and fast components of the reduced K^+ currents were still present, and E_K remained negative to rest as indicated by spike afterhyperpolarization. Similar behavior could also be obtained by tetraethylammonium (TEA) injection which blocks the fast K^+ current in addition to decreasing E_K. It is concluded that a relatively small reduction of I_K in the presence of a normal I_i is sufficient to cause bursting. This may be one mechanism by which normal neurons are recruited into seizure activity. (Supported by VA Research Grant #MRIS 1610.)

422 ICTAL MECHANISMS AND CORTICAL AUDITORY PROCESSING. D.D. Daly, D.M. Daly, J.A. Wada*, M.E. Blaw. Dept. Neurology, UT Health Sci. Ctr., Dallas, Texas 75235, and Div. of Neurol. Sci., U. British Columbia, Vancouver, Canada.

Focal epileptic seizures disrupt function of the cortical area in which they occur; postictally, paralysis of cortical function persists for periods varying from minutes to hours (e.g. Todd's paralysis). Frequently recurring focal seizures may cause more enduring focal deficits in cortical functions. In previous reports, we have shown that sparse acoustic stimuli (SAS) provide a measure of cortical auditory processing (Neurosci. Abstr. 2:5, 2:6, 1976). We now report the use of SAS in the study of cortical ictal processes.

A 9 year-old boy developed partial seizures characterized by formed auditory perceptions (voices, sound of drums); music seemed to precipitate some seizures. Six months before study, he had had progressive inability to understand speech ("word deafness") without impairment of reading and writing; two weeks before study, aphasia evolved. Retrospectively, his parents recalled increasingly frequent, short intervals of altered responsiveness (brief seizures). Perception of SAS both monaurally and binaurally was profoundly altered although early click-evoked potentials (BSR) were normal, and he localized sounds correctly. Carbamazepine was added to his drug regimen. The frequency of seizures decreased associated with slow, steady improvement in comprehension and aphasia. After one month, his perception of SAS had improved significantly although during testing, short episodes of greater impairment intervened (brief seizures). In two months, aphasia had resolved and speech perception was virtually normal, paralleled by improved perception of SAS, even when best-performance testing time was not fixed to control for diurnal variation.

In contrast, studies of a patient during partial status epilepticus of mesial frontal origin and also during intoxication with phenytoin showed normal speech comprehension and sharply defined SAS identification functions (IF). Studies of another patient with focal seizures arising in visual area showed sharp IF except when ictal propagation to the other hemisphere impaired level of consciousness and caused transient fixed responses to stimuli.

Focal seizures in auditory cortex *alter* perception of SAS, whereas focal seizures elsewhere do not. In contrast, propagation of focal ictal discharge can *impair* perception of SAS. We conclude that in focal epilepsies serial SAS testing can provide a useful measure of recovery in function and a sensitive index of effective treatment.

423 CONVULSIVE DOSES OF PENICILLIN DO NOT REDUCE A MONOSYNAPTIC GABA-MEDIATED IPSP IN THE INTACT, ANESTHETIZED CAT. J. Davenport,* P.C. Schwindt* and W.E. Crill. VA Hosp. and Depts. Physiol. Biophys. and Med., Univ. Wash., Seattle, WA 98195.

A number of recent studies using a variety of model neural systems have shown that penicillin (PCN) can greatly reduce GABA-mediated IPSPs and hyperpolarizing inhibitory responses to externally applied GABA. It has been proposed that reduction of GABA-mediated inhibition may be the mechanism by which PCN causes seizures. To test this hypothesis in an intact mammalian system, we examined the effect of I.V.-administered PCN on the monosynaptic IPSP, believed to be GABA-mediated, evoked in Deiters' neurons by stimulation of the anterior vermal cerebellar cortex.

Ten cats were anesthetized with pentobarbital (6) or α-chloralose (4). The cortical EEG was continuously monitored to indicate the effectiveness of incremental PCN doses causing progressively increased EEG activity, interictal spiking, and seizures. We specifically considered the possibility of a progressive decline of IPSP amplitude in parallel with the development of abnormal EEG activity as predicted from the above hypothesis. Intracellular recordings with KCit. filled microelectrodes were obtained from 105 Deiters' neurons. PCN doses ($\geq 2 \times 10^5$ IU/Kg) sufficient to cause frequent interictal spiking or seizures were associated with spontaneous high frequency firing of Deiters' neurons, "depolarization shifts," and large, spontaneous IPSPs. We found that in normal cells the IPSP may decrease over time concomitant with a decrease of resting potential and input resistance, probably due to injury from impalement. Therefore, we closely monitored these quantities and to ensure the stability of the IPSP before evaluating the effects of PCN. Our data are based primarily on 5 cells which fulfilled the above criteria and were held for 9-53 minutes. Although the IPSPs in these cells varied considerably with each stimulus, no consistent change in mean IPSP amplitude could be detected after single or multiple doses of PCN; in particular, there was no consistent IPSP decrement as EEG or Deiters' cell activity progressively increased. Analysis of IPSPs in populations of neurons before and after PCN in 9 experiments also suggested that PCN caused no significant change in IPSP amplitude in the cell populations. These results cast doubt on the hypothesis that reduced inhibition due to PCN-GABA antagonism is necessary to cause seizures. It is possible that cortical GABA receptors are much more sensitive to PCN than those of Deiters' neurons. Alternatively, mechanisms other than reduced GABA-mediated inhibition may produce seizures at the brain PCN concentrations resulting from I.V. PCN administration. (Supported by VA Research Grant MRIS 1610 and NINCDS Grant NS05082.)

424 REDUCTION OF POSTSYNAPTIC INHIBITION BY PENICILLIN IN THE IN VITRO HIPPOCAMPAL SLICE. Raymond Dingledine and Leif Gjerstad*. Institute of Neurophysiology, Univ. of Oslo, Oslo, Norway.

Reduced synaptic inhibition may play a role in epilepsy by allowing the unrestricted expression of excitatory synaptic events. The epileptogenic agent Na benzyl penicillin has been found to reduce presynaptic inhibition in the spinal cord (Davidoff, Br.Res.,45:638,1972) and certain postsynaptic inhibitions in invertebrates (Wilson & Escueta, Br.Res.,72:168,1974; Hochner et al., Br.Res., 107:85,1976). The aim of the present study was to monitor IPSPs and EPSPs in a cortical pyramidal cell during the transition from a normal to an epileptic state. Intracellular recordings were made from CA1 pyramidal cells in transverse slices of guinea pig hippocampus maintained in vitro. Antidromic stimulation could elicit a pure IPSP, the transmitter of which is thought to be GABA. Subthreshold orthodromic stimulation yielded a mixed EPSP-IPSP, while stronger stimulation evoked a single action potential. Penicillin, applied as a small droplet (ca. 20 nl of 17 mM) near the recording electrode, quickly reduced the size of the IPSP, with recovery by 15-20 min. In parallel the response to suprathreshold orthodromic stimulation changed from a single action potential to a burst of spikes triggered from an underlying depolarizing potential. The membrane input resistance was not changed by penicillin. The IPSP reduction was shown to be due to a partial blockade of the IPSP conductance increase by comparing the voltage-dependent behavior of the IPSP before and after penicillin application. Penicillin also blocked the conductance and potential changes caused by iontophoretic GABA. Thus, penicillin appears to act directly on the pyramidal cell membrane, although it is not known whether the GABA receptor or iontophore is affected. Concurrent with the gradual reduction of the recurrent IPSP by penicillin, the mixed EPSP-IPSP elicited by subthreshold orthodromic stimulation was converted to a progressively larger and prolonged pure depolarizing potential. The initial rising phase of the EPSP was not altered by penicillin. This finding confirms the observation of Gjerstad et al (in Adv Epileptology, Meinardi & Rowan (eds), Swets & Zeitlinger, 1978), and is in accord with their finding that the presynaptic fiber volley and field EPSP are unchanged. Thus the processes underlying EPSP generation appear unaltered by penicillin. The timecourses of IPSP reduction and EPSP growth were similar, and the EPSP showed no further change after the IPSP was maximally inhibited. We conclude that the blockade of inhibition by penicillin may explain the enhanced response to weak orthodromic stimulation. We emphasize, however, that it is still unclear to what extent the removal of somatic inhibition contributes to the generation of intense burst discharges seen with stronger afferent stimulation.

425 ACUTE CARBON MONOXIDE HYPOXIA AND SEIZURES. Robert S. Dyer, Elizabeth Burden*, Karen Hulebak*, Nancy Schultz*, H. Scott Swartzwelder and Zoltan Annau. Neuroscience Prog., Nat'l. Ctr. Toxicol. Res., Jefferson, AR, and Dept. Environ. Health Sciences, Johns Hopkins Univ., Baltimore, MD.

Many attempts have been made to examine the consequences of exposure to various levels of CO hypoxia upon CNS function, yet disagreement still exists regarding appropriate methods for assessing toxicity. In the present report we describe attempts to develop a seizure model for assessing the neurobehavioral response to CO hypoxia. Exposure to CO is monitored according to the gas concentration, but the probable parameter of physiological significance is %HbCO. In the rat, exposure to a constant concentration of CO produces equilibration of HbCO in about 90 min. The %HbCO values for concentrations of 1000, 500, 250 and 0 ppmCO are about 55%,38%,22% and 0%. Rats were exposed to either 1000ppm or 0ppm CO for 2 hr and injected with either 30, 40 or 50 mg/kg pentylenetetrazol. No differences in seizure severity or duration were observed between the exposed and control groups at any dosage. In the second experiment animals were exposed for 2 hr to either 1000, 500 or 0 ppm CO and tested for 6 Hz electroshock responses. Again no differences were observed between exposed and control groups. Finally, animals were implanted with stimulating and recording electrodes in the dorsal hippocampal formation (HPC) and exposed to either 1000, 500, 250 or 0 ppm CO. HPC afterdischarge (AD) properties were studied. CO hypoxia did not significantly change the duration of the AD, nor did it significantly increase the duration of the postictal depression of the EEG. As concentration of CO increased, the spike frequency within the AD and the probability of a rebound AD decreased. Rebound AD's occurred in 100% of the 0ppm, 80% of the 250ppm, 60% of the 500ppm and 0% of the 1000ppm CO groups. The significance of the rebound AD's and their disappearance under hypoxia is not known. It may be pointed out that rebound AD's decrease in frequency with dosages of sodium pentobarbital as low as 10mg/kg. We conclude that the HPC AD model is the most reliable of those tested for studies of CO-induced hypoxia.

426 **ANTICONVULSANT ACTIVITY OF DIPROPYLACETIC ACID AND DIPROPYLACETAMIDE IN THE BABOON PAPIO PAPIO.** **C. L. Ehlers*, L. W. Mulbry* and E. K. Killam* (SPON: C. H. Sawyer). Department of Pharmacology, School of Medicine, UC Davis, Davis, CA 95616.**

The ability of dipropylacetic acid (DPA) and dipropylacetamide (DPM) to modify seizures induced by intermittent light stimulation (ILS) was assessed in 4 female and 3 male Papio papio. Whereas repeated oral doses of 100-200 mg/kg of DPA given over an 8-hr period, which produced blood levels of 19-44 ng/ml were not effective in modifying ILS-induced seizures, repeated doses of DPA (200-300 mg/kg) given over a 32-hr period produced blood levels of 62-372 ng/ml and were highly protective against ILS.

Similar results were obtained following the administration of DPM. Repeated oral doses of 200-400 mg/kg given over an 8-12 hr period produced blood levels of 18-91 ng/ml and caused a mild reduction in seizure activity, whereas repeated doses of 200 mg/kg given over a 36-hr period produced blood levels of 124-137 ng/ml and were completely effective in blocking ILS-induced seizures. Both agents produced a quieting effect upon behavior without severe depression.

Background EEG in response to DPA and DPM showed evidence of a striking increase in relative power in the 10-15 Hz range primarily in the parietal cortex.

(Supported in part by NS 08935)

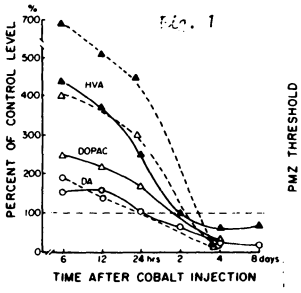
427 CHANGES IN RETICULAR FORMATION NEURONAL RESPONSES TO SENSORY STIMULI INDUCED BY STRYCHNINE. C.L. Faingold and J.D. Stittsworth, Jr. Division of Pharmacology, Dept. Medical Sciences, Southern Illinois University, School of Medicine, Springfield, Illinois, 62708

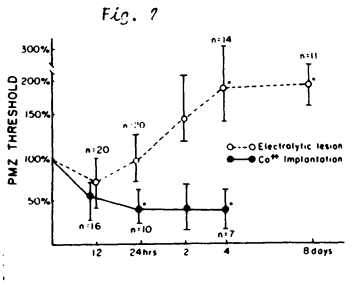
Strychnine administration has been shown to enhance sensory-evoked field potentials in the brainstem reticular formation (RF). Response enhancement in the RF is considerably greater than that seen in primary sensory sites and occurs at a lower dose of strychnine than that which enhances the responses in other non-primary sensory sites (Faingold, Neuropharm. 16:73-81, 1977). This study was undertaken to elucidate the neuronal events associated with this response enhancement in the RF utilizing locally anesthetized, paralyzed cats. The response patterns of RF neurons to auditory, visual or somatosensory stimuli were characterized using poststimulus time histograms. Many neurons which were unresponsive to sensory stimuli before strychnine became responsive to the stimuli after strychnine administration (i.v. 0.025 mg/kg/min). Other neurons which were responsive to the stimuli became more responsive after strychnine administration. However, most of these neurons became temporarily unresponsive at the onset of strychnine-induced 10-20 Hz regular spiking in the EEG of the lower brainstem. At this time many of the neurons began to fire tonically at the same 10-20 Hz frequency. Sensory responsiveness returned and/or was enhanced when the EEG pattern either proceeded to the next stage of strychnine-induced activity (occasional high amplitude brainstem EEG spikes) or if the EEG recovered to normal. The doses of strychnine which produced these effects were considerably less than those which cause seizure generalization. With time, response patterns returned to those observed before drug administration. Strychnine-induced enhancement of the sensory response of RF neurons is quite extensive and very similar to that observed with pentylenetetrazol (Faingold and Caspary, Neuropharm. 16:143-147, 1977). In contrast, previous evidence indicates that these convulsants induce only minimal changes in sensory-evoked field potentials (Faingold, Neuropharm. 16:73-81, 1977) and single unit responses in primary sensory sites (Faingold and Stittsworth, Neurosci. Abs. 3:139, 1977). These data suggest that the enhancement of neuronal responses to sensory stimuli may be a general action of convulsant agents but may be a specific effect on non-primary sensory neurons such as those in the reticular formation.

(Supported in part by Southern Illinois University Foundation.)

428 *METRAZOL (PMZ) THRESHOLD IN RATS WITH LESIONS OF THE SUBSTANTIA NIGRA (SN).* *R. G. Fariello and O. Hornykiewicz. Depart. of Neurology, University of Wisconsin, Madison, Wis. USA and Clarke Institute of Psychiatry, Toronto, Canada*

Three groups of preselected rats with a stable PMZ threshold for generalized convulsions (less than 20% variation in three tests in different days) were used for the study. Group A was injected with 2µg cobalt HCl in 0.6µl of saline in the left SN. Group B received left nigral electrolytic lesion and group C were sham operated. As we have previously reported (Shibuya et al, Exp. Neurol, 58, 486-499, 1978) such lesions induce changes in DA and metabolite content in the ipsilateral neostriatum. The time course and the magnitude of these changes are illustrated in fig. 1 where the continous line refers to group B and the dotted line to group A. Rats of the three groups were retested for PMZ threshold at various times after surgery. At the end of the experiments they were sacrificed and histologically examined and likewise the subjects that died during the experiments. PMZ threshold in group A and B changed as shown in fig. 2. Cobalt lesioned rats showed a steady decrease in threshold. Electrolytically lesiond rats showed changes in the opposite direction to DA changes in the neostriatum. Group C did not show variations. Cobalt caused a progressive necrotic lesion extending over time beyond the SN, whereas electrolysis induced a small stable lesion of the SN. It is therefore likely that the continous decay of the PMZ threshold in group A is unrelated to DA changes and linked to the epileptogenic action of cobalt on extranigral structures. Results from group B indicate that in rats increase of DA in the neostriatum is associated with decreased resistance to PMZ threshold and vice versa. The change in seizure susceptibility may be linked to the different functional state of the caudate nucleus.





429 CHOLINERGIC MODULATION OF INTERICTAL SPIKING IN KINDLED RATS J. Gregory Fitz* and James O. McNamara. Epilepsy Center, Durham VA Hospital, Duke Univ. Med. Center, Durham, N.C. 27705

Spontaneous interictal spiking (SIS) is the electroencephalographic (EEG) hallmark of epilepsy. Kindling is a recently developed animal model of epilepsy induced by periodic electrical stimulation of the brain. In response to successive stimulations there is a progressive intensification of stimulus induced epileptic activity. This culminates in a fully kindled seizure consisting of rearing, falling, and clonic limb movements. The goal of this study was to determine the role of muscarinic cholinergic synaptic mechanisms in the regulation of SIS in kindled rats.

Multiple depth electrodes were stereotaxically implanted into the brains of male Sprague-Dawley rats. Group I rats received no stimulations, Group II rats were fully kindled, and Group III rats were fully kindled and then treated with drugs known to affect the muscarinic cholinergic system. All kindled rats received hourly stimulations (400 µA, 1 sec duration) until a single fully kindled seizure was elicited. Thereafter, all animals were electroencephalographically monitored for > 1 hour per day for 7 days. A field effect transistor circuit eliminated movement artifact, and permitted quantitation of SIS in awake, unrestrained animals.

SIS was never observed in Group I animals. In Group II animals, SIS developed and declined in a highly reproducible temporal and spatial pattern. The frequency of SIS was maximal prior to completion of kindling, and disappeared 3-4 days after cessation of stimulation. In Group III animals: a) physostigmine (0.3 mg/kg IP) caused a 73% reduction in the frequency of SIS ($p < .01$) whereas saline had no effect; b) atropine (25 mg/kg IP) given to kindled rats with no SIS 7 days after kindling caused a rapid reactivation of SIS ($p < .001$); c) this atropine induced reactivation was reversed by physostigmine ($p < .005$); d) scopolamine (10 mg/kg IP) also reactived SIS, and e) neither atropine, physostigmine, scopolamine, nor saline caused SIS in control rats. Taken together, these results are consistent with the notion that muscarinic cholinergic synaptic transmission is capable of modulating SIS in kindled rats.

439 THE EFFECTS OF OPIATE-ANTAGONISTS ON THE SEIZURE PATTERNS OF AN ANIMAL MODEL OF EPILEPSY. H. Frenk, L. Pauly J. Diaz, and B. Bailey*(SPON: R. J. Schain). Dept. Psychology, Tel Aviv University, Ramat Aviv, Israel, Depts. Psychology, Neurology, Psychiatry, UCLA, Los Angeles, CA 90024.

Strains of the Mongolian gerbil (Meriones unguiculatus) which spontaneously exhibit severe generalized motor seizures have proved to be excellent models of epilepsy. Cortical EEG's recorded during these seizures show continuous hypersynchronous bursting of high amplitude similar to those observed in human grand mal seizures. Conventional anticonvulsants (i.e., phenobarbital, phenytoin) protect the adult gerbil from seizures.

Recent studies of endogenous opiate peptides in the brain have shown that these substances are potent neuromodulators and that alterations in these systems produce bizarre behavior such as prolonged muscular rigidity and catalepsy (Blum, et al, Science, 194, 1976). The findings that these peptides also cause pronounced and sustained epileptic electroencephalographic activity (Frenk,et al, Science, 200, 1978) have led some investigators to speculate that endogenous peptides may play a role in epilepsy. The purpose of the present study is to examine the effects of opiate antagonists upon the seizure behavior of gerbils.

Adult gerbils with reliable seizure behavior were treated with opiate antagonists in four separate experiments: 1) chronic administration of naltrexone, 10 mg/kg; 2) acute treatment with naltrexone, 10 mg/kg; 3) examination of a dose response curve of acute naltrexone injections (0.1, 1, 5, 10, 50 mg/kg); and 4) acute naloxone treatment in conjunction with phenobarbital (20 mg/kg) administration.

At all dose levels examined, neither opiate antagonist affected the intensity of the seizures, the duration of the individual seizures, or the latency for the animal to seize.

These data strongly suggest that endogenous opiate systems do not mediate the seizure phenomena of this model of epilepsy.

431 NEURONAL INTERACTIONS IN EXPERIMENTAL EPILEPTOGENIC FOCI. Richard N. Harner, Otto M. Sgro*, Department of Neurology, Graduate Hospital, University of Pennsylvania, Philadelphia, Pa. 19146

Large, extracellular units are recorded from a linear array of four insulated tungsten microelectrodes (impedance > 10 Megohms at 1 KHz) spaced at 100 micron intervals in the forepaw area of S_1 during 1-2/sec stimulation of the contralateral median nerve before and after topical application of Penicillin (100,000 U./ml) in rats anesthetized with 6 mg/100 g pentobarbital. Prior to penicillin, units had a post-stimulus latency of 6-18 msec. with infrequent and highly variable interactions between units recorded from nearby electrodes. After penicillin, latency and PST histograms showed (1) increased frequency and decreased variability of unit responses, (2) development of "tight connections" at latencies of 0.5-5.5 msec between nearby units and (3) bursts of unit activity at frequencies up to 500 Hz, correlated with the surface cortical potential. Nearby units may be heterogenous with respect to sensory modality, response to electrical stimulation, and burst response to Penicillin. Intensification and development of 3-6 msec interactions between nearby units produced by Penicillin suggests a possible role for long-loop and/or polysynaptic mechanisms in the epileptogenic process.

432 OPERANT CONDITIONING OF 12 - 16 HZ SENSORIMOTOR RHYTHM REDUCES MOTOR SEIZURES MORE THAN PSYCHOMOTOR SEIZURES. William J. Jackson, Arden V. Nelson*, and June O. Kearns*. Depts. of Physiology and Biomedical Engineering, Medical College of Georgia, Augusta, GA. 30901.

Four previously intractable psychomotor epileptics were compared to a group of previously intractable epileptics with focal motor or generalized convulsive disorders following 75 operant training sessions. The patients had learned to increase the density of 12 - 16 Hz activity recorded between Cz and either C3 or C4. All patients showed significant increases in the density of 12 - 16 Hz activity following training, but not all the patients realized improvement in their seizures as a result. Improvement was greater in the group with focal motor and generalized convulsions, with all of these patients showing some improvement in their seizure condition. Improvement included fewer seizures and sometimes less severe seizures. Only one of the four patients with temporal lobe foci showed significant seizure reduction following training, although the improvement in this one patient was substantial. One of the psychomotor patients reported increased numbers of seizures during training.

An additional factor involved the design of the devices which analysed the signal prior to feeding a cue back to the patients. Half of the patients in each of the two diagnostic categories were trained by use of one or the other of two signal analysis techniques. One signal analysis method utilized zero crossing techniques adjusted to trains of 12 - 16 Hz activity. A feedback cue was provided to the patients when at least 5 of the previous 8 zero crossings were within the 12 - 16 Hz band. The other method of signal analysis utilized eliptic filters to separate the 12 - 16 Hz band from the 0.2 - 40 Hz band. A computer then compared the two bands and a feedback cue was provided the patient if the power within the 12 - 16 Hz band was at least 20% of the power within the 0.2 - 40 Hz band for the previous second. Good learning curves were achieved using both devices. Each has its own sets of advantages and disadvantages, but the zero cross system is less expensive. (Supported by NIH-NINCDS Contract No. NO1-NS-6-2340)

433 ANTICONVULSANT ACTIVITY OF DIPROPYLACETIC ACID (DPA) IN EPILEPTIC FOWL. D.D. Johnson, H.L. Davis* and R.D. Crawford*. Depts. of Pharmacol. and Animal & Poultry Science. Univ of Sask. Saskatoon, Sask. Canada S7N 0W0

The seizure process in epileptic fowl is sensitive to the anticonvulsant actions of phenobarbital (Pb), phenytoin (Ph) and primidone (Pr) but not to ethosuximide. With Pb and Ph anticonvulsant activity occurred within the range of plasma concentrations achieved in the control of grand mal seizures in humans. Although Pr itself had anticonvulsant activity, metabolically derived Pb contributed to the anticonvulsant effect. This data indicates that epileptic fowl represent a potential pharmacological model of grand mal epilepsy. To further characterize this model, dose-response studies have been conducted with DPA, an agent whose major clinical value appears to be in absence seizures although it appears to have some efficacy in other epilepsies. DPA in doses of 25, 50, 75, 100 and 125 mg/kg were administered (i.v.) to groups of 8 epileptic chickens using a cross-over experimental design. Seizure susceptibility in response to stroboscopic stimulation was determined 1, 3, 6, 12 and 24 h later and compared to that in a control group treated with saline. At 1 h DPA 125 mg/kg produced complete protection and statistically significant protection occurred with all doses above 25 mg/kg for a 3 h period. No anticonvulsant effect was observed at 6 h. Only a minimal amount of sedation occurred at the highest dose level.

Supported by the MRC of Canada

434 TRIMETHADIONE REDUCES POSTSYNAPTIC POTENTIAL AMPLITUDES IN NEURONS OF THE ABDOMINAL GANGLION OF APLYSIA. W.M. King* and N.R. Kreisman (SPON: P.S. Guth). Dept. of Physiology, Tulane U. Sch. Med., New Orleans, LA 70112.

Trimethadione (TMO) is the prototypical anticonvulsant agent effective in suppressing petit mal epilepsy. To gain some insight into its mechanism(s) of action at a cellular level, we have investigated the effects of TMO on synaptic transmission between identified neurons in the abdominal ganglion of Aplysia californica.

Simultaneous intracellular recordings were made from the cholinergic interneuron L10 and two monosynaptically connected follower neurons, L5 and R16, which receive an ipsp and epsp, respectively. We found that TMO (1-10 mM) reduced the amplitudes of both psp's as well as L10's action potential in a dose-dependent manner. In control observations, however, it was noted that activation of an unidentified neuron or set of neurons ("interneuron II") produced effects similar to those of TMO on both L10's action potential and its followers psp's. This observation, coupled with an earlier finding that TMO increases "interneuron II" activity (Kreisman et al. Comp. Biochem. Physiol., in press), implies that the effects of TMO on synaptic transmission from L10 may be the consequence of actions at other sites.

In order to avoid the problem of recruited synaptic input as well as to assay TMO's effect on presynaptic functions, we examined the cholinergic epsp produced in R15 by stimulation of the right connective. A 1 per sec. train of stimuli to the right connective produces an initial depression of epsp size followed by facilitation and post tetanic potentiation (PTP) (Schlapfer et al. Brain Res. 76:267-280, 1974). We found, that TMO (1-10mM) produced a dose-dependent decrease in the size of all epsps of a train without affecting the relative degree of depression, facilitation, or PTP. Simulation of "interneuron II" input to R15 by stimulation of the branchial nerve prior to the train did not alter the amplitude of any of the epsp's in the train, essentially ruling out any influence of "interneuron II". Bath application of acetylcholine (ACh) was conducted in the presence and absence of TMO to test for a possible postsynaptic site of action. TMO in doses up to 10mM had no effect on the depolarization produced in R15 by ACh.

These results are consistent with a presynaptic action of TMO leading to reduced transmitter release. The mechanism of the reduction in transmitter output will be the subject of further investigation. (Supported by grants from NIH NS-12419 and the Schleider Foundation.)

435 COMPUTER-ASSISTED ELECTROMICROPHYSIOLOGY OF THE DEVELOPMENT OF PENICILLIN INDUCED FELINE GENERALIZED EPILEPSY. George Kostopoulos*, Pierre Gloor, Andrea Pellegrini* and Jean Gotman*. Dept.Neuro.& Neurosurg.McGill Univ.MNI, Montréal,Canada H3A 2B4.

In acute preparations of 42 awake, immobilized cats we studied the activity of neurons located in the deep layers of the medial suprasylvian gyrus. The EEG was recorded by the extracellular microelectrode and at the point where it entered the cortex. Recordings lasting 3-5 min were stored on tape in regular intervals during the time in which the cortical response to single medial thalamic volleys was transformed from spindles (initially) to spike wave bursts(SW) (1-2 hours after penicillin administration). Analysis of data included unit-EEG correlations, wave-unit correlations and laminar profiles using a PDP-12 computer. Our results reaffirm the important role of synchronization of neuronal discharges in rhythmical EEG phenomena. Statistically appreciable neuronal excitation was often associated with negative waves of type I spindles (of Spencer and Brookhart, 1961) but very rarely with those of type II spindles. During SW, neuronal excitation coincided with the first part of the large negative-going transient of the "spike" while an increasing inhibition during the "wave" was the most characteristic phemomenon of this form of epilepsy. During either of the above conditions the increased probability of neuronal discharge was associated with transients which connect negative and positive peaks of biphasic surface EEG waves, and especially when the positive wave came first as is more often the case in SW than in spindles.

Observations like the above in conjunction with previous studies from this laboratory provide clues which may explain how thalamocortical volleys responsible for the production of spindles can produce SW under conditions of altered neuronal excitability induced by penicillin.

436 LOCAL CHANGES IN PO_2 INSIDE AND SURROUNDING EPILEPTOGENIC FOCI PRODUCED BY PENICILLIN OR FREEZING. Norman R. Kreisman, Thomas J. Sick* and D. Woodrum Pierson*. Dept. Physiology, Tulane U. Sch. Med., New Orleans, LA 70112.

It is well known that generalized epileptiform activity is accompanied by increases in brain blood flow and oxygen consumption. However, little is known about local changes in these parameters in the vicinity of focal epileptogenic lesions. Local changes in PO_2 were recorded with polarographic microelectrodes from two separate loci in the superficial layers of the bullfrog telencephalon. The first polarographic microelectrode was placed inside the boundaries of the region which was to become the epileptogenic focus and the second was placed 2-3mm adjacent in the same hemisphere or in the homotopic region of the opposite hemisphere. The ECoG was recorded from bipolar electrodes on each hemisphere. After placement of all electrodes focal epileptiform activity was initiated by topical application or microinjection of Na penicillin G. An increase in local PO_2 consistently occurred within seconds in all recorded areas and preceded the onset of ECoG signs of epileptiform activity in the primary focus in 4 of 13 experiments. Paroxysmal ECoG spike onset occurred within 5 min in the treated hemisphere and was associated with phasic decreases in PO_2 which were superimposed upon the initial PO_2 increase. The PO_2 decreases began simultaneously with each interictal discharge but outlasted the discharge by 30-60 sec. As the frequency of interictal discharges increased, the phasic PO_2 dips summated, driving the PO_2 level down toward 0 mm Hg. Similar PO_2 changes were recorded adjacent or homotopic to the focus but the onset of paroxysmal discharges and associated PO_2 decreases were absent or delayed. The onset of paroxysmal discharges in regions adjacent to the focus may have been the result of contamination by diffusion of penicillin. In order to avoid complications of penicillin diffusion, experiments were repeated with epileptiogenic foci produced by focal freezing. An increase in PO_2 was observed in adjacent and homotopic points but epileptiform activity and transient PO_2 dips were confined to the region of the freezing focus.

We interpret the increase in PO_2 to represent an increase in local blood flow, and the superimposed phasic PO_2 decreases to represent increases in O_2 consumption. Further study is required to elucidate the mechanism of the increase in blood flow. This blood flow increase in the "surround" may serve to aid in restricting the epileptogenic focus by preventing accumulation of diffusing metabolites. (Supported by USPHS grant NS 12419.)

437 CORTICO-CAUDATO-THALAMIC INTERACTIONS IN EXPERIMENTAL FOCAL EPILEPSY. John A. Kusske. Div. of Neurological Surg., UCI-Irvine, and VA Hospital, Long Beach, CA 90822

To study the interaction of corticothalamic, corticocaudate and caudatothalamic networks during the propagation of seizures neuronal activity has been recorded from the anterior sigmoid gyrus of cats with a tungsten microelectrode placed 1-2 mm from the site at which 0.03 to 0.05 ml of tungstic acid gel was injected subpially to produce acute recurring model seizures. Two microelectrodes were also placed in the head of the ipsilateral caudate nucleus, the lateral most being positioned in a site somatopically related to the cortical focus; a fourth electrode was placed in the ipsilateral ventral anterior thalamus. Both multineuronal unit activity and slow waves were recorded on magnetic tape; the time course of cortical and subcortical events was studied by transcribing the magnetic tape records on paper by means of a Honeywell Visicorder. Frequency spectra were computed, using a PDP-12 computer, on each channel of data. Initially burst firing patterns and large field potentials were recorded at the focus; these events were followed by field potentials in the thalamus and both caudate sites at latencies appropriate for multi-synaptic transmission. An increase in the rate of unit discharge in the lateral caudate was evident with each burst recorded at the focus; this was not apparent in recordings from the medial caudate. Recurring seizures, with propagation of afterdischarge to all recording sites, characterized the final maturation stage of the models. Frequency spectra derived from the four recording sites demonstrated sequential, progressive involvement at each location. There was spread of afterdischarge from the cortical epileptogenic focus to the thalamus and then to both caudate recording sites; delays ranged from 5-10 sec before there was an increase in the energy levels in the caudate nucleus following thalamic activation; usually there was an abrupt rise at both sites simultaneously. This study suggests that cells in the caudate nucleus which are somatopically related to the cortical focus can be driven by rapidly discharging cells in the seizure focus. Even though cells in lateral caudate are readily activated by hypersynchronous cortical neuronal activity it is apparent, here at least, that thalamic activation must occur before seizure afterdischarge can be recorded in the caudate nucleus.

438 ELEVATED AMINO ACIDS IN AN AUDITORY NUCLEI OF EPILEPTIC RATS. Hugh E. Laird and Ryan J. Huxtable* Depts. Pharmacol and Toxicol. and Pharmacol., Colleges Pharm. and Med., Univ. Ariz., Tucson, AZ 85721

There are several reports associating deficiencies in glutamic acid, γ-aminobutyric acid and taurine with experimental and human epilepsies. Recently, van Gelder (In: Taurine and Neurological Disorders, pg. 387, Raven Press, 1978) has suggested that a loss of taurine accompanied by an alteration in the glutamine/glutamate balance in the brain are causative factors in some types of epilepsy. Brain amino acid analyses in our laboratory on the genetically seizure susceptible rat (GS), indicate that this theory is not a general explanation of the CNS biochemical defect of epilepsy. GS rats show lower thresholds for seizure in the inferior colliculus (IC) than do non-GS rats of the same strain (Laird & Huxtable, Neurosci. Abst. 3:42, 1977), whereas no difference in cortical thresholds were found between the strains.

Table I shows selected and total amino acid concentration in the IC and cortex (C) of GS and non-GS rats. The concentration of the amino acids are uniformly higher in the IC of GS subjects whereas there is no difference in amino acid levels in the C. Also, if amino acid ratios are compared in IC and C of GS and NGS rats there are no differences in ratios between these rat strains in either brain area. These data do not support the suggestion that epileptogenesis is the result of loss of neuronal taurine in conjunction with a derrangement in the glutamine/glutamate balance.

TABLE I

Brain Amino Acid Concentrations (μmoles/gm)

	Inferior Colliculi		Cortex	
	GS (n=12)	NGS (n=10)	GS (n=11)	NGS (n=9)
Tau	2.25(0.45)	1.94(0.07)	5.60(1.05)	5.96(0.87)
Asp	3.49(0.80)	2.94(0.94)	3.56(1.59)	3.61(0.64)
Gln	2.35(0.55)	1.99(0.52)	2.46(0.58)	2.38(0.48)
Glu	6.36(1.41)	5.33(1.56)	11.32(3.90)	10.70(1.80)
Gly	1.57(0.44)	1.33(0.47)	0.70(0.29)	0.70(0.22)
GABA	2.94(1.41)	2.75(1.33)	1.50(0.65)	1.72(0.69)
other amino acids	2.50(0.38)	2.20(0.34)	3.56(0.51)	3.63(0.56)
TOTAL amino acids	21.46(1.83)	18.48(1.55)	28.70(3.38)	28.70(3.23)

Values represent means ± S.D.

This work was supported by USPHS grants HL 19394 and HL20087.

439 EFFECTS OF ANESTHETICS AND ANTICONVULSANTS ON THE ACTION OF KAINIC ACID INFUSED INTO THE RAT HIPPOCAMPUS. Mark F. Nelson, Robert Zaczek* and Joseph T. Coyle. Dept. Pharmacology, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205

The injection of 2 µg of the potent excitatory neurotoxin, kainic acid (KA) (Coyle and Schwarcz, Nature 263:244, 1976), into the hippocampus causes degeneration of the intrinsic neurons while sparing cholinergic and adrenergic afferents. During the 4-6 hrs. after injection, the rats exhibit epileptiform behavior with dystonic posturing (DYS), hyperactivity (HA) and generalized convulsions (CNV). The effects of various anesthetics and anticonvulsants on the behavioral and neurotoxic actions of a threshold dose of KA (0.5 µg) injected into the hippocampus were examined.

After anesthesia with Equithesin [EQ; 40 mg/kg pentobarbital (PB) plus 200 mg/kg chloral hydrate (CH)] rats exhibited DYS and HA; 4 days after the injection glutamate decarboxylase specific activity (GAD-A) was reduced to 80% of control. After ether anesthesia, rats displayed DYS, HA and CNV, and GAD-A was reduced significantly further to 65%. Similarly, treatment with the short-acting anesthetic, hexobarbital, potentiated the epileptiform behavior and the neurotoxic action of KA. In contrast, 6 hr. anesthesia with EQ prevented the appearance of DYS, HA and CNV upon awakening and significantly limited the fall in GAD-A to 90%. Anesthesia with PB alone (65 mg/kg) provided some protection while CH alone (400 mg/kg) was ineffective against KA. Pretreatment of ether-anesthetized animals with the anticonvulsants diazepam (10 mg/kg), phenobarbital (50 mg/kg), phenytoin (50 mg/kg), or carbamazepine (75 mg/kg) attenuated the epileptiform behavior and reduced the fall in GAD-A as compared to rats anesthetized with ether alone.

The protective effect of non-sedative anticonvulsants and the lack of such an effect with CH suggests that sedation is not the fundamental factor altering the action of KA. Rather, the ability of drugs to limit neuronal excitation is more likely the primary mechanism. Hippocampal kainate injection may offer an animal model for temporal lobe epilepsy. (Supported by USPHS Grants NS 13584, RSDA KO2-MH 00125, The McKnight Foundation and a Pharmaceutical Manufacturer's Association Fellowship).

440 INTRINSIC NEURONAL MEMBRANE ABNORMALITIES IN HUMAN EPILEPTIC FOCI AS INFERRED FROM EFFECTS OF FOCAL COOLING. George A. Ojemann, William H. Calvin and Arthur A. Ward, Jr. Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195

Focal cortical cooling has been used as a technique for studying neuronal membrane abnormalities in animal models of epilepsy (Reynolds, Ojemann & Ward, Exp. Neurol. 46:583, 1975). Compared to normal cortex, intracellular recordings from bursting neurons in epileptic foci demonstrate a more rapid rate of action potential (AP) widening with cooling, and a tendency to decreased bursting (normal cortical neurons become burstier with cooling). The rate of widening of the rising and falling phases of the AP with cooling differed in the alumina and penicillin models of epilepsy. In the penicillin model, the rising phase widens more rapidly than the falling; the alumina model shows the reverse. These data were interpreted as suggesting different neuronal membrane changes in the two models: abnormalities of passive Na+ exchange and Na-K pumping in the penicillin focus; altered K+ flux (and accelerated pumping) in the alumina focus.

The present study is directed at determining whether either of these models fits human epileptic foci. Although intracellular recording during cooling in man is not presently feasible, extracellular microelectrode recordings can be obtained from human epileptic foci in patients undergoing craniotomy and cortical resection (Calvin, Ojemann & Ward, EEG 34:337, 1973). The changes in bursting and AP width in units that can be followed through a cooling cycle are likely to be independent of geometric changes that might interfere with extracellular recordings. These were obtained with tungsten microelectrodes; focal cooling to temperatures of 26°-30°C was accomplished through a silver footplate attached to a heat pipe; temperature was monitored by a thermistor advanced in parallel with the microelectrode.

Recordings during cooling of bursting neurons in human epileptic foci have been obtained from seven patients at the time of writing this abstract. These neurons show the tendency to decreased bursting with cooling characteristic of the epileptic models rather than normal cortex. But the average rate of AP widening has been close to that of neurons in normal cat cortex. Most commonly, the falling phase of the AP has widened substantially more rapidly than the rising phase similar to neurons in alumina foci, and qualitatively different from neurons in penicillin foci. This finding suggests that intrinsic neuronal membrane abnormalities in human epileptic foci are qualitatively similar to those of the alumina focus, modelled as involving alteration in K+ flux.

Supported by NIH Grants NS 04053 and NS 09677.

441 POSSIBLE STRUCTURAL CORRELATES OF SPONTANEOUS SEIZURES IN MONGOLIAN GERBILS. L. Paul* I. Fried*, K. Watanabe*, J. Diaz, and A. B. Scheibel. Depts. of Psychology, Neurology, Psychiatry and Anatomy, UCLA, Los Angeles, CA 90024.

The Mongolian gerbil (Meriones unguiculatus) has been proposed as a model for the epilepsies (Loskota, et al, Epilepsia, 15, 1974) and strains of seizing and non-seizing gerbils have been established by several laboratories. Structural changes in the hippocampus of human temporal lobe patients have been reported (Scheibel, et al, Epilepsia, 15, 1974) and putative changes in hippocampal structures in kindled rats are presently under investigation in our laboratory. Following our experience in these areas, Golgi studies were performed on perfused and immersion-fixed brains of adult gerbils with known seizure histories.

Although our studies must be considered preliminary, several types of changes present in the actively seizing group seem worthy of description. 1) Mossy tufts of dentate granule cell axons appear enlarged in size and increased in number, most obviously at the transitional zone between CA3 and CA4. The tufts in non-seizing animals ranged in size from 2 to 7 micra, while those in seizure-prone gerbils were as large as 11 micra. 2) In many animals, the parent axons showed frequent beading. 3) Hippocampal pyramids in CA3 showed swelling of the cell body and proximal segment of the apical dendrite with patchy or even complete spine loss of many basilar shafts.

Selected electron microscopy studies are currently underway in our laboratory to confirm these changes.

442 INCREASED SEIZURE SUSCEPTIBILITY IN ADULT MICE FOLLOWING NEONATAL TREATMENT WITH GLUTAMATE OR ASPARTATE. William J. Pizzi, June E. Barnhart* and James R. Unnerstall*. Neuropsychology Lab, Northeastern Illinois Univ., Chicago, Il. 60625

Neonatal administration of the amino acids glutamate (GLU) and aspartate (ASP) has been shown to be neurotoxic in a variety of mammals. This report presents data demonstrating that neonatal administration of GLU and ASP can produce adult mice that have an increased susceptibility to pentylenetetrazol (PTZ) induced seizures. Evidence is presented to show that these seizures are of greater intensity than those seen in controls and are independent of the characteristic obesity seen in GLU- and ASP-treated animals.

Neonatal mice were given subcutaneous doses of GLU, ASP or bacteriostatic saline from days 2-11 after birth according to a dose schedule which started at 2.2 mg/g b.w. and increased to 4.4 mg/g b.w. by the last day of injection. The dose of PTZ which induced seizures in 50% (CD_{50}) of the species of mouse (Ha:ICR) used in this study was determined on a population of normal controls. GLU- and ASP-treated mice were challenged at various stages during development with the CD_{50} of PTZ.

Obese GLU-treated males and females ($p < 0.001$) showed an enhanced susceptibility to PTZ ($p < 0.01$, chi square). An analysis of the severity of seizures showed that both male and female GLU-treated groups experienced seizures that were more severe ($p < 0.002$, Mann-Whitney U). A second group of GLU-treated mice were allowed to become obese and then reduced in weight to within 11% of a control group before being challenged by PTZ. These animals demonstrated the same pattern of behavior with both GLU-treated males and females showing increased seizure susceptibility ($p < 0.01$ & $p < 0.02$ respectively, chi square), and increased severity of seizures ($p < 0.002$, Mann-Whitney U). A third experiment was conducted on GLU-treated and ASP-treated mice just prior to their becoming obese (equated weights). Again, these animals showed greater seizure susceptibility ($p < 0.05$ GLU-treated, chi square; $p < 0.002$ ASP-treated Binomial test), and seizures were of greater severity than controls ($p < 0.02$ GLU-males and $p < 0.002$ GLU-females, Mann-Whitney U). All probability values are two-tailed.

Whether this preparation will serve as a useful model of convulsive disorders will most likely depend upon the elucidation of the particular mechanism underlying the lowered seizure threshold. The mechanism is far from clear and could involve any of the following: a) permanent disruption of the blood-brain barrier; b) endocrine dysfunction; c) impairment of GABA synthesis; d) neurodevelopmental abnormalities; e) lowered metabolic efficiency.

443 CONVULSANT ACTION OF HYPOGLYCEMIA: CORTICAL DISINHIBITION. W. Raabe. Dept. Neurol., VA Hospital, Minneapolis, Minn. 55417.

Hypoglycemia is a well known cause of convulsions. However, the detailed mechanism of the convulsant action of hypoglycemia is not clear. Since neurons derive their energy supply solely from oxygen and glucose, it was a surprise to find that hypoglycemia produced a neuronal dysfunction, e.g. seizures, at a time when the overall energy state in the CNS was normal. Since hypoglycemia increases cerebral ammonia concentrations prior to convulsions, it was suggested that the effects of ammonia, abolition of postsynaptic inhibition, initiate hypoglycemic convulsions.

The effects of insulin-induced hypoglycemia (IIH) and i.v. ammonium-acetate (AA) on cortical postsynaptic inhibition and cerebral ammonia-concentration were studied. Pentobarbital anesthetized and artificially respirated cats were used. Extracellular recordings were obtained from pyramidal tract cells. Cortical postsynaptic inhibition was measured as the efficacy of recurrent postsynaptic inhibition of pyramidal tract cells to suppress antidromic action potentials of pyramidal tract cells. As soon as the efficacy of inhibition was abolished the widely exposed cortical hemispheres were frozen with liquid nitrogen. Cerebral ammonia concentrations were determined with the Conway microdiffusion method.

IIH (100 I.U. insulin/kg i.p. or i.v.) abolished action potential suppression by cortical postsynaptic inhibition as the first sign of neuronal toxicity. This disinhibition occurred at blood glucose levels of 47-54 mg% and cerebral ammonia concentrations of 416± 75 S.E. nmol/gm wet weight. Although cerebral ammonia-concentrations during IIH-induced disinhibition were slightly increased when compared to values obtained from sham animals (284± 55 S.E. nmol/gm) the difference was statistically not significant. Intravenous infusion of AA (1.1-2.8 mmol/kg) abolished action potential suppression by cortical postsynaptic inhibition at cerebral ammonia-concentrations about three times higher (742± 80 S.E. nmol/gm) than in sham-operated animals.

IIH abolishes the efficacy of cortical postsynaptic inhibition like ammonia does. The effect of IIH occurs independent of IIH-induced increases of cerebral ammonia concentrations. IIH-induced cortical disinhibition occurs at blood glucose levels known to produce preconvulsive changes in the EEG. Cortical disinhibition may account for the convulsant action of hypoglycemia.

444 SEIZURE ACTIVITY OF ANTIBODIES TO G_{M1} GANGLIOSIDE PURIFIED BY AFFINITY CHROMATOGRAPHY. Maurice M. Rapport, Sahebarao P. Mahadik, and Stephen E. Karpiak. Div. Neurosci., N. Y. State Psychiatric Inst. and Depts. Biochem. and Psychiat., Columbia Univ. Coll. Phys. & Surg., New York, N. Y. 10032.

We have previously shown[1] that a single intracortical injection of antiserum against total brain ganglioside into the sensorimotor cortex of rats will induce recurrent epileptiform seizure activity lasting as long as several weeks. The specificity of the antibody was established by showing that removal of anti-G_{M1} ganglioside antibodies by absorption of the antiserum with small quantities of pure G_{M1} ganglioside eliminated the biological activity. However, in view of the variability in both titer and avidity of antibodies in individual antisera, the reproducibility of the reagent can only be assured by demonstrating the effectiveness of pure antibodies. Very highly purified antibodies to G_{M1} ganglioside were prepared from two different antisera by affinity chromatography on G_{M1}-containing gels[2]. Intracortical injection into the sensorimotor cortex of 6.5 and 6.8 µg, respectively, of protein purified by affinity chromatography from these two antisera resulted in the onset of epileptiform discharges within 1 to 5 hrs. With native antisera, induced discharges were not seen earlier than 24 hours after injection. Intensity of response with purified antibody was greater than with antiserum judging by the increase in spike amplitude and discharge frequency. No convulsions were seen. We also observed that unilateral injection of purified antibody into the ventral hippocampus through a bipolar electrode cannula induced epileptiform activity within 30 min. Discharges were sustained (3 to 5/sec) and lasted for the duration of the experiment (6 to 8 hrs). Injection of antiganglioside serum into an identical preparation usually was followed by the following sequence over a 5 to 6 hr. period: depression of EEG activity, return to normal EEG patterns, then infrequent discharges of low amplitude and low frequency. It is concluded that pure anti-G_{M1} antibody is able to induce seizure activity without participation of other exogenous substances present in the antiserum and should be a reproducible agent. The fact that it is more reactive than antiserum suggests that there are factors in serum that inhibit the epileptiform activity of the anti-G_{M1} ganglioside antibody. Supported by NIH Grant NS-13762.

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445 ALTERED PHOSPHORYLATION OF CORTICAL MEMBRANE PROTEINS AFTER ECS. M. V. Reddy*, Y. H. Ehrlich, L. G. Davis, J. Daugherty* and E. G. Brunngraber. Mo. Inst. of Psychiatry, Univ. of Mo.-Columbia, St. Louis, Mo. 63139.

Seizure activity has been often related to changes in the levels of cAMP in the brain. Previous findings in our laboratory have indicated that such changes are accompanied by alterations in the phosphorylation of specific membrane-bound proteins, presumably of synaptic origin (Brain Research Bull. in-press). In the present study, the effects of electroconvulsive shock (ECS) on this enzymatic activity have been investigated. Rats (120-140 gms) were sacrificed by body immersion in liquid N_2 during the tonic (9-11 sec after the shock) and clonic (28-32 sec) phases of electroshock-induced convulsions and after recovery (2 min and 4 min). Controls were fitted with the same ear-clip electrodes but were not shocked. Membrane fractions were prepared from the cortices of frozen rats as described (Pharm. Biochem. Behav. 6: 169, 1977). Endogenous phosphorylation reactions were carried out by incubating the membranes for 10 sec with gamma-^{32}P-ATP (Neurochem. Res. 2: 533, 1977) and the reactions were stopped by solubilizing the membranes in a detergent (1% SDS). Incorporation of radioactive phosphate from ATP into specific protein components was determined by autoradiography of reaction products separated electrophoretically in gradient (7-18%) polyacrylamide gel-slabs. Over twenty specific protein bands that incorporate radioactive phosphate under these assay conditions were identified in the preparations. Of these, the phosphorylation of one group of bands, designated H (MW 15-20K) demonstrated temporal relationships with the treatment. Compared to the above controls, phosphate incorporation into H increased in membranes from shocked rats, peaked at the clonic phase and then gradually decreased. Examination of cytosol fractions from the same animals did not reveal such time-dependent changes. The possibility that phosphorylation of membrane-bound proteins is involved in mechanisms underlying seizure activity will be discussed. Supported in part by a grant from the Epilepsy Foundation of America and by intramural funds from the Missouri Institute of Psychiatry.

446 AMYGDALOID KINDLING AND CENTRAL ENZYME ACTIVITY. D. Peter Reedy*, Edith G. McGeer, William A. Staines*, and Michael E. Corcoran. Division of Neurological Sciences, University of British Columbia, Vancouver, Canada.

Intermittent application of localized electrical stimulation to the amygdala or certain other areas of brain results in the development of generalized convulsive seizures (kindling). In an attempt to identify possible neurochemical correlates of kindling, we measured the regional activity of neurotransmitter-synthetic enzymes in the brains of amygdaloid-kindled rats and of yoked control rats that either received nonepileptic low-frequency stimulation of the amygdala or carried amygdaloid electrodes and were handled but not stimulated. In addition to measuring the activity of enzymes involved in the synthesis of GABA, glutamate, acetylcholine, catecholamines, and taurine, we also measured the regional high-affinity uptake of glutamate in kindled and control rats. All rats were killed 24 hours after the fifth fully generalized seizure in the kindled group.

The results were negative: There were no changes in enzyme activity or glutamate uptake that were reliably related to amygdaloid kindling. The mechanisms underlying the lasting increase in seizure susceptibility produced by kindling thus may not be expressed at the levels of enzyme activity or uptake of glutamate.

447 HISTOPATHOLOGY OF FERRIC-INDUCED EPILEPTOGENIC FOCI: A GOLGI SURVEY IN CATS. S.A. Reid*, W.M. Boggs*, G.W. Sypert and L.J. Willmore. (SPON: F.A. King). Dept. of Neuroscience, Univ. of Fla. Coll. of Med., Gainesville, FL 32610

Domestic cats were rendered epileptic via sub-pial injection of 20 microliters of saturated ferric choloride ($FeCl_3$) solution into the regions of either the left sigmoid or marginal gyri. Serial EEG's, recorded from a permanent montage of bone screw electrodes, confirmed the development of focal epileptiform activity which continued unabated until the animals were sacrificed six months later. Brain tissue was prepared for histopathological evaluation using the tungstate modification of the Golgi-Cox method. The cortical region exposed to $FeCl_3$ was compared to the homotopic contralateral cortex. Changes noted in the $FeCl_3$ exposed cortex include: 1) striking neuronal depopulation; 2) a relative increase in astrocytic forms; 3) segments of poor impregnation; 4) reduction of dendritic branching; 5) dendritic nodulation and swelling; and 6) marked loss of dendritic spines. These pathological changes are similar to those observed in human epileptic foci obtained at neurosurgery. Hence, this study suggests that sub-pial injection of ferric chloride solution in cats may be an accurate model of human focal epilepsy. (Supported by VA Hospital Medical Research Service.)

448 GABAergic AXON TERMINALS DECREASE AT EXPERIMENTAL SEIZURE FOCI IN MONKEY CEREBRAL CORTEX. C. E. Ribak, A. B. Harris, L. Anderson*, J. E. Vaughn and E. Roberts. Division of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010 and Department of Neurological Surgery, University of Washington, Seattle, WA 98195.

Using an immunocytochemical method for the localization of the GABA synthesizing enzyme, GAD, GABAergic nerve terminals were found to be distributed homogeneously in all layers of normal monkey sensorimotor cortex. This distribution in the neocortices of both Macaca mulatta and M. fascicularis was similar to that previously described in rat neocortex where GAD-positive terminals arising from aspinous and sparsely-spinous stellate neurons made symmetric synapses with all cortical somata and formed extensive pericellular plexuses with the somata of pyramidal neurons in layers III and V. GAD-containing axon terminals in monkey neocortex also formed symmetric synapses with somata and dendrites suggesting that they arise from the same types of stellate neurons.

In order to determine if GABAergic neurons are altered at seizure foci, specimens were obtained from the sensorimotor cortex of five monkeys made epileptic by either intracortical injections or subarachnoid applications of alumina gel. EEG tracings from these monkeys were normal initially, but changed as epilepsy developed. Electrocorticography verified epileptic foci prior to fixation. Frozen sections of the specimens were incubated in GAD immunocytochemical reagents and examined by light microscopy. At low magnification, sections at sites of alumina gel (A) displayed a staining intensity for GAD which was lower than both (B) ipsilateral sections further away from the alumina gel and (C) control sections from contralateral homotopic cortex. Sections from these sites (A, B and C) taken from each of the subjects were examined at high magnification, and the numbers of GAD-positive terminals were counted in 38 contiguous 100 μm^2 areas from the bottom of layer VI to the middle of layer V. The mean numbers of GAD-positive terminals per 100 μm^2 for sites A, B and C were 5.8, 11.4 and 16.7, respectively. An analysis of variance and a comparison of means by the Newman-Keuls method showed that the differences among these mean values were all highly significant ($P<.01$). These results indicate a numerical decrease in GABAergic axon terminals at sites of seizure foci (A and B), and this decrease may be due to a selective loss of GABAergic, cortical neurons. Such a loss of GABAergic inhibition at seizure foci could be responsible for the observed epileptic activity.

Supported by USPHS grants NS01016, NS04053, NS09037 and NS12116.

449 EFFECT OF PHENYTOIN ON THE ACTION POTENTIAL OF A NEURON IN THE LAMPREY SPINAL CORD. Michael E. Selzer. Dept. Neurol., Sch. Med., University of Pennsylvania, Philadelphia, PA 19104.

Dorsal cells are primary sensory neurons within the spinal cord of lampreys. They receive no synaptic input and are easily impaled under direct microscopic vision. Conventional techniques were used to stimulate these cells through intracellular microelectrodes, and record their resting membrane potentials and action potentials in various bathing solutions. The action potentials were abolished in 10^{-7}M tetrodotoxin and greatly reduced or eliminated by replacing Na^+ with choline. Removal of Ca^{++} and addition of 1mM Mn^{++} did not reduce the action potential. Thus the action potential of dorsal cells is generated by increased Na^+ conductance. A total of 146 dorsal cells were studied in normal lamprey solution (N = 45), in phenytoin (PTN) 20 uM/ml (N = 60) or in washout (N = 41). PTN did not significantly affect the average resting membrane potential (50.9 mV) or the input resistance (21.9 megohms). It did reduce the maximum rate of rise of the spike (from 110.7 to 50.6 V/sec.), the spike overshoot (from 40.9 to 29.5 mV), and the spike undershoot (from 15.8 to 12.3 mV). PTN increased the spike width, and both the current and voltage thresholds for spike initiation. Most of these changes were at least partially reversible, although both the drug effect and the washout effect were usually not maximal after more than 1 hour of perfusion.

The results are best explained by the hypothesis that PTN partially blocks the sodium conductance increase of the dorsal cell action potential. The long delay in onset cannot be explained by diffusion time from the bath to extracellular space, and suggests the possibility that PTN requires diffusion into the cell or partitioning into the membrane for its effect.

450 CEREBROSPINAL FLUID AND REGIONAL BRAIN KINETICS OF γ-HYDROXYBUTYRATE. Jack S. Shumate* and O. Carter Snead. Dept. of pediatrics and Medicine, USAF Med Ctr., Keesler AFB, Ms 39534.

Gamma hydroxybutyrate (GHB) is a naturally occurring metabolite of gamma-amino butyric acid (GABA) which possesses the ability to precipitate profound electroencephalographic (EEG) and behavioral changes in animals. These changes closely resemble human petit mal epilepsy and can be aborted by anticonvulsant drugs used specifically for these types of seizures (Godschalk et al, Neurosci. Lett. 3:145, 1975). The present study was undertaken to ascertain the time relation between the administration of an epileptogenic dose of GHB and its appearance in spinal fluid, plasma and specific areas of brain. In addition, the relationship of these kinetics to the EEG and behavioral effects of the drug were studied. GHB was administered to dogs intravenously in varying dosages with continuous EEG and temperature monitoring. Dosages of GHB in excess of 500 mg/kg produced ataxia, stupor and myoclonic jerks with occasional spiking and paroxysmal slow waves on the EEG. In addition there was a mild hypothermia produced by the GHB. These changes were evident within 15 to 20 minutes of administration. Single pulsed doses of 500 mg/kg of GHB sodium in an aqueous solution were then administered to dogs intravenously and timed serum, CSF, and brain samples obtained for determination of GHB concentration. Regional brain distribution of GHB was determined 60 minutes after the pulse dose. All assays for GHB were done by a modification of the gas liquid chromatographic method of van der Pol et al (J. Biopharmacokinet. Biopharm. 3:99, 1975). Brain and plasma concentration of GHB peaked within ten minutes of administration at 120 mcg/gm and 3000 mcg/ml respectively. The CSF concentration of GHB peaked at 180 minutes post infusion at 170 mcg/ml. The highest concentration of GHB at 60 minutes post infusion was in the white matter of temporal lobe at 100 mcg/gm. The amount of GHB in the frontal and temporal lobes exceeded that in thalamus, cerebellum, caudate and pons. These results demonstrate that the behavioral and EEG effects produced by GHB can be correlated with the concentration of this substance in brain as well as plasma. Further an active uptake of GHB into brain with subsequent passive diffusion into spinal fluid is suggested by our data. Finally, the low concentration of GHB in subcortical structures suggest that these areas are inordinately sensitive to GHB, since the GHB-induced EEG changes have their origins in subcortical areas (Snead et al, Neurology 26:51, 1976).

451 HIPPOCAMPAL AFTERDISCHARGES AND SODIUM PENTOBARBITAL. H. Scott Swartzwelder and Robert S. Dyer. Psych. Dept. American Univ., Washington, D.C. and Neuroscience Prog. Nat'l.Ctr. Toxicol. Res., Jefferson, AR.

Because of its apparent sensitivity to a variety of toxicants, the hippocampal formation (HPC) is a potentially useful structure for studies of neurotoxicity. In work reported at this meeting this year and last year we have characterized some of the normal properties of afterdischarges (AD's) elicited by stimulation of the HPC of the rat, and shown how these properties are altered by exposure to toxicants. The present experiment was performed to provide a standard of comparison for previous and future studies. Sodium pentobarbital (SP) was used because of its well known effects upon the central nervous system. The influence of various dosages of SP upon the ictal and postictal manifestations of HPC AD's was explored. Twelve hooded male rats were implanted with bipolar stimulating and recording electrodes in the dorsal HPC. AD thresholds were determined by a method similar to that described by Pinel (1972), and the properties of the threshold AD were measured. Testing occurred on alternate days. On test days the animals were injected, 30 min before testing, with either 0,10, 20 or 30 mg/kg SP. Each animal received each concentration once, but the order of presentation was counterbalanced across animals. At 10 mg/kg there was a small decline in AD duration, and a decreased incidence of rebound AD's. At 20 mg/kg there was an increase in threshold for producing an AD, a large drop in the number of AD's followed by a rebound AD, and a further decline in AD duration. At 30 mg/kg the threshold increased further and the spike rate during the AD declined significantly. Using the Pinel method of threshold determination instead of the Racine method increases the probability that threshold AD's will not be followed by depression of electrical activity. About 90% of threshold AD's determined by the Racine method are followed by depressions. In this study only 50% of threshold AD's determined by the Pinel method were followed by a depression at the 0 mg/kg dosage, and this incidence was reduced further to less than 10% at the 20 mg/kg dosage. These results provide a profile of the effects of a CNS depressant upon AD activity. The most sensitive measures of AD activity in this study were AD duration and incidence of rebound AD's.

452 POSTICTAL EXCITABILITY AND POSTICTAL DEPRESSION IN THE HIPPOCAMPAL FORMATION. Steven T. Wegner*, H. Scott Swartzwelder, Craig T. Johnson*, and Robert S. Dyer. Towson State Univ., Baltimore, Md. and NCTR., Jefferson, ARK. (SPON: ZOLTAN ANNAU)

Afterdischarge (AD) and postictal depression (PID) resulting from electrical stimulations of the hippocampal formation (HPC) depend upon the properties of the eliciting stimulus. Stimulation at 400% of threshold produced short ADs and long PIDs compared to those elicited by stimulation at 115% of threshold. It is not yet known to what extent the PID of the HPC EEG is an accurate reflection of depressed HPC excitability. If PID duration accurately reflects depressed excitability then high intensity stimulation should be followed by a longer period of inexcitability than low intensity stimulation. On the other hand, if depression of excitability is better reflected by duration of the AD, then low intensity stimulation should be followed by a longer period of inexcitability than high intensity stimulation. The purpose of the present experiment was to determine the excitability of the HPC after an AD and it's relationship to AD and PID duration. Male hooded rats were each implanted with a bipolar electrode for stimulation and recording in the right dorsal HPC. Animals were then divided into 3 groups, matched according to their threshold values. Two days later ADs were induced at stimulus intensities of 115% threshold for group 1, 200% for group 2, and 800% for for group 3. Postictal excitability was assessed by introducing a stimulus 115% of threshold within 2 sec of AD termination, and once every 30 sec thereafter until a second AD was elicited. As expected, high intensity stimulation produced short AD durations and long PID durations. Animals receiving high intensity stimulation also required fewer postictal stimulations to elicit a second AD than did animals receiving low intensity stimulation. These results indicate that the duration of the PID following HPC AD is not positively correlated with the time it takes for a subsequent near threshold stimulus to elicit an AD. The significance of the postictal EEG flattening for HPC electrical excitability therefore remains to be determined.

453 ALTERED PROTEIN SYNTHESIS WITHIN A RECURRENT SEIZURE FOCUS IN THE ALBINO RAT. L.J. Willmore, A.J. Dunn, J.J. Rubin*, and G.W. Sypert, VA Hospital and Departments of Neurology, Neuroscience and Div. of Neurosurgery, Univ. of Fla. Coll. of Med., Gainesville, FL 32610

Intracortical injection of microliter quantities of isotonic ferrous or ferric chloride into rat sensorimotor isocortex will induce a focus of recurrent seizure discharge. Electroencephalographic recordings with platinum needle electrodes confirm the presence of an active epileptiform discharge in experimental animals. Histological assessment after transcardiac perfusion with neutral buffered formalin shows an epileptic cortical focus containing a meningocerebral cicatrix, neuronal ferrugination and astroglial proliferation accompanied by moderate neuronal loss.

Six groups of 10 each of 200-300 gm albino rats were prepared with a single 5 ul injection of 100mM ferric chloride. Each of 5 animals from each group was injected with [^{3}H]lysine, 1 uCi/g body weight. At 30 minutes the brain was removed and a 3mm core was punched perpendicular to the pial surface at the site of focal epileptiform discharge, from the contralateral homotopic cortex and from the cerebellum. Tissue processed for liquid scintillation counting showed increased total uptake of [^{3}H] in the actively discharging epileptic focus, but the relative incorporation of [^{3}H]lysine into protein was decreased. Animals not developing epileptiform discharge showed diminished uptake of [^{3}H] without alteration in relative incorporation of [^{3}H]lysine into protein compared to control.

We propose that active epileptiform discharge results in alterations in either blood-brain barrier and/or focal blood flow while inhibiting cellular protein synthesis. Altered uptake of [^{3}H] in the developing or inactive epileptic focus and remaining brain may indicate a generalized change in uptake of amino acids during the process of epileptogenesis in the rat isocortex. (Supported by VA Hospital Medical Research Service.)

454 MEDIAL FOREBRAIN BUNDLE LESIONS PROLONG AMYGDALAR AFTERDISCHARGE WITHOUT CHANGING THE ONSET OF BEHAVIORAL SEIZURES IN THE KINDLED RAT. W.C. Wooten*, J.E. Walker*, and I.L. Crawford. Southwestern Regional VA Epilepsy Ctr. and Univ. Texas Health Sci. Ctr. at Dallas, TX. 75235

Brief, daily electrical stimulation of the amygdala develops sequential stages of epileptiform activity that progresses from partial complex seizures to generalized convulsions. The kindling effect occurs concomitant with trans-synaptic changes in electrophysiological responsiveness, yet it has not been determined whether kindling is dependent on specific pathways and neurotransmitters. We studied the effects of directly reducing forebrain monoamine levels on amygdaloid kindling by making electrolytic lesions in the posterior medial forebrain bundle (MFB).

The left MFB was lesioned (5 mA, 10s) and bipolar electrodes were implanted in the ipsilateral basolateral amygdala of albino male rats. Control rats were implanted with electrodes but were not lesioned. After 5 days recovery the rats were stimulated once daily (400 μA, 1s, 60 Hz). Afterdischarge, a proposed electrophysiologic index of the kindling process, was recorded from the stimulation electrodes in each rat. The maximal seizure stage of kindling, characterized by the onset of a bisymmetrical clonic-tonic motor convulsion, was attained by day 7 ($\pm$ 0.3 days, SEM) in the non-lesioned rats. A similar number of days (7.8 $\pm$ 0.5) were required to develop fully expressed seizures in lesioned animals; however, stereotypical seizure behavior was attenuated, particularly in its early stages of development. 24 hrs after the third maximal seizure, tissues from the frontal cortex, hippocampus, and caudate-putamen were sampled on the lesioned and unlesioned sides of each rat brain. Norepinephrine, dopamine, and serotonin were assayed by a fluorometric method. The concentration of dopamine was significantly less ($p < .001$, N=8) on the lesioned side (756 $\pm$ 158 ng/g/wet wt) compared to the contralateral side (7540 $\pm$ 610 ng/g). Norepinephrine and serotonin levels were reduced 25% to 40% and 40% to 60%, respectively in tissues from the lesioned side. The average duration of afterdischarge was significantly ($p < 0.02$) longer for rats with MFB lesions (49.5 $\pm$ 4.5s) than for non-lesioned control animals (28.8 $\pm$ 3.2s).

The data supports the concept that monoamines influence the duration of afterdischarge. Our results also suggest that the temporal sequence and behavioral expression of kindled amygdaloid seizures are independent of intact monoamine pathways in the medial forebrain bundle. Reduction of forebrain monoamines levels may dissociate electrophysiological events from kindled seizure behavior. (Supported by a grant from the Epilepsy Foundation of America and by the VA Medical Research Service).

EVOKED POTENTIALS AND EEG

455 EVOKED POTENTIAL CORRELATES OF CONCEPTUAL TEMPO IN ADOLESCENCE.[1] Ernest S. Barratt, James H. White*, and Perrie M. Adams. Dept.of Psychiatry and Behavioral Sciences, Univ. of Texas Medical Branch, Galveston, Texas 77550

Performance on the Matching Familiar Figures Test (MFFT) was used to assess the conceptual tempo of adolescent males and females between the ages of 13 and 16. Those subjects scoring at the low end of the distribution (N=33) were classified as reflective (low error/long latency ratio). Those scoring at the high end of the distribution (N=28) were classified as impulsive (high error/short latency ratio). Those subjects in the middle of the distribution of ratios were classified as moderates (N=14). Auditory evoked potentials recorded from the vertex were found to be significantly lower in amplitude for the N100 and N100-P180 components for the reflective subjects. In addition, the reflective subjects demonstrated a significantly greater tendency to show a reduction in the P100-N140 component of the visual evoked potential across increasing flash intensity. The impulsive subjects showed a tendency to augment (increase in amplitude) across intensity levels. These findings were taken as further evidence in support of a hypothesized difference in central nervous system processes as the basis for the observed range in conceptual tempo.

[1]This research was supported in part by a Grant from the Office of Naval Research, Physiology Branch.

456 IDENTIFICATION OF VOLUME-CONDUCTED EARLY EVOKED POTENTIALS IN THE SOMATOSENSORY SYSTEM OF CAT. Philip C. Bechtel* and Robert J. Sclabassi. Biomedical Engineering Program, Carnegie-Mellon University, and Department of Neurological Surgery, University of Pittsburgh, Pittsburgh, PA 15213.

Early volume-conducted (far-field) evoked potentials have been observed in the somatosensory system of the rat, cat and human. Ambiguities have existed in identifying these components and their origins. This study demonstrates the components (some not previously reported) and clarifies their origins in the cat.

Evoked EEG activity was recorded at multiple cranial epidural locations using screw electrodes and selected points along the neuraxis using needle electrodes. All were referenced to a frontal screw. Filter bandpass was 1-3000 Hz (-3 dB). Using a photoelectric stimulus isolation unit, constant current impulses (width .1 msec) were applied directly to the exposed sciatic nerve unilaterally via platinum electrodes. Computer-generated stimuli were randomly produced (mean rate 2 per sec, minimum interstimulus interval 100 msec). Each stimulus epoch lasted 60 sec with 4 to 8 epochs per run. Averaged evoked potentials were computed using cross-correlation techniques.

Selective enhancement of the early components was achieved by variation of recording electrode positions along the neuraxis. The earliest component occurred at 1.5 msec and disappeared with section of the sciatic nerve proximal to the point of stimulation. The second component appeared at 3.5 msec; it was removed by section of the nerve both above and below the stimulation site. Lesions of the somatosensory system at higher levels were also performed and their effects on the form and latency of subsequent components were evaluated.

The initial primary negative displacement in the cortical somatosensory evoked potential in cat appears to be a resultant complex produced by the distributed volume-conducted components. Further, these results establish a basis for evaluating the functional and anatomical interaction of the somatosensory system. While subserving a localizing capability, they provide an objective view of this system as a continuum from the periphery to the cortex.

(Partially supported by NIH Training Grant 5-T32-GM07477.)

457 INCENTIVE-RELATED CORTICAL UNIT ACTIVITY DURING THE M-WAVE AND CNV. E. H. Boyd, E. S. Boyd and L. E. Brown*. Dept. of Pharmacology and Toxicology, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642.

When squirrel monkeys (Saimiri sciureus) have learned that two tone cues (a warning cue followed in 1 sec by an imperative cue) signal the availability of a food reward if they bar-press within 1 sec, a short latency (150-200 msec to peak), short duration surface-negative waveform, the M-wave, develops in post-arcuate and post-central cortex following the cues (Boyd et al., EEG Clin. Neurophysiol. 42: 341, 355, 364, 1977), as well as a longer latency, surface-negative potential, sustained during the interstimulus interval. Since this sustained potential resembles the contingent negative variation (CNV) evoked in humans and in rhesus monkeys performing in a similar paradigm, it has been called a CNV (Boyd et al., submitted for publication). In the squirrel monkey, both the M-wave and the CNV reflect the conditioned, positive incentive value of the cues. Investigation of the activity of single cortical neurons in relation to the M-wave, the CNV, and the behavioral paradigm, compared to activity during a baseline period, has revealed at least six categories of units (Boyd and Boyd, Fed. Proc. 37: 617, 1978). We report here that the changes in activity of some, but not all, cortical neurons whose activity changes during the M-wave or CNV, reflect, like the M-wave and CNV, the conditioned, positive incentive value of the cues, in that 1) these changes were greater for trials in which the monkey responded correctly, and was rewarded, than for trials in which the monkey failed to respond; 2) they were greater for trials early in the session than for trials late in the session, after the monkey had consumed a number of pellets; and 3) they were greater when the food reward was of a more preferred flavor than when the reward was of a less preferred flavor. (Supported in part by USPHS BRSG, to the University of Rochester.)

458 NEUROMAGNETIC SEGREGATION OF FUNCTIONALLY DIFFERENT NEURAL POPULATIONS IN THE HUMAN VISUAL CORTEX. D. Brenner, L. Kaufman*, S.J. Williamson*. Neuromagnetism Lab., Dept. of Physics and Psychology, N.Y.U., N.Y., N.Y. 10003.

Magnetic fields associated with current flow in the human visual cortex were measured at various positions on the scalp with a high resolution superconducting detector. Spatio-temporal mapping of the steady-state visually evoked field revealed the existance of at least two populations of cells that respond differently to grating stimuli. Because of the detectors high resolution, the two populations can be studied separately merely by repositioning the probe.

Responses from the two areas were studied as a function of the spatial and temporal frequencies of the grating as well as the area of retinal stimulation.

Comparisons with evoked potentials have been made.

459 EFFECTIVENESS OF THE WIENER FILTERING METHOD OF IMPROVING EVOKED POTENTIAL ESTIMATION. E. Carlton* and S. Katz. Medical University of South Carolina, Charleston, South Carolina 29403.

Two recursive and two a posteriori Wiener filters were applied to somatic evoked potentials from monkeys anesthetized with N_2O (70%) delivered by face mask. Sterile stainless steel electrodes were aseptically inserted into the skull overlying the cortical forelimb and hindlimb projection areas. A reference electrode was placed in the nasal bone. Bipolar stimulating electrodes were placed on the skin over the median or radial nerves in the forelimb and the peroneal or posterior tibial nerves in the hindlimb. Square wave pulses having a duration of 0.1 - 0.3 msec were routinely delivered at a frequency ranging from one every 3-5 sec. The cortical evoked responses produced by stimulation of the peripheral nerves were amplified, filtered (0.1 - 500 Hz), and recorded on analog tape for subsequent playback and analysis on a LSI-11 computer.

Effectiveness of the Wiener filtering method of improving evoked potential estimation was ascertained by comparing filtered and unfiltered averages of the same and greater numbers of sweeps. The different filters were found to be effective in different ways. Both a posteriori filters gave smoother averages in fewer sweeps than produced by unfiltered averaging. However, high frequency transient components were suppressed by both a posteriori filters. The recursive filters had opposite effects from each other. One enhanced all components present in the unfiltered averages, while the other produced flat averages. Components not yet apparent in unfiltered averages were clearly delineated in averages produced from the same number of sweeps by one method of recursive Wiener filtering. The recursive Wiener filter which emphasized evoked potential components may be particularly useful in distinguishing evoked potentials produced under different experimental conditions.

(Supported by NINDS Grant #NS-11066)

460 FUNCTIONAL POWER SERIES ANALYSIS OF VISUAL EVOKED EEG SUGGESTS PARALLEL LINEAR AND NONLINEAR PATHWAYS. Richard Coppola*. (SPON: S.A. Cohen), *Biological Psychiatry Branch, NIMH, Bethesda, MD20014*

Visual evoked potentials (VEP) are clearly nonlinear in nature since in many cases a sine wave modulated light stimulus produces its major EEG response at twice the frequency of stimulation. It has previously been shown that nonlinear analysis may be applied to the human VEP by collecting VEPs to white noise modulated light (Trimble, Neurosci. Abstracts, 2:241, 1976). In that study, no significant contribution to the VEP from kernels higher than second order was noted.

In the present study an attempt is made to elucidate the mechanisms contributing to transient and steady state VEPs. EEG in response to three types of visual stimulation was recorded in a group of normal volunteers: 1) white noise modulated light (WNML) 2) sine wave modulated light (SML) in one Hz steps from 1 to 20 Hz and 3) half second light flashes at several intensities. Functional power series analysis was performed on the WNML data to yield first and second order Wiener kernels, i.e., the linear and nonlinear kernels. Frequency response characteristics for the SML EEG were obtained by spectrum analysis. The response amplitude at the fundamental and first harmonic were plotted versus stimulation frequency. Because in some cases the harmonic response is larger than the fundamental a single frequency response characteristic is not possible. Having obtained the system kernels, the prediction of the system response to any temporally modulated light signal is possible. In this case, the linear and nonlinear kernels give the fundamental and harmonic response. The frequency characteristics computed in this way agree favorably with the spectrum analysis of the SML data. The complete predicted response also agrees favorably with the actual steady state VEP (SSEP). Using this method of analysis the variation in wave shape of the SSEP is seen to be a function of the differential gain and latency of the linear and nonlinear parts. The kernels may also be used to predict the transient visual response (averaged VEP). The different responses seen to increasing intensities of stimulation seem to be related to the different gains of the linear and nonlinear components of the response revealed by this type of analysis.

Although the addition of the linear and nonlinear parts is intrinsic to the form of the functional power series, the comparison to actual responses in terms of wave shape suggests that parallel linear and nonlinear pathways might exist in the visual system and that a combination of the potentials due to each pathway may account for the variation in frequency and intensity response seen in VEPs.

461 CONTRIBUTION OF THE SPEECH MUSCULATURE TO APPARENT HUMAN EEG ASYMMETRIES PRIOR TO VOCALISATION. Merlin W. Donald and Barry H. Brooker. Queen's University, Kingston, Ontario, Canada.

The origins of slow surface EEG asymmetries preceding speech have remained unresolved because of conflicting claims regarding the importance of myogenic artifact at the electrode sites commonly used in these studies. This study reports an attempt to quantify the contribution of the major muscles involved in articulation to the EEG electrode sites used in previous speech potential studies. The procedures used by previous studies claiming slow wave asymmetries of neural origin were replicated. Twenty-eight female, right-handed college students served as subjects. The first experiment replicated the procedures and analysis of McAdam and Whitaker (Science, 1971; 172: 499-502), involving bilateral scalp recordings over inferior frontal and other relevant scalp loci. Tongue glossokinetic activity was recorded at the cheek. Off-line backward averages were triggered by the subjects' self-paced vocalisations (voice key). The second experiment was a more extensive study of the relationships between various parts of the speech musculature and averaged inferior frontal slow potentials. The design included repetitive, as well as variable vocal responses, under both language and non-language conditions. Temporalis, masseter, obicularis and myelohyoid muscles, as well as tongue movement activity, were monitored and averaged along with the anterior scalp sites.

Results

Three different procedures were used in quantifying the slow potential amplitudes, each indexing a different point in time prior to vocalisation. Averaged glossokinetic potentials were significantly correlated with the inferior frontal slow potentials but not with the vertex (C_z) recordings. Other muscles involved in articulation also showed significant correlations with averaged inferior frontal steady-potential shifts, depending on their timing relative to vocalisation.

Significant left-right differences between some of the speech and non-speech conditions did occur at inferior frontal electrode sites, confirming in principle previous claims, of EEG hemispheric asymmetries related to speech. However, these effects were reduced to statistical non-significance by analysis of covariance using the averaged muscle potentials as covariates. Since the muscle averages far exceeded in amplitude the inferior frontal averages, it appears likely that the apparent EEG asymmetries were myogenic in origin, suggesting that previous reports of EEG asymmetries preceding speech reflected a combination of several myogenic confounds.

462 FAR-FIELD POTENTIALS FROM TRIGEMINAL NERVE STIMULATION. Michael Feely*and Chi-ming Huang* (SPON: Susan Hoppe), Depts. of Neurosurgery and Physiology, Col. of Medicine, Univ. of South Alabama, Mobile, AL 36688.

Electrical stimulation of the infraorbital nerve in the cat resulted in a series of minute evoked potentials at the vertex detectable by computer averaging. The waveform of these potentials is similar to the auditory brainstem evoked response and the somatosensory far-field response; i.e., it is multicomponent, the amplitudes are of the order of 0.5 μV, the latencies are within 8 msec. The anatomical origins of these evoked potentials were first investigated by mapping with bipolar electrodes in the brainstem area near the trigeminal complex. Surgical lesions in the trigeminal afferent pathway were also attempted. Results indicated that: (1) The primary generator loci of the far-field potentials were within the trigeminal complex with main contribution coming from the spinal trigeminal and the principal nucleus. (2) The earliest electrical activity detectable in the thalamus had a latency of >10 msec. (3) These potentials were not affected by flaxedil. However, myogenic potentials were recordable at a latency of 7-10 msec but were abolished following administration of flaxedil. It was concluded that the multi-component far-field potential in this study was due to neural activity generated by the brainstem trigeminal structures. The relevance to clinical studies in trigeminal neuralgia will be discussed.

463 EEG CORRELATES OF "COGNITIVE" ACTIVITIES

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As a prerequisite to possible clinical use of the EEG for the assessment of subtle patterns of cortical dysfunction, two studies were conducted. Study 1 used a battery of relatively complex sensory - "cognitive" - motor tasks. Study 2 employed simpler tasks without overt continuous motor activity, and controlled for gross sensory and task-load related differences.

In Study 1, 23 subjects performed two to three 1 min replications of reading (RE), writing from memory (WR), Koh's block design (KB), mental paper folding (FO), fixation on a spot (FO), eyes closed (EC), and several control tasks. In Study 2, thirty subjects (21 were retained based on behavioral analyses) each performed thirty 5-15 sec replications of mental rotation of block structures (BR), serial addition of a column of decimal digits (AR), substitution of letters with subsequent word recognition (LE), and fixation on a spot (EO).

In each study EEGs from F4, F3, C4, C3, P4, P3, O2 and O1 were Fourier-analyzed, and measures based on estimation of spectral intensity in the theta, alpha, $beta_1$ and $beta_2$ bands were retained.

Pattern recognition was performed using a nonlinear, two-layered algorithm.

Task-to-task discrimination, strong in Study 1, was weak in Study 2 (Table I), suggesting that sensory-motor and arousal-related factors can be more prominent in EEG signals than factors relevant to the "cognitive" processes under study.

Other conclusions which followed from these studies were: (1) When decreased arousal can be ruled out, increased theta band spectral intensity, accompanied by decreased alpha and beta band intensity, may reasonably be associated with use of an underlying cortical region; (2) changes in alpha band spectral intensity are less important in distinguishing complex tasks; and (3) intertask differences in patterns reflecting asymmetry were smaller than in other topographically-organized patterns.

TABLE I

Average % Performance on Independent Validation Data

	Study 1					Study 2		
	EO	WR	RE	FO		EO	AR	BR
EC	92				AR	67		
KB	94	85	85	75	BR	67	58	
RE	88	84		81	LE	69	55	61

Supported in part by USPHS Grants NS10471 and RR05755.

464 TRANSIENT NEURONAL DYSFUNCTION FOLLOWING MECHANICAL HEAD INJURY IN CATS EVALUATED WITH EVOKED POTENTIALS.

Richard P. Greenberg, Marti S. Hyatt* and Donald P. Becker. Div. of Neurosurg., Medical College of Virginia, Richmond, VA 23298.

In an animal model of head trauma based on the fluid percussion mechanical brain injury model of Rinder and Olsson (Acta Neuropath. 11:183-200, 1968), multimodality evoked potentials were recorded to evaluate cortical and brainstem function immediately after brain injury and serially thereafter. Detailed physiological and pathological correlates of pressure transients from 0.4 - 5.0 ATM have been made in this model and found to be reproducible. Injury forces between 1.5 and 2.5 ATM consistently produce the appearance of coma in the cat for up to 16 hours, thereafter the animals recover without detectable physiological or pathological evidence of brain injury. Above 2.5 ATM the neurological and pathological findings increase directly with the magnitude of injury force.

Visual, auditory and somatosensory cortical and brainstem responses, multimodality evoked potentials (Greenberg et al. J. Neurosurg. 47:150-177, 1977) were recorded in cats (respiratory function was controlled and B/P, ICP and EEG monitored) within minutes after impact and continuously for 3 hours after injury forces from 1.7 - 4.1 ATM. Animals receiving mild injury, < 2.0 ATM, demonstrated transient loss of cortical function. Above 2.0 ATM both cortical and brainstem evoked potentials were transiently absent. The EEG although abnormal was present throughout the period of evoked potential recording. Time to recovery of the evoked potentials following impact increased directly with the magnitude of peak pressure. At 2.0 ATM cortical and brainstem potentials returned to 50% of baseline by 40 minutes after injury. At injury forces of 3.0 ATM or greater neither somatosensory nor auditory brainstem potentials recovered by 3 hours. However, visual evoked potentials did recover within 1 hour of impact at 3.0 ATM or more. Overall the somatosensory evoked potential was most sensitive to mechanical brain injury recovering more slowly than either the visual or auditory evoked potentials.

The appearance of coma following 1.7 - 2.0 ATM injuries coincided with cortical dysfunction as the brainstem evoked potentials were unchanged from baseline. (Supported in part by NIH grant NS 12587 and a Southern Medical Association Training Grant).

465 RECOVERY CYCLES OF SCALP-RECORDED EVENT-RELATED POTENTIALS FOLLOWING HEMI-RETINAL STIMULATION.

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Checkerboards flashed to the upper hemi-retina elicit event-related potentials (ERPs) which, over the first 75-200 msec, are composed of several components differing in latency, polarity, and scalp distribution. Moreover, the same stimulus flashed to the lower hemi-retina elicits ERPs in which the components are opposite in polarity and, in some cases, different in scalp distribution from corresponding components in the upper hemi-retina ERP. Jeffreys and Axford (Exp. Brain Res., 1972, 16:22-40) have attributed these ERP differences to activity occurring in the different areas of visual cortex to which the upper and lower hemi-retina are known to project.

If checkerboards presented to upper and lower hemi-retina engage different neuronal populations, then two successively presented checkerboards should interact more if presented to the same rather than to different hemi-retinas. Thus measuring the "recovery cycle" of the ERP elicited by the second of two hemi-retinal checkerboards can serve to test Jeffrey's formulation. Furthermore, a determination of the extent to which the three distinct ERP components within a given hemi-retinal ERP show different recovery cycles, will help in assessing the functional significance of these components.

Checkerboard patterns, 25 msec in duration and subtending 3^0 x 6^0 of visual angle, were tachistoscopically presented, with no overall luminance change, to either the upper or lower hemi-retina. Each of the possible stimulus pairs -- upper-upper, upper-lower, lower-upper, and lower-lower -- was presented at 8 inter-stimulus-intervals (ISIs) ranging from 50 to 800 msec. Single stimuli were also presented to either the upper or lower hemi-retina. On each trial, subjects reported whether a single stimulus, a pair of "same" hemi-retinal stimuli, or a pair of "different" hemi-retinal stimuli had occurred. EEG was recorded from 7 evenly spaced mid-line electrodes, each referred to the linked earlobes.

The data indicate that the ERP elicited by a checkerboard presented to a hemi-retina is largely unaffected by the preceding presentation of a checkerboard to the other hemi-retina. However, the amplitude of this ERP is quite depressed if the same hemi-retina has been stimulated prior to its presentation, the amount of amplitude depression depending on the time interval between the two stimuli. Moreover, the recovery of the various components within a given hemi-retinal ERP occurred at different rates with increasing ISIs. These data are consistent with the suggestion that the components both within and between hemi-retinal ERPs are manifestations of distinct brain processes.

Supported in part by DARPA through ONR contract N000-14-76-C-0002.

466 THE COMPARATIVE EFFECT OF ISCHEMIA ON CAT VISUAL, AUDITORY AND SOMATOSENSORY CORTICAL AND BRAINSTEM EVOKED POTENTIALS.

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Observations on the behavior of individual evoked potential modalities either visual, somatosensory or auditory in response to brain ischemia have been reported by others. Some of these authors found a progressive loss of amplitude and waveform as well as a delay of latency of the evoked potentials with increasing degrees of ischemia (Chang. J. Neurophys. 13:305-318, 1950; Meldrum et al. Brain Res. 13:101-118, 1969, etc.). Others noted an ischemic threshold above which the evoked potential remains unchanged while below this level the evoked potential rapidly disappears (Branston et al. Exp. Neurol. 45:195-208, 1974). A comparative study of the behavior and sensitivity of the visual, auditory and somatosensory cortical and brainstem evoked potentials following progressive ischemia has not been reported. Therefore, we recorded multimodality evoked potentials in cats subjected to stepwise decreases of systemic blood pressure and measured the ICP, CPP and CBF to evaluate the comparative response of each evoked potential modality to the ischemic insult. An assessment of the relative sensitivity of the cortex and brainstem to ischemia was also made with evoked potentials.

An aortic reservoir was utilized for controlled stepwise decreases in systemic blood pressure. Each animal had respiratory function controlled and its ICP, CPP, EEG and CBF (hydrogen clearance technique) measured throughout the experiment.

Somatosensory cortical and brainstem potentials were the most sensitive of the modalities to ischemia, progressively declining and finally disappearing at a mean CPP of 45 mmHg and CBF of 24 ml/100 g/min. The sequential nature of the waveform deterioration was similar for all modalities and progressed from afterwaves to primary complex with increasing levels of ischemia. Auditory cortical and brainstem potentials were the most resistent to ischemia often peristing at a CPP of 30 mmHg, a level at which both visual and somatosensory potentials had disappeared. (Supported in part by NIH grant NS 12587 and a Southern Medical Association Training Grant).

467 TEMPERATURE INDEPENDENT ALTERATION OF BRAINSTEM AUDITORY EVOKED RESPONSES BY ENFLURANE. Timothy A. Jones, James J. Stockard, and Kenneth R. Henry. Dept. Animal Physiol. UCD, Davis CA 95616

Temperature profoundly alters latencies of far field recorded brainstem auditory evoked responses (BAERs)[1]. Few drugs (if any), have been shown to have temperature independent influences on the latencies of BAERs. Enflurane (2-chloro-1,1,2-trifuoroethyl difluoromethyl ether) was found in the present study to have potent influences on BAERs in contrast to other general anesthetics, anticonvulsants, and tranquilizers tested to date.

Cats were anesthetized with and equilibrated at enflurane concentrations producing relatively flat background EEG tracings accompanied by large amplitude spike-wave paroxysms. Tympanic, esophageal, and rectal temperatures were monitored and strictly controlled in order to assess the temperature independent effects of enflurane. The same animals were curarized (awake) and stabilized at normal (38.6 to 38.9°C depending on animal) and reduced (37.5 to 37.6°C) temperatures. End tidal CO_2 concentrations were maintained between 4 and 5%.

Enflurane produced latency shifts (slowing) in positive peaks II through V($p<.001$), with the largest changes occurring in later waves. In addition, enflurane reduced the amplitude of waves PIII, PIV and PV relative to PII ($p<.001$). In curarized animals, reducing temperatures to 37.6°C was sufficient to produce latency shifts approaching those induced by enflurane alone, however, no decrease in relative amplitudes accompanied the shift. This latter finding may prove useful in distinguishing enflurane and temperature effects.

Many drugs modify systemic temperatures through central and/or peripheral actions and, therefore, will influence BAER latencies indirectly by that means. To our knowledge, this is the first evidence for the existence of a drug which is capable of altering BAER latencies through means other than hypothermia.

[1]Stockard et al: Effects of hypothermia on the human brainstem auditory evoked response. Annals of Neurology 3;368-370,1978

468 EFFECTS OF SPINAL CORD LESIONS ON SOMATIC EVOKED POTENTIALS ALTERED BY INTERACTIONS BETWEEN AFFERENT INPUTS. S. Katz, H.F. Martin, J.G. Blackburn*, R. Simpson*, and E. Carlton*. Medical University of South Carolina, Charleston, South Carolina 29403

In cats anesthetized with alpha-chloralose, somatic evoked potentials (SEP) were recorded in response to electrical stimulation of surgically isolated peripheral nerves. Selected surgical lesions were made at T_9-L_1 spinal cord and were histologically verified. Two stimulus magnitudes were used to activate peripheral nerves, one only exciting the large fibers and another exciting the small fibers as well.

Control SEPs were recorded in response to stimulation of both large and small fibers of the radial nerve. The later components (latencies greater than 40 msec) of this SEP are suppressed when evoked 100 msec after application of a conditioning stimulus (CS) to the large fibers of either peroneal nerve. Evidence indicates this interaction occurs supraspinally. Bilateral transection of the dorsal columns and spinocervical tracts eliminates these effects. Increasing the CS intensity to include small diameter fibers again resulted in reduction of the later components of the SEP. This interaction was largely eliminated if the transection was extended to include mid-lateral cord tracts.

These results suggest that the SEP can be influenced by small fiber afferent activity conducted in mid-ventrolateral spinal cord in the absence of the dorsal columns and spinocervical tracts. Alterations in the forelimb-evoked SEP by a conditioning hindlimb stimulus is a sensitive indicator of spinal cord integrity. This method may be used to assess whether low spinal injury spares ventrolateral columns.

(Supported by NINDS P-5P81-NS-11066)

469 EVOKED POTENTIAL INDICES OF HABITUATION AND GENERALIZATION. Andrea L. Megela and Timothy J. Teyler. Sect. Neurobiol. Behav., Cornell Univ., Ithaca, N.Y. 14853 and Neurobiol. Div., Northeast. Ohio Univ. Col. Med., Rootstown, Ohio 44272.

This study was designed to investigate whether scalp-recorded evoked potentials exhibit habituation and generalization, forms of neural plasticity related to behavioral learning.

Auditory and visual evoked potentials were recorded in a habituation/generalization paradigm from left hemisphere frontal, temporal and occipital scalp placements in 26 normal, right-handed adults. Stimuli were either loud and soft tones or bright and dim flashes of light. These stimuli were presented in series of repetitive (habituation) stimuli of one intensity which were interrupted in random, unpredictable positions by test (generalization) stimuli of another intensity. Evoked potential averaging was performed on a stimulus-by-stimulus basis across series. Changes in the amplitudes and latencies of the N1P2 and P2N2 components across the repetitive stimuli were examined according to the defined operational criteria of habituation (response decrement as a result of repetitive stimulation) and generalization (similarity of responses to test and repetitive stimuli). Direction and degree of intensity generalization of habituation were analyzed according to the differential predictions made by the dual-process and stimulus comparator models of habituation.

Auditory evoked potentials showed decreases in amplitudes and latencies in response to both loud and soft repetitive stimuli at all three electrode sites, but most consistently at the temporal. Visual evoked potentials exhibited decrements in response to repetitive bright and dim light flashes at frontal and temporal sites but not at the occipital. Both auditory and visual evoked potentials habituated in response to repetitive stimuli in a manner consistent with the operational definition of habituation. These results indicate that evoked potentials can be used as electrophysiological indices of learning in humans.

For both modalities of stimulation, intensity generalization of habituation occurred in the manner predicted by the dual-process theory of habituation: Less intense generalization stimuli evoked less response recovery above the habituated level than more intense generalization stimuli.

470 PERIPHERAL VERSUS CENTRAL PROCESSES AS REFLECTED BY AUDITORY EVOKED FREQUENCY FOLLOWING AND BRAINSTEM RESPONSES. T. Mendelson*, J. Gardi*, A. Ortiz* and A. Salamy (SPON: N. Peterson). Brain-Behavior Research Center and Letterman Army Medical Center, Sonoma State Hospital, Eldridge, CA 95431

Developmental studies of the brainstem auditory click-evoked response (BSER) in humans have shown that the P1 (VIII nerve) component attains adult latency within a few weeks of birth, whereas P5, believed to represent summated activity from the region of the inferior colliculus, does not mature until after the first year. The differential rates of maturation have been presumed to reflect separate peripheral and central processes (EEG clin. Neurophysiol. 40:418, 1976). The tone-evoked frequency following response (FFR) has been investigated in efforts to provide frequency-specific information on auditory function. Recent findings by Gardi (manuscript in preparation) have suggested that the FFR is generated primarily by the cochlea and cochlear nucleus and is mediated along the apical regions of the basilar membrane; thus it reflects peripheral activity. In this investigation FFRs were recorded from 22 neonates and 10 normal-hearing adults in an attempt to assess the level of maturity of peripheral structures in the neonate. FFRs were recorded to 10 msec duration tones of 250, 500 and 1000 Hz presented monaurally at levels ranging from 65dB HL downward to threshold. Electroencephalic activity was recorded from vertex to ipsilateral ear and summated on line. Responses were evaluated to determine amplitude, waveform configuration and threshold. Easily identifiable FFRs were obtained from all subjects at 65 dB HL. Neonatal responses were similar in configuration to those of the adults; responses at 250 and 500 Hz were of greater amplitude than those at 1000 Hz. Neonates typically gave responses which were somewhat smaller in amplitude and approximately 15 dB higher in threshold than those of adults. In both groups, response amplitude decreased as a function of stimulus intensity. The recording of essentially mature FFRs from neonates confirms that peripheral mechanisms in audition are highly developed at birth. This supplements the contention that the developmental trend witnessed for P5 of the BSER must represent a process which is not primarily peripheral.

Supported by NIH grant NS12424.

471 ELECTROPHYSIOLOGICAL PREDICTORS OF THE ROTATIONAL DIRECTION IN RATS. Michael S. Myslobodsky, Yehuda Shavit* and Jeffrey Rosen* Psychobiol. Res. Unit, Dept. Psychol., Tel-Aviv Univ., Ramat-Aviv, Israel.

A general principle of the rotational behavior states that normal rats or animals with lesions in the nigrostriatal dopamine (DA) system circle away from the more active side of the brain. An increase in DA content was found to correlate with the side contralateral to the preferred direction (Life Sci. 18: 889, 1976). The present study was designed to compare bilaterally recorded secondary components of the visual evoked potentials (i.e. slow negative wave, sensory afterdischarge) and Metrazol-induced wave-spike afterdischarges with rotational side preference as assessed in the rotometer. 80% of naive undrugged rats displayed reliable interhemispheric asymmetry of the secondary components of the visual evoked potetials. The unilateral facilitation of these components was associated with corresponding unilateral synchronization of the EEG which displayed a 40% increase in the duration of spindles in the 6-8 Hz frequency band. The rats with reliably asymmetric EEG and evoked potentials showed rotation after intraperitoneal amphetamine (1.5 mg/kg) administration in the direction opposite to the side with more suppressed electrocortical activity. Electrocortical asymmetry was emphasized and sometimes reversed after subconvulsive (10-20 mg/kg) and convulsive (20-40 mg/kg) intraperitoneal Metrazol (1% solution) injections which transformed sensory afterdischarges into wave-spike complexes. With the convulsive dose of Metrazol the asymmetry of wave-spike discharges was observed during the recovery phase after a generalized electrographic fit. Administration of amphetamine on the background of the Metrazol effect elicited the rotational behavior in the direction away from the hemisphere with more suppressed wave-spike activity. A decrease of the synchronized and hypersynchronized electrocortical (evoked and spontaneous) activity over the hemisphere opposite to the preferred rotational side is believed to reflect the higher DA content in the more active nigra. These findings will be discussed with respect to the DA nigrostriatal participation in the control of seizure activity.

472 AMPHETAMINE-INDUCED ALTERATIONS OF EVENT-RELATED SLOW POTENTIALS IN THE RAT ARE DEPENDENT ON DOSE AND CORTICAL AREA. James H. Pirch. Department of Pharmacology and Therapeutics, Texas Tech University School of Medicine, Lubbock, Texas 79409.

Amphetamine alters event-related slow potentials recorded from the rat cortex in response to a warning stimulus which is always followed by food reinforcement (Pharmacol. Res. Comm. 9: 669, 1977; Pharmacol. Biochem. Behav. 6: 697, 1977). The present experiments were conducted to investigate the effect of d-amphetamine on slow potentials (SPs) associated with operant performance. Two seconds after the onset of a 20 msec auditory warning stimulus a retractable lever began to move into the chamber. Once the lever was activated (1.9 sec after extension was initiated) the rat had 2 sec to press for a food pellet. The lever was inactivated and retracted either at the end of this 2-sec period or after a lever press. Cortical slow potentials were recorded by means of d.c. amplifiers and permanently implanted silver-silver chloride electrodes in contact with the brain or dura via agar-saline pools. In some rats an "active" surface electrode was located 2-3 mm anterior to the bregma and 2-3 mm lateral to midline while a "reference" was placed 2-3 mm anterior to the parietal-interparietal suture and 3 mm lateral to midline (A-P recordings). In others, surface electrodes were located at the same anterior and posterior sites while a depth electrode (1-1.5 mm below surface) was placed approximately 1.5 mm lateral to each surface electrode, thereby allowing surface-to-depth recording (S-D recordings). After conditioning, the warning stimulus elicited negative SPs in the A-P recordings and surface-negative SPs in the anterior and posterior S-D recordings. Posterior S-D SPs were generally smaller than anterior S-D SPs. SPs were averaged with a computer and peak amplitudes (μV) and areas (μV sec) were obtained from control and drug sessions (40 trials per session presented at variable intervals of 18-70 sec). Amphetamine (0.25 to 1.0 mg/kg) caused a dose-related depression of SP amplitudes and areas in A-P and anterior S-D recordings. Peak amplitudes of posterior S-D SPs were slightly depressed. However, the areas of posterior S-D SPs were enhanced by some doses during the same time that anterior S-D SPs were markedly depressed. These results show that the effects of amphetamine on cortical event-related slow potentials in the rat depend upon dose, cortical area and method of analysis. Amphetamine and other drugs may prove useful in the elucidation of mechanisms of generation of event-related slow potentials. (Supported by USPHS MH29653 and by the Tarbox Parkinson's Disease Institute at Texas Tech University School of Medicine.)

473 SELECTIVITY OF ATTENTION TO MULTI-FEATURE GRATINGS: EVOKED POTENTIAL AND BEHAVIORAL MEASURES. Fred H. Previc,* M. Russell Harter,* and V. L. Towle* (SPON: R. G. Eason). University of North Carolina, Greensboro, N. C. 27412.

The mechanisms underlying selective attention to four gratings, varying in spatial frequency (0.83 vs 3.33 c/d) and orientation (vertical vs horizontal), were explored using visual evoked potentials (VEPs). The problem investigated was whether the facilitory effects of attention on VEP amplitude to gratings of varying spatial frequency and orientation will be specific to 1) those gratings with either feature identical to those of the attended grating (feature-specific attention), or 2) only that grating identical to the attended grating (pattern-specific attention). The four gratings were stroboscopically presented sequentially (about 1/780 msec) in random order. Selective attention was manipulated by requiring the subject to give an RT response to one of the four gratings. The results indicated that the specificity of the facilitory effects of attention depended on which point in time after stimulation the VEP amplitude was measured. Between 175 and 225 msec post-stimulus, the facilitory effect occurred when the flashed grating had either feature in common with the attended grating (feature-specific attention). After 225 msec post-stimulus, the facilitory effect occurred primarily when the flashed grating had both features in common with the attended grating--that is, was identical to the attended grating (pattern-specific attention). The behavioral measure of attention (median latency of less than 375 msec) also reflected a pattern-specific attention effect and was most related to the effects of attention on VEP amplitude measured at 375 msec post stimulus. Both the VEP and behavioral RT measures indicated facilitation was greater when the flashed grating had the same spatial frequency, as compared to same orientation, as the attended grating. These results were related to information processing and neurophysiological models of spatial vision.

474 LINGUISTIC PERFORMANCE IN THE CONTEXT OF LANGUAGE NEURO PHYSIOLOGY. Armando F. Rocha* and E. Françozo* (SPON: C. Timo-Iaria), Dept. of Physiol. and Biophysics, UNICAMP, Campinas 13100, SP . BRASIL

The purpose here is to study the neurophysiological correlates of the intonational system of Portuguese, by means of recording the scalp Evoked Potentials (EP) during speech recognition. Intonation,in Portuguese as in many other languages, renders Given&New information in a sentence.New is the information the speaker (S) expects the hearer(H) not to have present at the moment of communication.Given&New, in Linguistics, are related to the concept of theme(the structure of the communicative act as related to the discourse framework). Also, the notion of Communicative Dynamism,assuming the sentence as a continuum between two extremes,the topic and the comment(G&N and the thematization structure), was considered.A 3 minute tape was presented twice, with an interval of 30 min.,to right-handed people,and was composed by:i)a short text (from a newspaper)read with no Intonation Emphasis(IE)so as to allow us to study the intonational pattern H imposes on the test,and ii)a set of 25 sentences,in which IE varied according to a) different sentences and b)different intonational patterns of the same sentence,in order to reveal the patterns S imposes upon H.The linguistic performance was tested by asking H to point to the IE in a written copy of the tape.Our results clearly indicate that 1)both in the text and in sentences,the underlined words evoked easily recognizable EEG configurations;2)in the text, all thematic words induced EEG modifications,even if not chosen by H as IE, and the EEG pattern remained similar for the words well uttered and sharply changed with hesitation,stuttering,etc;3)changes of IE and the type of information in the same sentence were accompanied by correlate EEG changes;4)for a fixed position of IE in different sentences there was a fixed relation for the EP onset,accompanied by modifications in the EP pattern; finally 5)there was a high correlation between the EEG pattern and the linguistic performance,both regarding success and failure.Our work demonstrates that language neurophysiology and that relevant questions regarding linguistic knowledge can be studied with the aid of the EEG,and the results also support our theoretical model of language performance, presented elsewhere. In this context,both experimental and theoretical language neurophysiology can be realized.

475 RELATIONSHIP BETWEEN EVOKED POTENTIALS AND PSYCHIATRIC SYMPTOMS. Richard A. Roemer and Charles Shagass *. Eastern Pennsylvania Psychiatric Institute and Temple University Health Sciences Center, Philadelphia PA 19129.

We have reported a number of evoked potential differences between different psychiatric populations. The clinical populations in these studies were derived from discharge diagnoses arrived at by two senior psychiatrists. The question examined here deals with the extent to which symptom patterns, indicated by the Brief Psychiatric Rating Scale (BPRS), are associated with alterations in evoked potentials. BPRS ratings and evoked potential records were obtained during the second week of hospitalization while patients were free of medications. Stimuli were pseudorandomly presented left or right median nerve shock (LSEP, RSEP), clicks (AEP), or checkerboard pattern flash (VEP). Recordings were obtained from 14 scalp and one EOG lead.

The BPRS ratings of 150 hospitalized psychiatric patients were subjected to principal component factor analysis with varimax rotation. Individual factor scores were computed for each of the resulting 8 components in the factor structure. The resultant arrays for the 150 patients were subjected to a hierarchical cluster analysis to identify the "natural" ordering of the rating profiles. Three age and sex matched groups of 29 patients each were derived from the respective thirds of the original 150 patients. This provided three patient groups, each group composed of patients with mathematically similar BPRS ratings. The first and third groups tended to reflect symptomotology associated with psychoses.Composite evoked potentials for each stimulus mode and lead location were computed for each of the three groups and statistically compared.

The key findings were with LSEP and RSEP recordings. EP differences were observed during three time periods: 1) 40-50 msec; 2) 70-110 msec; 3) 200-300 msec post-stimulus.

The nonpsychotic group 2 had lower amplitudes than the other two groups over the contralateral hemisphere during the 40-50 msec period and higher amplitude responses during the 200-300 msec period over most of the scalp. Group 1 differed from group 3 with lower amplitudes during the 70-110 msec period over the contralateral posterior scalp.

Previously, the EPs of nonpsychotic inpatients were shown to be essentially like those of nonpatient controls; by inference, the second group, defined by BPRS rating profiles, appears to be a nonpsychotic subpopulation and their EPs appear similar to those of controls. Consequently, the results appear to be in accord with our previous reports comparing nonpatient controls and psychiatric populations.

476 POWER SPECTRAL DENSITY METHODS FOR THE DETECTION OF WEAK EVOKED POTENTIALS. Bernard Saltzberg. Information Analysis Section, Texas Research Institute of Mental Sciences, Houston, TX 77030.

Coherent averaging is frequently inadequate for the detection of weak evoked potentials because of the waveshape and/or latency instability of the evoked responses. Waveshape and latency of the evoked potential is commonly state dependent and therefore constancy of response may be limited to a single state which persists over a time period so brief that it is not possible to present a sufficient number of replications of the stimulus to make averaging an effective detection procedure. In some experimental designs averaging as a detection method is precluded because the stimulus events are spontaneous and therefore knowledge of stimulus timing is not available to accomplish coherent addition. The alternative to averaging in these circumstances is based on the morphology of high resolution power spectral densities from which the presence of evoked responses can be inferred. Aperiodicity of the signal background is required in order for the power spectral density to unambiguously reveal the presence of evoked responses. Noninvasive detection of spontaneous deep brain electrical spikes which produce weak scalp evoked potentials is an especially useful application of the proposed approach.

477 EFFECTS OF TRIETHYLTIN INDUCED NEUROPATHY ON BRAIN STEM AUDITORY EVOKED POTENTIALS IN RATS. S.N. Shah*, R.C. Johnson*, A. Amochaev* and A. Salamy, Langley Porter Neuropsychiatric Institute, UCSF, Brain-Behavior Res. Ctr., Sonoma State Hosp., Eldridge,CA 95431

In a recent study we reported that the latencies of the brain stem auditory evoked potential (BEP) increased when myelination was impaired in the developing rat (Exptl. Neurol. 58:111, 1978). During maturation, however, changes in structural, metabolic and functional processes occur in parallel. Thus, the demonstration of a correlation between myelin content and electrophysiological parameters is not necessarily indicative of a causal relationship. In the present study we therefore examined the effects of demyelination induced by triethyltin (TET) intoxication on the BEP in young adult rats. Two groups of Sprague-Dawley rats (average body wt. 200g) drank either normal tap water or water containing TET (20mg/liter) for a period of 12-15 days. At the end of this treatment period, BEPs to auditory stimuli were recorded as described earlier (Exptl. Neurol. 58:111, 1978). An average of 400 BEPs was summated with a computer of average transients and written out on an X-Y plotter. The peak latencies were then computed from these tracings. Immediately after testing the animals were sacrificed, brains removed and weighed. Two hemispheres were dissected and one from each rat was frozen immediately, later lyophillized for lipid analysis. The remaining hemispheres from three or four rats of each group were pooled and myelin and synaptosomes prepared using discontinuous sucrose gradient procedure. Results of these experiments showed that the rats receiving TET water had a lower body wt, increased brain wt, as well as an increase in moisture content of brain tissue. The lipid content per g of wet brain and the amount of myelin recovered was reduced in TET treated rats. But the amount of synaptosomes recovered remained normal. The decrease in myelin content in CNS of TET treated rats was accompanied by not only a significant increase in the latencies of all BEP components, but also an increase in the difference between the latencies of waves IV and I. The latencies of the BEPs and the amount of myelin recovered became normal within 15 days when TET was removed from drinking water. These results indicate that when the myelin content of CNS is reduced, there is an increase in the latencies of the BEP and vice versa. Since the synaptosomal content of CNS remained unaltered in TET treated rats, it is reasonable to suggest that BEP latency changes are associated with alteration in the CNS myelin and may serve as a measure of myelin defect.

Supported by NIH Grants NS11670 and NS12424.

478 THE UNCONDITIONED FELINE SENSORIMOTOR RHYTHM AND AUDITORY INFORMATION PROCESSING: AVERAGED EVOKED RESPONSE AND SPECTRAL STUDIES. J. Marc Simard*, Calvin C. Turbes and Gerald T. Schneider* (SPON: James Maskin). Creighton University School of Medicine, Omaha, NE 68178

Electroencephalographic activity (EEG) was recorded from the sigmoid gyrus, the nucleus accumbens and the basolateral amygdala in freely moving cats using telemetric methods. Free field auditory stimulation consisted of 10 ms bursts of a 2 KHz tone, repeated every 10 sec for 20 min, each day for 5 consecutive days. Cats were rerun using the same paradigm one month later. (Amphetamine was administered between runs.) Ten sets of data were thereby obtained for each cat. All data were analyzed digitally. For each set of data, amplitude spectra of the sigmoid EEG were computed for the 500 ms immediately preceding each of the 120 auditory stimuli. The statistical parameters of each of these groups of spectra were then used to sort each set of responses into 2 classes: responses associated with high prestimulus SMR; responses associated with low prestimulus SMR. The subcortical responses were sorted according to the concurrent classification of the sigmoid response. For each set of data, the average response in each class for the 3 brain areas was computed. In addition, the average of the power spectra of each response period and the power spectrum of the averaged evoked response (AER) for each class of each set for the 3 brain areas were computed.

Visual analysis revealed a high degree of stability, over the 1 month course of the experiments, of certain components of the AER's consistently associated with high SMR. Generally, differences between the 2 classes of responses were greatest for those responses obtained from the sigmoid gyrus, while lesser, though still distinct and stable differences between the classes were observed from the subcortical sites. Quantitative analysis of the P250-350 wave of the sigmoid AER's sorted for high SMR indicated a close relationship between the amplitude of this wave and the average power of the SMR preceding the response to the stimulus: in 3 of the cats, positive correlation coefficients of .94-.97 ($P<.01$) were obtained for this relationship. The spectral analyses demonstrated the absence of coherence between the SMR and the AER, thereby excluding the high amplitude, highly synchronous SMR as a direct contributor or contaminant of the AER.

The significance of these data in a re-evaluation of current theories on the SMR will be discussed.

Supported in part by a doctoral dissertation research fellowship from the Benevolent Foundation of Scottish Rite Freemasonry, Northern Jurisdiction to JMS.

479 INTERACTION BETWEEN SPATIALLY SEPARATE PERIPHERAL NERVE FIBERS AS MEASURED BY ALTERATIONS OF THE SEP: A FUNCTION OF ALGEBRAIC ADDITION OF WAVE COMPONENTS. R.K. Simpson, Jr.,* E.H. Carlton*, and S. Katz (SPON: L.D. Middaugh). Dept. of Physiology, Medical University of South Carolina, Charleston, South Carolina, 29403.

Interaction between spatially separate peripheral nerves in monkeys anesthetized with 70% N_2O and 30% O_2, was investigated using as measurement, alterations of the somatosensory evoked potential (SEP). The SEP's from forelimb peripheral nerve stimulation were observed 100 msec after a stimulus was applied to a hindlimb peripheral nerve and compared to control forelimb SEP's. The resultant alterations were consistently demonstrated in forelimb SEP early wave components (within 40 msec.). Interaction observed by this study demonstrated different SEP alterations than those seen using cats anesthetized with chloralose. This may reflect fundamental differences in CNS structure or mechansims responsible for interaction. In this study, decreased amplitudes of negative potentials and enhanced amplitudes of positive potentials occured. Hindlimb SEP's, recorded from the forelimb receiving area, resulted in long duration, long latency, positive potentials. This SEP when algebraically added to the control forelimb SEP, corrected for the 100 msec delay, resulted in duplication of the SEP derived from interaction. These data suggest that interaction between spatially separate peripheral afferent inputs may be due to the algegraic summation of voltages. A physiological mechanism for interaction is suggested by this study based on the observation that later wave components of one SEP may influence early wave components of another SEP when these potentials occur at the cortex simultaneously. This is further supported by previous investigations showing a correlation between different components of the SEO and activation of different diameter peripheral nerve fibers. The observed interaction may take place as a result of summed electrical events occuring at the postsynaptic level. A simple model fitting the observed SEP alterations and established theories of SEP wave formation would be that of inhibitory potentials invluencing excitatory potentials of neurons in the upper cortical cell layers. This could occur as a result of activity from late arriving small fiber systems affecting the activity of earlier arriving large fiber systems in an inhibitory manner. This may be reflected in the observed alteration of the SEP. (Supported by NINDS grant P-5P81-NS-11066).

480 SINGLE-TRIAL AND SMALL-N VERTEX BRAIN POTENTIALS RELATED TO SENSORY THRESHOLD AND RELATIVE DURATION ESTIMATE FOR TWO MODALTIES IN HUMANS. Hilton Stowell. Sensory Neurophysiol., Rivers Lab, CSH, GA 31062.

For psychophysical correlates single-trial and small-n records of event related brain potentials (ERBP) have advantages over conventional averages. This study is part of a plan to evaluate the functional significance of the vertex component of somatosensory and acoustic ERBP for sensoriperception, with reference to exogenous and endogenous aspects of central processing. Vertex-focussed ERBP may confound activity from both "nonspecific" thalamic nuclei and specific contra- and ipsilateral cortex. Moreover, the relationship of P200 phenomena to the vertex component is unclear. Also, there is evidence for the functional importance of amplitude changes in the vertex component for pain perception in man.

Intrasubject studies of a small sample, using self-administered and exogenously administered stimulation with control of subjective expectancy and attention, suggest that the vertex component (80-400 ms after stimulus onset; 50-200 ms after stimulus offset) of somatosensory and acoustic ERBP is an indicator of subjective readout on (a) sensory detection threshold; and (b) discrimination of relative stimulus durations (off responses); also that this component is unlikely to reflect only stimulus/sensory-nonspecific, endogenous variables, since the vertex scalp electrode, during intermodality stimulation, seems to differentiate either the spatial or the temporal patterns of cerebral activity, or a combination of both, with respect to input modality.

The single-trial data of this study are "raw" ERBP. That is to say, they have not been subjected to adaptive filtering, template matching, or any processing other than the usual on-line amplifier bandpass and filter time constant used for small-n averaging.

This work was supported by the Research Section of PERT, Division of Mental Health and Mental Retardation, Dept. of Human Resources, Central State Hospital, Milledgeville, GA 31062.

481 STUDIES ON BRAIN RHYTHM INTERRELATIONS (THETA, SENSORIMOTOR [SMR] AND 40 Hz) IN THE SIGMOID GYRUS, NUCLEUS ACCUMBENS AND AMYGDALA OF THE CAT. Calvin C. Turbes, J. Marc Simard*, Gerald T. Schneider*, Dept. Anat., Sch. Med., Creighton Univ., Omaha, NE 68178; and R. John Morgan, Colorado State Univ., Ft. Collins, CO 80521.

Recordings are made on cats with chronic electrode implants and using radiotelemetry and hardwire methods. Analog data are collected on FM tape and analyzed digitally using Varian V-72 minicomputer. The discrete time data are transformed using a Fast Fourier Transform (FFT) algorithm. Spectral estimates are plotted sequentially out to a frequency of 50 Hz.

Special consideration is given to these CNS rhythms as they relate with normal behavioral states and altered behavior induced by d-amphetamine and l-amphetamine.

Spectral analysis of auditory evoked potentials during normal behavior and d-amphetamine and l-amphetamine altered behavior is compared with ongoing spectral arrays of the same experimental states. These data indicate that amphetamine altered cerebral cortical activity resulted in changes in the averaged auditory evoked potentials and the spectral profiles. These changes and how they relate to deficits in auditory information processing are discussed.

Long term ongoing spectral arrays show that amphetamine induced behavior shows dissociation of rhythmic activity at the cortex and these subcortical nuclei. There is also a "fixation" of rhythmic processes at the cortex and the subcortical areas that deviates from the rhythmic activity of normal behavior.

482 WAKEFULNESS-SLEEP MODULATION OF THE THALAMIC MULTIPLE UNIT ACTIVITY IN HUMANS. Francisco Velasco, Marcos Velasco, Carlos Cepeda*, Héctor Muñoz* and Xavier Almanza.* Sci. Res. Dept., Natl. Med. Ctr., IMSS and Service Neurol. Surg., General Hosp. SSA, México, D.F.

This is a study of the changes in spontaneous and evoked multiple unit activities from different thalamic nuclei during wakefulness and sleep of Parkinsonian patients with implanted electrodes as part of their surgical treatment of tremor:

Spontaneous multiple unit activity (number of units /min) of all studied thalamic nuclei (VPL, VL and NcM) showed large variations according to the state of wakefulness and sleep: It was high during distraction and paradoxical sleep, it was moderate during quiet wakefulness and attention and was low during habituation and slow wave sleep (stages I, II, III, IV). In contrast, multiple unit activity evoked by single shock stimulation of the median nerve (number of units/0.2 msec) and recorded from VPL nucleus (mean latency of 18 msec) showed no significant changes during various states of wakefulness and sleep.

483 CEREBRAL POTENTIALS PRECEDING SPEECH PRODUCTION. Donald H. York and Thomas W. Jensen. Dept. of Physiology and Communication Disorders Unit, University of Missouri, Columbia, MO 65212.

Earlier studies have examined the EEG for hemisphere assymetries during vocalizations. Other studies have looked at computer averaged slow waves which precede speech. The present study addressed the question of whether there are potentials preceding speech which are utterance specific. If such potentials can be demonstrated, do they represent a cortical program for the vocalization?

Experiments were conducted on 26 female subjects, mean age 23.6 years, who were right-handed and not taking any medications. Standard EEG electrodes were placed at the midline intra-aural position (Cz) and on the left mastoid process. A ground electrode was placed on the right forearm. Electrodes were connected to a preamplifier and then averaged in a computer of average transients (CAT1000). A stimulator provided timing pulses to one oscilloscope which served as a visual target for the subject to start a vocalization. It was also used to trigger the averager at various times preceding the vocalization. The initial run (control) consisted of the subject fixating on the oscilloscope screen, at a precise point where a black arrow was placed, while 100 sweeps were obtained in three groups of 33 sweeps. A short break inbetween was undertaken to minimize fatigue. The subsequent runs consisted of the subject voicing a mono-syllablic production when the oscilloscope trace reached the arrow. Various time periods preceding the vocalization were examined for consistencies in the topology of the averaged waveforms. At least five utterances were evaluated on each subject. Recordings of EMG from muscles overlying the larnyx were also obtained during vocalizations and always occurred after the time period over which EEG signals were averaged. The results demonstrate that consistent waveforms between subjects for certain vocalizations were obtained only during selected time periods preceding the vocalization. A most interesting observation was the production of a waveform "identical" to a voiced waveform by thinking the word, but not voicing it. The potential for such studies in speech pathology is presently being explored.

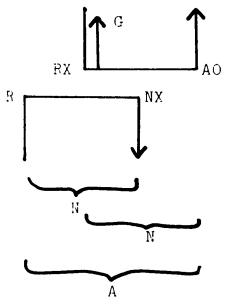
(supported by Research Council, School of Medicine)

EXTRAOCULAR MOVEMENT

484 LATENT EVENTS PRIOR TO HUMAN SACCADES. B.D. Adams* and P.E. Hallett. Dept. of Physiology, Univ. of Toronto, Toronto, Canada.

The purpose of this study was to examine some timing parameters of the human saccadic oculomotor system. Targets were presented as blue-green oscilloscope dots (100 x foveal threshold) which stepped horizontally to one of 6 or 8 randomly selected positions in the range ± 12 degrees. The subject's left eye was monitored in the dark by a special near infra-red tracking device, the right eye being occluded by an eyepatch. Seven subjects were asked to perform two tasks - to follow the target with their eye (normal saccade N) or to make an equal and opposite eye movement (anti-saccade A) - and feedback of latency and error was given automatically (Hallett, Vision Res., in press). "Reflex" responses to the target (direction errors) occurred in only 5% of A trials. The mean A latency was typically prolonged and had a larger standard deviation than the N. Reaction times N or A, or differences such as A-N, vary considerably from subject to subject. However, the regression line of A on N is simply $A = 2.01N - 143$, with $r^2 = .98$ and $\sigma(A) = \sqrt{2}\,\sigma(N)$. This implies that the definition of the A goal occurs a fixed time in advance of the missing N saccade.

The diagram symbolically interprets the regression equation $A = 2N - 143$ as two identical overlapping processes, each of mean duration N, where R is the retinal image step and NX would be the start of the cancelled reflex saccade. Event AO is the observed anti-saccade, which originates, not from a fictitious retinal input RX, but more plausibly from an internal redefinition of the goal at some fixed time G in the overlap region.



Similarly, we interpret the experiments of Lisberger et al. (Vis.Res. 1975) as showing that cancellation of a saccade occurs a fixed time prior to the missing saccade. 'Fixed time' in the present context could be 'fixed place' neuroanatomically.

485 MODIFICATION OF THE PATTERN OF SACCADIC EYE MOVEMENTS FOLLOWING ABLATION OF THE MONKEY SUPERIOR COLLICULUS. Joanne E. Albano and Robert H. Wurtz, NIMH, Bethesda, Md. 20014.

A striking aspect of the ongoing behavior of the alert rhesus monkey is the high frequency of eye movements and fixations that are made as he surveys his visual environment. EOG recordings in normal monkeys with head restrained show that while viewing a complex visual field animals display a repertoire of large and small saccadic eye movements; the smaller eye movements of less than 15 degrees predominate. Some of these smaller eye movements take the form of a glance where the intervening fixation may be no longer than the latency for a saccade of the same size. When in the dark, the alert animal no longer executes the smaller eye movements or glances with such high frequency but the rate at which larger saccades occur is the same as in the light, suggesting that the smaller eye movements are visually-elicited.

Two monkeys were studied: one with a unilateral and the other with a bilateral ablation of the superior colliculus made under visual inspection. There is a selective reduction in the frequency of the smaller saccades and glances made in the damaged visual field. This effect is limited to saccades made in the lighted environment; there is no major change in the frequency of saccades made in the dark. This effect is not a result of a simple sensory or motor loss, since these animals could perform detection and saccade tasks to points within the central 15 degrees.

While performing a visual fixation task normal monkeys are readily distracted by an irrelevant and unrewarded stimulus onset and movement occurring in the peripheral visual field. This distraction is expressed by either a glance or less frequently by a saccade toward the stimulus. If the stimulus is presented repeatedly or predictably the animal no longer glances at the stimulus. Following ablation of the superior colliculus, monkeys are no longer distracted by onset and movement of a stimulus in their damaged field although the unilaterally lesioned monkey continues to be distracted by a stimulus in the visual field contralateral to the remaining superior colliculus.

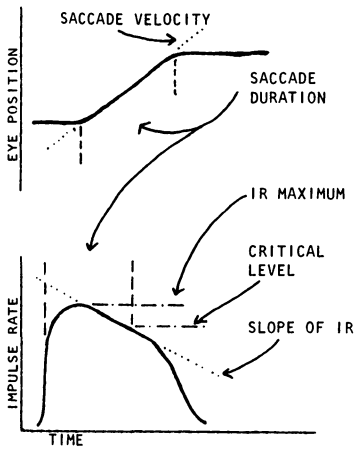
Previous experiments on visual-oculomotor function following ablation of monkey superior colliculus concentrated on a learned saccade task and found only increases in saccadic latency or slight errors in saccadic accuracy in the central visual field. Our observations suggest that in a more complex visual environment the consequence of colliculus ablation is to dramatically modify the normal pattern of visually elicited saccadic eye movements.

486 FINE STRUCTURE OF SACCADE BURST UNITS IN THE MACAQUE BRAINSTEM. S.M. Blair*, R. Eckmiller and M. Gavin*. Dept. of Physiology-Anatomy, University of California, CA 94720.

Single unit recordings from the brainstem of the alert macaque show a population of neurons whose bursts are closely correlated with saccadic dynamics.

Juvenile macaques were allowed to make random saccades, or were trained to execute a stereotyped series of saccades in the horizontal meridian. Neuronal discharges were recorded along with vertical and horizontal electro-oculograms. For units which burst in relation to saccades we determined the time course of the impulse rate (IR) in each burst in relation to the dynamic properties of the accompanying saccade.

We identified a class of neurons that began firing about 12 msec before each saccade, and stopped firing about 2.5 msec after completion of the saccade. During the last 2/3 of the burst the IR showed a near linear decrease with a slope varying from 127 to 1270 ips/sec. This slope varied inversely with velocity of the saccade. The duration from onset of burst to the time when IR fell to a constant critical level was correlated with duration of the saccade. Maximum IR occurred in the first 1/3 of the burst, was nearly constant for all saccades in the "on" direction, but was inversely correlated with the slope of the last 2/3 of the burst.



These findings are consistent with a view which links the dynamics of the saccade to the time course of IR in the bursting unit, but separates these from the neural input which initiates the saccade. Such an interpretation implies that saccadic dynamics are due in part to a neural input, available when the saccade starts, which codes the position in which the saccade will end.

Supported by Grant EY-00592 from the Nat. Eye Inst., U.S. P.H.S. Eckmiller is supported by Ec43/4 from DFG/ Germany.

487 CONNECTIONS OF A VERTICAL EYE MOVEMENT AREA IN THE ROSTRAL MESENCEPHALIC TEGMENTUM OF THE MONKEY. J.A. Büttner-Ennever* and W. Lang*
(SPON: M.E. Anderson). Brain Research Institute, University of Zürich, 8029 Zürich, Switzerland.

Recent anatomical and physiological studies indicate that not only the interstitial nucleus of Cajal (iC), which lies caudal to tractus retroflexus, but also the rostral interstitial nucleus of the medial longitudinal fasciculus (rostral iMLF), lying rostral to tractus retroflexus, is involved in the generation of vertical eye movements. On the other hand our results suggest that nucleus Darkschewitsch does not participate in oculomotor control. The neural connections of iC and rostral iMLF were studied using anterograde ([^{3}H]proline and [^{3}H]leucine) and retrograde (horseradish peroxidase) tracer substances in macaque monkeys.

HRP studies revealed that both cell groups send fibers to the oculomotor nucleus but the projections from rostral iMLF were mainly ipsilateral while those of iC were mainly contralateral. The anterograde tracer experiments revealed that these efferent fibers supply the trochlear and oculomotor nuclei, bilaterally, with the exception of the subgroups of the oculomotor nucleus which receive an input from the abducens nuclei, that is the medial and inferior rectus divisions. The contralateral terminal labelling after an injection of [^{3}H] amino acids into iC and rostral iMLF arose from fibers that crossed within the posterior commissure, and also included rostral iMLF, iC and its adjacent reticular formation. Control injections in n. subfascicularis, the fields of Forel, the hypothalamus, the red nucleus and the n. Darkschewitsch labelled of none of the above structures. Long pathways which descend within the ipsilateral MLF were shown to originate from both iC and rostral iMLF. Both cell groups receive vestibular afferents, although the pontine reticular formation, a center for vertical and horizontal gaze projects only to rostral iMLF.

Rostral iMLF and iC certainly subserve different functions, but there are some striking similarities in their anatomy. These results emphasize their importance in the control of vertical eye movements.

Supported by the Swiss National Science Foundation Grant 3.636.75 and the Dr. Eric Slack-Gyr Foundation.

488 THE ROLE OF VELOCITY STORAGE IN VISUAL-VESTIBULAR INTERACTIONS IN HUMANS AND MONKEYS. Bernard Cohen, Volker Henn*, Theodore Raphan, and Victor Matsuo*. Depts. of Neurology, Mt. Sinai Sch. of Med., CUNY, New York, N.Y. 10029, and Univ. of Zurich, Zch, SW

In the monkey a velocity storage mechanism in the VOR is important for production of optokinetic nystagmus (OKN), optokinetic after-nystagmus (OKAN), and vestibular nystagmus, and provides a focus for visual-vestibular interactions (Raphan, Cohen & Matsuo, 1978). The purpose of this study was to compare OKN, OKAN and per- and post-rotatory nystagmus in humans and monkeys and to determine the role of velocity storage in producing visual-vestibular interactions in man. As previously reported the gain of OKN in humans is 0.8-1 to 60-90°/sec. Similar gains were found for rotation in light, but for rotation in darkness vestibular gains were lower, varying between 0.35-0.75. In the monkey gains for rotation in dark and light are close to 1 to 180-240°/sec. During OKN in the monkey eye velocity rises rapidly and then slowly reaches peak values. In humans eye velocityrises immediately and there is no slow rise to a steady state value. OKAN in monkeys saturates at 90-120°/sec; in humans OKAN is weak and saturates at about 20°/sec. Rotation in light is followed by a decrease in intensity of after-nystagmus in both humans and monkeys. Consistent with saturation levels of OKAN, after-nystagmus could be reduced by 90-120°/sec in monkeys, but only by about 20°/sec in man. Visual fixation during OKAN and post-rotatory nystagmus causes a loss of activity responsible for the nystagmus. In the monkey fixation times of 2-3 sec abolish OKAN and 5-8 sec abolish vestibular nystagmus. In humans, the effect of visual fixation was much less than in the monkey; even 30 seconds of fixation failed to block the reappearance of vestibular nystagmus in darkness. Perception of motion was affected by visual fixation to a much greater extent than nystagmus. Evidence for velocity storage is the presence of OKAN, and the ability to reduce post-rotatory nystagmus by stored activity associated with OKN. In monkeys the visual system is capable of powerfully activating velocity storage to reduce after-nystagmus. The visual system has much less access to this storage mechanism in man. The response can be modeled using a similar scheme to that in the monkey. The model indicates that direct pathways from the visual system to the oculomotor system are more important for mediating OKN in man than in monkey and that the integrator plays a relatively minor role. Consequently the velocity storage integrator seems to be more closely related to enhancing the low frequency characteristics of the VOR than in supporting ocular following. It could also be important in mediating the perception of motion.

Supported by NINCDS Grant NS 00294 & Fellowship NS 05297(T.R.)

489 ASYMMETRIES IN BINOCULAR COUNTERROLLING IN HUMANS DURING DYNAMIC ROTATION. Shirley G. Diamond*, Charles H. Markham, Norman E. Simpson* and Ian S. Curthoys*. Dept. Neurol., Sch. Med., UCLA, Los Angeles, CA 90024.

Seven subjects ranging in age from 18 to 66 years underwent 360° rotation about a naso-occipital axis at a constant velocity of 3°/sec in a specially constructed rotating chair. Subjects were securely held by a series of straps. The head was stabilized by means of a bite bar adjustable to permit precise horizontal alignment of the centers of the two pupils. A camera was mounted on the chair and rotated with the subject. A line etched on the view finder passed through the centers of both pupils, enabling verification of pupil alignment during rotation. Photographs of the whole upper part of the face were taken at each 10° of rotation. Most subjects were given 4 trials, with rotations beginning left ear down or right ear down in random order.

Dual projectors were used to measure counterrolling. The first projector contained a slide of the eyes taken while the subject was upright (0°). The second projector contained the film strip of the eyes at each 10° of rotation, and was fitted with horizontal and vertical adjustments and a calibrated rotating device. The image from the second projector was aligned and rotated until it was exactly superimposed on the image from the first projector. Repeated interruption of one light beam induced apparent motion of the iris when superimposition was not precise. The extent to which the second image had to be rotated to achieve superimposition was the measure of counterrolling at that position. This measurement system has a mechanical accuracy of one minute of arc; practical accuracy is between 15 and 30 minutes. Right and left eye in each trial were measured independently.

Results showed the extent of counterrolling was greater than most earlier studies have indicated. Mean range of counterrolling (maximum on one side to maximum on other side) was about 20° with individual means varying from 9° to 35°. Each subject showed a high degree of consistency from trial to trial.

Although mean range of counterrolling was 20°, this was not distributed symmetrically around the upright position. Maximum counterrolling when subjects were right ear down averaged 12° and left ear down averaged 8°, regardless of which direction was presented first. The two eyes did not counterroll in perfect conjunction, sometimes being as much as 4° different. The downward eye almost invariably rolled more than the upward eye. Amount of counterrolling appears to decrease with age.

490 VELOCITY CODED NEURONS: A NEW CLASS OF PRE-MOTOR NEURONS IN THE PRIMATE OCULOMOTOR SYSTEM DURING PURSUIT Rolf Eckmiller and Manfred Mackeben*. Smith-Kettlewell Institute, San Francisco, CA 94115

Initially, Duensing and Schaefer(Arch.Psychiat. Z. ges.Neurol.196:402,1957)working with rabbits, and since then several authors working with monkeys described neurons in the reticular formation, which were only "loosely coupled" with eye movement(EM) parameters. Therefore, they were not considered in the description of oculomotor functions.

We found a population of such neurons in a circumscribed region 0.5 to 1.5 mm caudal to the abducens nuclei and discovered that they reveal their correlation with EMs only during pursuit. Single unit recordings were made in 2 Java monkeys trained to pursue a light spot(4 min.of arc in diameter), which was sinusoidally moving in the horizontal plane. EMs were recorded form both eyes simultaneously as DC-EOG using 3 implanted electrodes. During spontaneous EMs this neural activity was only loosely related to large saccades and to far eccentric eye positions. However, during pursuit EMs these neurons showed a good correlation with eye velocity. The neural activity was characterized by the following features:
a) impulse rate IR during spontaneous EMs was zero or very low and irregular in the range of ± 10 deg.
b) IR of neurons caudal to the right abducens nucleus increased with pursuit velocity to the right and vice versa for neurons located on the left side.
c) IR_{max} during sinusoidal pursuit(f= 0.1 to 1.5 Hz) in the on-direction typically did not exceed 100 impulses per second. d) IR_{max} during sinusoidal pursuit occurred in the phase range of ± 30 deg. around the maximum stimulus velocity v_{max}(in comparison, IR_{max} of motoneurons during sinusoidal pursuit lagged 40 to 80 deg. behind v_{max}). Therefore we suggest to designate this class of neurons as velocity coded (VC) neurons.

We assume that these VC-neurons provide the velocity component for the activity of motoneurons(dur. pursuit).

(Supported by NIH, Ey 01474 to P. Bach-Y-Rita and by DFG/Germany, Ec 43/4 to R.Eckmiller)

491 VISUAL RESPONSES OF OMNIPAUSE NEURONS IN THE AWAKE CAT. C. Evinger*, C.R.S. Kaneko, and A.F. Fuchs. Dept. Physiol. and Biophysics, and Regional Primate Center, Univ. WA., Seattle, WA 98195.

Omnipause neurons (OPN) in the cat cease their tonic discharge just before and during all saccadic eye movements made in either the light or dark. In addition, microstimulation near OPN suppresses all saccades, but does not interrupt smooth eye movements. Models of saccade generation propose that OPN inhibit neurons that exhibit a burst of activity during saccades and thereby control saccade duration. However, we have shown that OPN are excited by visual stimuli and it is not clear how this response fits into models of saccade generation. To understand the role of the visual input, we tested these neurons with a variety of visual stimuli. Stimuli were presented at locations within the visual field of alert cats trained to track or fixate a moveable target spot.

Omnipause neurons respond with a transient burst of activity to changes in luminance. Movement of a 45x55 deg striped background (0.6 cycles/deg) at velocities ranging from 1 deg/sec to step changes elicits an excitatory response regardless of the direction of movement. Turning a full field light stimulus either on or off evokes a brisk excitatory response. The cells respond over a four log unit range of light intensity with latencies ranging from 35 msec (0 log units) to 94 msec (4.0 log units).

The receptive fields of OPN were mapped with a 2 deg light spot. The spot was maintained at different constant positions on the visual field by using eye position to control spot position. With these techniques, each OPN studied (N=20) had a receptive field within which either a 2 deg spot displacement or turning the spot on and off elicited a vigorous discharge. The strongest response usually occurred for stimuli within 5 deg of the area centralis; however, significant responses occurred over a larger area ranging from 20 to over 40 deg in diameter for different neurons. The receptive fields showed no evidence of inhibitory areas. The strength of the visual response was inversely proportional to receptive field size.

In one cat visual stimuli were presented during saccades to test their effect on saccade properties and OPN activity. Visual stimuli triggered to occur during a saccade appeared to interrupt some saccades in midflight. During the interruption, the OPN resumed its tonic firing. Nevertheless, our data do not suggest an obvious role for the visual response of OPN in the generation of saccades.

492 RAPID CHANGE IN GAIN OF VESTIBULO-OCULAR REFLEX IN CHICKENS. Alan E. Green* and Josh Wallman. Biology Department, City College of City University of New York, New York, N.Y. 10031.

The vestibulo-ocular reflex (VOR) helps stabilize the visual world by means of eye movements that compensate for rotations of the head. If the horizontal movements of the visual world relative to the head are reversed, as by wearing reversing prisms, the normal VOR destabilizes the visual world, since the eye movements produced now increase, rather than decrease, retinal image slip. In this situation, the amount of eye rotation for a given head rotation (that is, the gain of the VOR) is gradually reduced, thereby improving visual stability. This adaptive plasticity of VOR gain has been shown in humans, monkeys, cats, rabbits, and goldfish. There is evidence for involvement of the cerebellum in this gain plasticity. Since at hatching chicks have apparently normal visual function, and the cerebellum is relatively immature, we were interested in whether there is a post-hatching developmental change either in VOR gain, or in the degree of plasticity of VOR gain.

Eye position and head position were measured with the magnetic field-search coil technique. The VOR gain (eye velocity/head velocity) was measured in the dark. Reversed movements of the visual field relative to the head were produced by rotating the animal sinusoidally in the horizontal plane (0.25 Hz, 7° peak-to-peak), while a surrounding vertically striped cylinder rotated in phase with the animal, but with twice the angular amplitude. This simulates the effect of reversing prisms without restricting the visual field.

Normal VOR gain was about 0.4 in two-week-old birds (n=4), but only 0.24 in two one-day-old chicks. After two hours of sinusoidal rotation in the reversed visual movement situation, the gain was reduced to about 0.1. The time constant of this gain reduction was approximately one hour. The gain reduction could be reversed with a similar time course by rotating the birds within a stationary visual field. VOR gain was higher at higher frequencies (0.5 Hz and 1 Hz) and showed less change as a result of the reversed visual experience. These results suggest that this form of motor learning is present at hatching and improves with age. The rate of the VOR adaptation to reversed visual movement seems more rapid than reported for other species.

493 AFFERENTS TO THE MEDIAL PONTINE RETICULAR FORMATION AS DEMONSTRATED BY HORSERADISH PEROXIDASE (HRP) RETROGRADE TRANSPORT. R. W. Greene*, M. Schulder* and G. B. Stanton* (SPON: A. I. Kobrine). Armed Forces Radiobiology Research Institute, Bethesda, MD 20014 and Howard University, Washington, D.C. 20059.

Projections have been demonstrated from the superior colliculus (SC) to the caudal pole of the contralateral nucleus reticularis pontis caudalis (RPC) using anterograde degeneration techniques (Kawamura et al., Exp. Br. Res. 19:1, 1974). In order to identify the cells of origin to this projection, the floor of the IVth ventricle was exposed by aspiration of the midline cerebellum and HRP was pressure-injected through micropipettes into the RPC and the VI nucleus. Control injections were made in the rostral part of the nucleus reticularis gigantocellularis (RGc) and anterior to the VI nucleus.

Injection of the RPC and the VI nucleus produced labeled cells bilaterally in the deep and intermediate layers of SC but none in the superficial layers. Labeled neurons in the contralateral SC were most numerous in the rostral half (12 large and 14 medium-sized neurons/60 µm section). Moreover, labeling intensity of the large cells was greatest in the medial third of SC which suggests that these soma have the largest number of axon terminals within the injection site. On the ipsilateral side labeled neurons were few and faintly labeled. No projections from the SC to the VI nucleus were seen following HRP studies by Maciewicz et al. (Br. Res. 123:229, 1977). Furthermore, our control injections resulted in relatively few labeled cells in SC, confirming the localization of terminals of SC-RPC projection to the caudal pole of the RPC.

Another major projection to the RPC was from the vestibular nuclei. Cells were most numerous in the ipsilateral medial, inferior, and ventrolateral vestibular nuclei, but were also seen in these nuclei on the contralateral side. Other nuclei labeled by injection in the RPC include on the ipsilateral side, the nucleus of the field of Forel, the nucleus of Darkschewitsch, the interstitual nucleus of Cajal, the mesencephalic reticular formation and the spinal nucleus of V. Nuclei labeled bilaterally include intermediate sized cells in the caudal part of the nucleus of III, the pontine raphe nuclei, the pontine lateral tegmental fields, the RPC and the RGc.

These results demonstrate that the cells of origin of the SC-RPC projection are the large and intermediate sized neurones in the deep and intermediate layers of the SC.

494 VERTICAL OCULOMOTOR DEFICITS FOLLOWING MLF LESIONS IN MAN AND MONKEY ARE SIMILAR. D. Guitton and T. Kirkham*, Montreal Neurological Institute, McGill University, Dept. Neuroophthalmol., 3801 University St., Montreal, P.Q. Canada H3G 1Y6.

Fibres of the monkey medial longitudinal fasciculus (MLF) can be grouped into two categories: (1) horizontal burst-tonic fibres that discharge with adducting saccades and have quantitatively similar characteristics as medial rectus motoneurones; and (2) vertical tonic-pause fibres that pause for vertical saccades and carry the full vertical vestibulo-ocular reflex, and a portion of both vertical pursuit and hold signals (King et al., J. Neurophysiol. 39:1135, 1976). Lesions of the MLF in monkey have revealed oculomotor deficits compatible with the elimination of these signals (Evinger et al., Exp. Brain Res. 28:1, 1977). In man, the syndrome of internuclear ophthalmoplegia (INO) has been associated with lesions of the MLF. The well documented horizontal oculomotor deficits are similar to those produced in monkey (Kirkam, T. and Katsarkas, A., Ann. Neurol., 2: 385, 1977).

In the present work we have examined vertical deficits. Vertical eye movements in INO patients have been recorded using the eye coil in magnetic field technique. An annular ring, with an embedded coil of fine wire, was held to the eye by suction and subjected to vertical and horizontal magnetic fields. The results are compatible with observations in monkey. Patients with presumed MLF lesions showed: (1) normal vertical saccades; (2) deficits in vertical pursuit and in the ability to hold the eye in an eccentric up or down position. We have not examined vertical VOR. However, we have measured optokinetic afternystagmus (OKAN) following the induction of vertical optokinetic nystagmus (OKN) with a 110°x110° moving bar pattern. OKAN is linked to activity in the vestibular nuclei (Waespe, W. and Henn, V., Exp. Brain Res., 27:523, 1977) and for vertical eye movements should be carried by the vertical tonic MLF fibres. Patients with INO had deficient OKN and this is compatible with deficient pursuit. OKAN was abolished.

In summary, vertical and horizontal oculomotor deficits associated with presumed lesions of the MLF in humans are compatible with recent neurophysiological and anatomical data obtained in monkey.

Partially supported by Killam funds.

495 QUANTITATIVE STUDY OF HUMAN SMOOTH PURSUIT TRACKING Michael J. Iandolo, A. Terry Bahill and B. Todd Troost, Biomedical Engineering Program Carnegie-Mellon University Pittsburgh, PA 15213

Random target movements with statistical properties approximating gaussian white noise were used to quantify human smooth pursuit system tracking behavior and to nullify the effects of the learning process. Bandlimited gaussian white noise and variations of Pseudo Random Binary Sequences (PRBS) were employed as the target waveforms. Because the power spectrums of the waveforms are flat from zero hertz to any predetermined frequency, the full frequency range of the smooth pursuit system can be characterized with these stimuli using a short test run.

Bandlimited gaussian white noise waveforms were discarded because the subjects found them to be too confusing. This confusion resulted in an increased number of saccades.

With the binary states of the PRBS corresponding to positive and negative velocities, a controllable, constant speed waveform was generated in which the direction of movement changed at random intervals. Because the smooth pursuit branch is a velocity sensitive mechanism, a complete test of the system would need to contain more than one velocity. Therefore another variation of the PRBS was implemented in which the target velocity was a random variable and the intervals at each velocity were constant. By using PRBS, some parameters can be easily constrained i.e. target velocities, target range and acceleration, so that system nonlinearities can be reduced. With these target waveforms, the system transfer functions were computed.

Also investigated was the interaction between horizontal and vertical eye movements using predictive (single sinusoids) target waveforms in one direction and random (PRBS) target waveforms in the orthogonal direction. It was found that the two channels of the system were independent with some crosstalk.

Eye movements were recorded with an infrared photoelectric measurement system. To eliminate head movement artifact, the subject's head was restrained. The target waveforms were generated in a digital computer and converted into an analog voltage. The target was a small phosphorescent dot located on a large oscilliscope screen.

Research was supported by NIH grant 1 R23 EY02382-01 and NIGMS grant T32 GM 07477

496 NECK POSITION MODULATES VESTIBULAR EYE NYSTAGMUS PARAMETERS. David W. Jensen. Dept. Neurosciences, UCSD, La Jolla, CA 92093.

Neck proprioceptive stimuli alter the eye nystagmus caused by a unilateral injection of 0.3cc of 2% Lidocaine HCl into the middle ear cavity of guinea pigs. Eye position was measured with Ag-AgCl ball electrodes chronically implanted into holes in the left and right orbital crests that communicated with the orbits. Electrode signals were differentially amplified and displayed on a D.C. recorder. The electrooculogram (EOG) was calibrated by either comparing a videotaped record of eye position during nystagmus with concommitant EOG amplitude, or by measuring saccadic EOG amplitudes and the magnitude of the associated horizontal voluntary head movements. Four parameters of nystagmus were measured as a function of maintained horizontal or vertical neck position: frequency of beating, mean eye position, mean slow phase velocity, and direction of beating. Direction of beating was measured by videotape analysis. Neck proprioceptive stimuli were delivered by moving the body about the fixed head. Vision was unobstructed in normal laboratory illumination.

The Lidocaine treatment produced a conjugate horizontal eye nystagmus within 5-40 minutes in all animals screened for normal vestibular reflexes. Vertical neck position did not alter this nystagmus. For a right-beating nystagmus (left ear block), a left (horizontal) neck deviation produced tonic increases in frequency and in mean slow phase velocity and a maintained left eye deviation, when compared to the situation with no neck deviation. Right neck deviation produced tonic decreases in frequency and in mean slow phase velocity and a smaller left eye deviation. The direction of beating was not changed. These effects were present in all 17 animals studied and were proportional to the amount of neck deviation imposed (maximum = 90° deviation). These influences persisted after a dorsal hemisection of the spinal cord just above C-1 (1 animal); after bilateral fastigial nuclear lesions (2 animals); and nodulectomy with extensive damage to the uvula and lobule VIIIb (1 animal). In two animals the entire vestibulocerebellar cortex was removed, along with the posterior vermis and postero-lateral hemispheres. Both these animals displayed a rightward eye deviation with right neck deviation, while all other neck-eye nystagmus influences remained as described above.

In conclusion, neck proprioceptive information via the ventral funiculi integrates with eye nystagmus activity in a spatially specific manner. The vestibulo-cerebellum does not participate in neck effects on mystagmus beat frequency or mean slow phase velocity. However, certain parts of the cerebellum, probably the flocculi, are involved in controlling the magnitude and sign of the gain of neck controlled mean eye position during nystagmus. Funded by a NASA grant to T.H. Bullock.

497 CONNECTIONS OF FELINE OMNIPAUSE NEURONS. C.R.S. Kaneko and A.F. Fuchs. Dept. of Physiol. and Biophysics and Regional Primate Center, Univ. WA, Seattle, WA 98195.

Omnipause neurons (OPN) are tonically active and pause before and during all saccadic eye movmeents. Because of this activity pattern, OPN have been implicated in the control of saccades. However, neither the afferent inputs nor the target neurons of OPN have been studied in detail. Therefore, we have begun experiments in Ketamine anesthetized cats which are sufficiently anesthetized to allow both surgical procedures and microstimulation but still make the saccades necessary to identify OPN.

Eye movements were measured by an X-Y photodiode which detected the light emitted by an LED affixed to the cornea. Stimulating electrodes were placed in the superior colliculus (SC), cerebellar vermis (CbV), fastigal nucleus (FN), and vestibular nerve (VN) since stimulation at these sites evokes eye movements, and in the optic chiasm (OX) to further evaluate our recently demonstrated visual input to OPN. The OX electrode was adjusted to record the maximal evoked response to a flash of light. The SC electrode was first placed 1 mm ventral to the area in which the maximal OX or flash evoked response was recorded and then adjusted to evoke eye movements at minimal currents by stimulation. The FN electrode was also placed to evoke eye movements. The VN electrodes were placed bilaterally on the 8th nerves which were exposed by ventral approach through the bullae. The CbV electrodes were placed on lobules 5 and 6 after exposing the lobules by partial craniotomy.

Neither FN or CbV stimulation resulted in OPN responses to single or multiple shocks with currents up to 1.5 mA. Similarly, ipsilateral or contralateral VN stimulation never resulted in a response in OPN even at current intensities which were sufficient to evoke a twitch of the globe and were several times threshold for the vestibular field. Optic chiasm stimulation produced a long latency (range 6-12 msec) excitation which was presumably polysynaptic since it was highly variable and did not become more stable with increasing current or multiple shock stimulation. As shown in the monkey, SC stimulation resulted in a short latency excitation (1-2 msec) and/or a longer latency inhibition with increasing current strength or multiple shocks. The inhibition occurred even at current strengths too low to evoke discernible eye movements and was proportional in duration to the number of stimuli.

Although the description of OPN afferents is probably incomplete the above data show that many structures believed to be associated with saccade generation do not project directly to OPN. Our preliminary microinjections of tritiated amino acids into the functionally identified OPN nucleus have suggested candidate target areas for OPN projections. Further anatomical analyses and evaluation of OPN efferents using microstimulation techniques should supply a broader basis for assessing OPN function.

498 LATE BURST PURKINJE CELL ACTIVITY IN THE VERMIS DURING EYE MOVEMENTS. Manabu Kase* and Hiroharu Noda. (SPON: S. Hagiwara). Brain Research Institute, Depts. Physiol. Anat., Sch. Med., UCLA, Los Angeles, CA 90024.

Single unit activity recorded from Purkinje cells in the vermis (lobules VI and VII) of the monkey during saccadic eye movements and fixation was analyzed.

The majority of saccade-related Purkinje cells were characterized by saccade-onset related bursts which continued throughout the duration of saccade. During fixation, the Purkinje cells fired continuously at a relatively high rate, which in some cells was a linear function of eye position.

In addition, we discovered that other Purkinje cells showed an increase in activity which was time locked with the end of saccades. When present, this increased activity (late burst) appeared regardless of the direction of the saccades. The late burst was not due to visual inputs, since it persisted even in complete darkness and Purkinje cells exhibiting late burst did not respond to visual stimuli. The burst started immediately prior to the end of saccade (average 21 msec) and lasted for approximately 150 msec. There was no relation between the durations of the burst and the saccade. The peak activity in these Purkinje cells appeared within 40 msec after the end of saccades and usually attained frequencies up to 2-4 times the level of background activity. In some cells the late burst was preceded by a slight decrease in activity which started with the onset of the saccades.

It is known from the literature that the impulses of Purkinje cells are the sole output signal from the cerebellar cortex and exert a powerful inhibitory action on postsynaptic cells. The late burst of these Purkinje cells indicates that the vermis sends inhibitory impulses which are time locked with the end of saccades. As a function of the vermis, therefore, we suggest that it helps control the terminal stage of saccades and participates in the mechanism concerned with holding a new eye position after a saccade. (Supported by NIH Grant EY01051)

499 FIRING PATTERNS OF CENTRAL VESTIBULAR NEURONS DURING ADAPTIVE MODIFICATION OF THE VESTIBULOCULAR REFLEX BY REVERSING PRISMS. Edward L. Keller and Wolfgang Precht*. Dept.Elect.Eng. and Computer Sci., Univ. of Cal., Berkeley, CA 94720 and Dept.Neurobiol., MPI for Brain Res., Frankfurt/M., FRG.

It has been reported that prism modification of normal image motion on the retina during head movement results in adaptive changes in the vestibuloocular reflex (VOR). The cerebellar flocculus has been suggested as the site of the synaptic modification implied by these adaptive behavioral changes. Since the flocculus does not project directly to oculomotor neurons, these supposed synaptic modifications would have to be expressed through other neuronal pools more directly connected to motoneurons. In the present study we looked for possible changes in vestibular nucleus neuron (Vn) discharge patterns induced during adaptive prism modification of VOR. We recorded in alert cats the response of a large sample of Vn to horizontal sinusoidal oscillations of the head in darkness over a frequency range from .025 to .25 Hz. In the same animals on random days a similar sample of Vn were recorded after the cats had been force rotated in the horizontal plane at a frequency of about 0.1 Hz while viewing a highly structured visual field through reversing prisms. Gain of the VOR was monitored during the forced rotation and neural recording was begun when it had adaptively decreased by more than 50%. In each animal a similar pattern of changes in the population of prism adapted Vn responses was observed. There was a small, but highly significant, shift toward lower gains (the ratio of peak unit modulation to peak head acceleration) of the overall sample of Type I cells. This shift was observed almost exclusively in the lower gain, large phase lag (re. head acceleration), Type I units. Small phase lag Type I units were relatively unmodified. Since the small phase lag cells have been shown in a number of other studies to be predominately monosynaptically connected to the vestibular nerve, this result suggests that second order Vn are relatively unaffected by prism adaption. Among Type II units, on the other hand, there was only a small shift toward lower gains, but this was accompanied by large (up to 90 deg.) phase shifts. This result suggests that adaptive changes in the VOR may be mediated through Type II units, which are known to provide commissural inhibitory pathways to Type I units.

500 IS THE INTERSTITIAL NUCLEUS OF CAJAL A PREMOTOR CENTER FOR VERTICAL EYE AND HEAD MOVEMENTS? W.M. King, W. Precht* and N. Dieringer*. Max Planck Institute for Brain Res., Frankfurt/M, W. Ger.

The interstitial nucleus of Cajal (INC) may be a premotor center for vertical and rotatory eye and head movements. Anatomical studies suggest that INC cells receive inputs from the vestibular nuclei, superior colliculi and cortical frontal eye fields (FEF), structures which play roles in eye and head movement. Other studies show that INC cells project monosynaptically to extraocular and neck extensor motoneurons. However, it is not known if any one INC cell projects both to neck and eye motoneurons, or if all INC cells receive similar synaptic inputs. Initially, we used intracellular recording techniques in nembutal anesthetized cats to determine the synaptic inputs to INC cells, and to test each cell for antidromic activation from the cervical spinal cord.

Stimulation of the contralateral (V_c) or ipsilateral (V_i) VIII nerves evoked characteristic field potentials centered in the INC. Most INC cells (68%) were disynaptically excited from V_c (EPSP latency 1.8 $\pm$ 0.6 ms) and inhibited from V_i (IPSP latency 2.1 $\pm$ 0.8 ms). Acute bilateral lesions of the medial longitudinal fasciculus rostral to the abducens nuclei abolished the field potentials and intracellular responses. Electrical stimulation of the FEF underlying the cruciate sulcus and in the depth of the presylvian sulcus excited 56% of the units tested (EPSP latency 2.6 $\pm$ 1.1 ms). 37% of these units received vestibular input, and 51% were antidromically or synaptically activated from the spinal cord. We were unable to demonstrate synaptic inputs from either superior colliculus or optic chiasm.

These results demonstrate differences among INC cells according to their connections. Can these differences in connectivity be related to differences in discharge pattern? In the second part of our study, we recorded extracellularly from INC cells in Ketamine anesthetized cats during spontaneous or induced eye movements. Unit firing was correlated with the left superior and lateral rectus EMG. Single unit response to electrical stimulation was used to determine connectivity. Preliminary results revealed two groups of INC cells: Group A exhibited a burst-tonic discharge pattern similar to the discharge pattern of oculomotoneurons. All Group A cells received a vestibular input and were polysynaptically excited from the FEF. In contrast, the discharge pattern of Group B cells was unrelated to the EMG, and no Group B cell was activated by vestibular stimulation. Group B cells were orthodromically excited by FEF stimulation, and antidromically activated from the spinal cord. Our results suggest that Group A cells are premotor to oculomotoneurons, and Group B cells are premotor to neck extensor motoneurons. However, other functionally significant groups of cells may exist within the INC.

501 INTERACTION OF THE EYE MOVEMENT AND LOCOMOTORY CONTROL SYSTEMS. Jose R. Latimer, A. Terry Bahill, and B. Todd Troost. Neurological Control Systems Laboratory, Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213.

There have been many studies of eye movements and of locomotion, but none were found on the effects of locomotion on the eye movement system. Human locomotion is usually guided by input from the visual system. To facilitate the information processing of this system, the eyes must be moved so that the foveae of the eyes are aimed at certain specific points. The head oscillates up and down, from side to side, and back and forth while walking. Thus, the head does not provide a stable platform for the eyes, and compensatory eye movements must be made in order to fixate the eyes on target while walking. Head turns of about one-half a degree, head sways of two cm, and compensating eye movements of $\pm$ five degrees were measured.

Inputs from at least three sensory systems are integrated to guide the eye movement control system: the visual system, the vestibular system, and the proprioceptive system. Normal human eye movements used while walking were studied with the feedback loops of each system "opened". A homeomorphic model of the human eye movement control system as used for while walking was developed. It incorporates the sampled data visual feedback aspects of the saccadic control system, the vestibular-ocular control system, the proprioceptive control system, and the sixth order nonlinear extraocular Reciprocal Innervation Plant (RIP). As a first step in intergrating these various models the nonlinear RIP model was linearized. Of the various methods for doing this, one of the best was by changing the nonlinear force-velocity relationship into a time varying relation. This allowed the use of canned computer programs to analyze and simulate the plant so as to correlate the input-output relation of the RIP and the behavior of our complex model. The model was justified qualitatively by comparing the shapes of the human and model movements, quantitatively by constructing Main Sequence diagrams plotting velocity versus magnitude and duration versus magnitude for both head and eye data from the human and model data, analytically by performing a sensitivity analysis to find which parameters are most important, which require further study, and which can be ignored, and heuristically by simulating movements that the model was not designed to do.

Research supported by NSF grant ENG77-22418 and NIH grant 1 R23 EY02382-01.

502 EYE MOVEMENTS IN PARALYZED CATS. R.A. Linsenmeier* and B. Gevene Hertz* (SPON: C. Enroth-Cugell). Biomedical Engineering Center, Northwestern University, Evanston, IL. 60201.

Most quantitative experiments in visual neurophysiology require stability of the eyes. We have found, however, that relatively large eye movements occur under some experimental conditions, even in paralyzed cats.

Experiments were performed on cats anesthetized with urethane and paralyzed with gallamine (Flaxedil, 50 mg/kg-hr). Small mirrors were glued to the corneae, and a laser beam was directed off these mirrors onto a screen. We obtained a record of eye position as a function of time by plotting the reflected beam.

Eye movements before the experimental manipulations were small--about 0.14 degrees in 10 minutes. However, when bicuculline (0.3 mg/kg), strychnine (0.1-0.4 mg/kg) or methoxamine (Vasoxyl, 0.25 mg/kg) was then given intravenously, or when the animals were made hypoxic by respiring them with 5% O_2, eye movements were larger. The displacement in eye position was 0.5 to 2.0 degrees within 5 minutes of the manipulation. The eyes slowly returned to their initial positions following hypoxia and bicuculline, but did not return after methoxamine or strychnine. Unilateral cervical sympathectomy reduced only those movements of the ipsilateral eye caused by bicuculline and strychnine. It did not prevent movements associated with any of the agents tested.

Supported by NIH 5-R01-EY00206 and 5-F32-EY05015.

503 SACCADIC AND VESTIBULAR VELOCITY COMMANDS: DO THEY ADD? G. Mandl and D. Guitton. Aviation Medical Research Unit, McGill University, 3655 Drummond St., Montreal, Canada H3G 1Y6.

Microelectrode recordings from the medial longitudinal fasciculus and vestibular nucleus of alert monkeys have suggested that the vestibulo-ocular reflex (VOR) is mediated by cells whose discharges pause during quick phases. This implies that there should be no linear addition of slow and quick phase velocities during head rotation. Consequently, the characteristics of vestibular quick phases, and saccades with head fixed, should be identical. This prediction has been confirmed in the monkey (Ron et al., Vision Res., 12: 2015, 1972). Recent experiments with cats by Haddad and Robinson (Neurosci. Abs., 3, 155, 1977) indicate that the velocity of quick phases with respect to the head is actually faster than the velocity of saccades.

The present experiments were undertaken to re-investigate the differences between spontaneous saccades and vestibular quick phases. Three normal cats were studied. Their heads were firmly attached to a turntable while eye movements were recorded with an implanted scleral coil in a magnetic field. Measurements were obtained both in the light and in the dark under the following conditions: (1) head stationary with the animal performing spontaneous eye movements; and (2) head and body oscillated sinusoidally at a peak-to-peak amplitude of 40°, and peak velocities ranging from 25°/sec to 75°/sec. VOR gain was 0.85-0.95. Saccade and quick phase amplitudes ranging from 2°-20° were plotted against: (a) peak velocities; and (b) durations. Regression lines were fitted to these data. Covariance analysis revealed no statistically significant differences between saccades and vestibular quick phases. This lack of linear addition would be consistent with the presence of VOR-mediating tonic-pause cells in the cat. However, the interaction between quick and slow phase may depend on visual context. This is implied by the experimental results of Bizzi et al. (Exp. Brain Res., 16: 492, 1973), who have shown that linear addition does occur during visually triggered eye-head movements in the monkey.

Supported by the Canadian Medical Research Council.

504 THE EFFECT OF ANESTHESIA ON PONTOMEDULLARY RETICULAR BURST INHIBITORY NEURONS RELATED TO NYSTAGMUS. Charles H. Markham, Shozo Nakao* and Ian S. Curthoys* (SPON: Thomas L. Babb). Dept. Neurol., Sch. Med., UCLA, Los Angeles, CA 90024.

Nystagmus is a sensitive indicator of arousal state and in this study we sought to identify the neural mechanisms responsible for this sensitivity. Under halothane anesthesia cats received a unilateral labyrinthectomy. Bipolar stimulating electrodes were placed on the damaged labyrinth. The activity of the ipsilateral abducens and oculomotor nerves was recorded in the orbit. Stainless steel crews over the frontal and parietal cortex were used to record EEG activity. After removal of the midline cerebellum extracellular recordings were made from reticular neurons in the vicinity of the contralateral abducens nucleus. Halothane was discontinued and Flaxedil and extensive local anesthesia were maintained for the rest of the experiment. Efficacy of anesthesia was assured by continuous monitoring of blood pressure, pupillary size and EEG.

Unilateral labyrinthectomy results in long-lasting horizontal nystagmus with the slow phase to the ipsilateral side. When cats so prepared are paralyzed with Flaxedil, it can be observed that the ipsilateral abducens nerve shows rhythmic discharges corresponding to each slow phase. Each discharge slowly increases in amplitude and is abruptly terminated under influence of a small group of burst inhibitory neurons (BINs), largely located caudal to the contralateral abducens nucleus (Hikosaka & Kawakami, 1977). Each BIN fires in bursts 50-100 msec long, reaching a maximum discharge rate of 200-800/sec. These cells are identified by their location, firing pattern as described above, and by their failure to fire when the direction of nystagmus is reversed by electrical stimulation of the damaged labyrinth.

For most BINs during nitrous oxide anesthesia (80% N_2O, 20% O_2) the maximum firing rate during the burst decreases, the number of bursts per second decreases, whereas the duration of each burst increases. These changes occur at about the same time, as soon as 20 sec after anesthesia onset, as the first detectable slowing of the EEG. These changes in BIN activity are accompanied by progressive rounding of the end of the abducens slow phase discharge.

Hikosaka and Kawakami have shown BINs have a major role in horizontal nystagmus; we have shown the activity of these cells is quickly affected by the relatively weak nitrous oxide anesthesia.

505 VELOCITY STORAGE DURING VERTICAL NYSTAGMUS. Victor Matsuo*, Bernard Cohen, Volker Henn*, Theodore Raphan, and Vianney deJong*. Depts. of Neurol., Mt. Sinai Sch. of Med., CUNY, New York, N. Y. 10029 and University of Zurich, Zurich, Switzerland.

Velocity storage plays an important role in producing horizontal nystagmus induced by optokinetic and vestibular stimulation in the monkey (Raphan, Cohen & Matsuo, 1978). Experiments were done to determine the function of velocity storage in producing vertical nystagmus. Vertical OKN was induced by rotating an optokinetic drum around monkeys lying on their sides. Vestibular nystagmus was induced both by rotating monkeys lying on their sides about a vertical axis, and by horizontal axis rotation (pitch). Regardless of the stimulus, upward nystagmus saturated at lower velocities than downward nystagmus, and neither reached the levels attained during horizontal nystagmus. The difference between upward and downward nystagmus was similar to that reported for vestibular nystagmus in man and other species, and was particularly apparent during OKN. Maximum downward slow phase velocities during upward OKN were less than 30°/sec, while upward slow phases of downward OKN could be 60°/sec or more. Saturation values of OKAN were similar to those of OKN. The addition of a rotating gravity vector during pitch caused nystagmus to be prolonged indefinitely. This is similar to effects during barbeque rotation.

The presence of OKAN indicates that velocity storage occurs for nystagmus in the vertical as well as in the horizontal plane. After-nystagmus following rotation in light was also weaker than after rotation in darkness, indicating that velocity storage during OKN had reduced the post-rotatory response. Reduced storage for upward nystagmus was reflected in a reduced ability to counteract post-rotatory downward nystagmus. Presumably during pitch the velocity storage integrator is continuously activated by the action of the rotating gravity vector on the labyrinth. The data suggest that the organization of the vertical and horizontal systems producing nystagmus is similar, but that there are differences in coupling to the velocity storage mechanism. Different coupling coefficients could also account for the observed differences between upward and downward nystagmus.

Supported by NINCDS Grant NS00294 and
Fellowship NS 05297 (T. R.)

506 PGO BURST NEURONS: EVIDENCE FOR PGO WAVE GENERATION. Robert W. McCarley, John P. Nelson, J. Allan Hobson. Lab. of Neurophysiol. Harvard Medical School, Boston, Mass. 02115.

PGO waves are the prominent monophasic potentials seen in Pons, lateral Geniculate body (LGB) and Occipital cortex just before (transition, T) and during desynchronized sleep (D). As a first step in defining the neuronal network involved in the chain of events leading to PGO wave generation, it is of particular importance to identify the set of neurons forming the last link of this chain, that is to identify a set of output neurons for PGO wave generation.

We now report on a class of neurons, PGO burst neurons, which satisfy the following correlational criteria for output cells for PGO wave generation: high discharge coherence with PGO waves (discharge bursts associated with most waves - range 60-93% for these 23 cells); high discharge specificity (relative absence of discharge at other times - range 79-99% for these cells); and a fixed phase lead and stereotyped discharge pattern (these cells discharge in bursts of 2-6 spikes, the first occurring with minimal variability 12 msec before PGO wave onset, 45 msec before PGO wave peak). These cells discharge single spikes in relationship to some eye movements and to startling stimuli during wakefulness (W).

In a series of explorations from A4 to P8, such neurons were recorded only at midbrain sites in close approximation to the brachium conjunctivum, an area in which there is HRP evidence of projection to LGB. However, we were unable to elicit antidromic activation from LGB. Orthodromically activated bursts could be elicited consistently by stimulating through LGB leads which gave the best PGO wave recordings. The evoked bursts were state-dependent, with a single spike at 37-70 msec in W contrasted to a typical burst at 25-30 msec latency in D. Another state-dependent result was that PGO waves could be evoked consistently in D by stimulation in the opposite LGB. Burst cell discharge had the same phase relation to the evoked PGO waves as to spontaneous PGO waves, and it is thus probable that these cells also act as output generators for evoked PGO waves.

In contrast, recent experiments intended to record cells in the nucleus abducens and praepositus hypoglossi (histology pending) have yielded a number of units whose discharges are highly correlated with eye movements and which show marked modulation in association with PGO waves, but not in the fixed, specific pattern of PGO burst neurons.

We postulate that PGO burst neurons are output cells for PGO wave transmission from brain stem to forebrain.

Supported by NIMH grant MH13923

507 NEURONS IN THE OCULOMOTOR, TROCHLEAR AND ABDUCENS NUCLEI PROJECT CAUDALLY IN THE MLF TO THE PREPOSITUS NUCLEUS. R. A. McCrea and R. Baker. Dept. Physiology & Biophysics, New York Univ. Med. Ctr., 550 First Ave., New York 10016.

Previous studies have shown that neurons in the oculomotor nucleus (Oc.n.) project to the abducens nucleus (Abd.n.), reticularis tegmenti pontis and cerebellum. In addition, neurons from the Abd.n. project to the Oc.n. and the cerebellum. Electrophysiological evidence has demonstrated that these neurons are not motoneurons. We now report that there are neurons in each of the oculomotor nuclei which project via the medial longitudinal fasciculus (MLF) to levels caudal to the Abd.n. and terminate in the prepositus nucleus. Following extracellular HRP injections in either the prepositus or the caudal MLF of the cat, labelled neurons are found bilaterally throughout the Oc.n. and Abd.n. Labelled cells are also observed in the rostral ipsilateral troclear nucleus. Many labelled neurons are also found in close proximity to each of the ocular nuclei as well as around, and within, the MLF. Most of the neurons were either small or medium sized, but a few large cells were always labelled.

The pathway from the ocular nuclei to the prepositus nucleus was demonstrated electrophysiologically by recording intra- and extracellularly from neurons in the Abd.n. while electrically stimulating the prepositus and Oc nuclei. Neurons in the Abd.n. which were antidromically activated following stimulation of the prepositus were not activated by stimulation of the VIth nerve or inferior cerebellar peduncle. Several of these neurons were stained by intracellular injection of HRP. Surprisingly, 36% of the caudally projecting Abd.n. neurons were antidromically activated following stimulation of both the prepositus and Oc.nuclei. Collision studies demonstrated that the axons of these neurons collateralized, one branch ascending and the other descending in either the ipsi- or contralateral MLF. In another set of experiments, neurons in the prepositus nucleus were recorded intracellularly while stimulating the Oc.n. Many of these cells were antidromically activated. In nearly all neurons, monosynaptic EPSPs were recorded following stimulation of the ipsilateral and, in most cases, the contralateral Oc.n. These data suggest the existence of reciprocally organized excitatory loops between the prepositus and each of the ocular nuclei. Given the wide profile of eye movement responses observed in the prepositus nucleus and that expected to be found in the neurons of the individual ocular nuclei, we propose that the aforementioned intrinsic pathways may be utilized to coordinate horizontal with vertical eye movements. (Supported by USPHS grants NS-05857, NS-13742 and EY-02007)

508 EFFECT OF PROLONGED OPTICAL REVERSAL OF VISION ON THE VESTIBULO-OCULAR REFLEX: SOME NEUROPHYSIOLOGICAL OBSERVATIONS. F. A. Miles and D. J. Braitman.* Lab. Neurophysiol., NIMH, Bethesda, MD 20014.

The vestibulo-ocular reflex (VOR) undergoes adaptive gain changes when the visual input associated with head turns is disturbed by reversing prisms. Single unit recordings were undertaken in the vestibular nerve and flocculus of 7 awake rhesus monkeys: 4 were normal and 3 had worn left-right reversing prism spectacles so that their horizontal VOR gain was low (<0.2) or even reversed. Animals were seated in a primate chair which could be oscillated about a vertical axis (providing controlled vestibular stimulation), and their heads secured to it through a platform which could be tilted 25° forward or backward. 437 semicircular canal fibers were analyzed, each being assigned to a given canal on the basis of its response to oscillation and tilting. Cosine functions relating oscillation sensitivity to tilt angle were constructed for individual fibers from each canal to determine apparent canal orientations and allow estimation of fiber sensitivities to sinusoidal oscillations when canal alignment was optimal. Statistical analyses revealed no significant differences between normal and low gain animals in the sensitivity, phase or resting discharge rate of their semicircular canal fibers. We conclude that VOR gain changes are not mediated by vestibular efferents. The discharge characteristics of 532 flocculus Purkinje cells were examined for vestibular and oculomotor relationships: 44% were related to gaze velocity during horizontal tracking, 23% to vertical tracking, 15% to eye position, 6% to miscellaneous combinations of head and eye movement, 4% to saccadic eye movements only and 8% were unresponsive. The discharges of horizontal gaze velocity neurons were examined during sinusoidal tracking both when the head was stationary--to provide a measure of "eye velocity" sensitivity--and when the head was moved exactly in concert with the target--to provide a measure of "head velocity" (vestibular?) sensitivity. Assessed in this way in the normal monkey, head and eye velocity signals were of similar strength (correlat^{n} coef. = 0.87; regression line has a slope not significantly different from 1 and an intercept not significantly different from zero). In the low gain animals, eye velocity signals were significantly greater than head velocity signals (correlat^{n} coef. = 0.72; slope of the regression line = 0.67 with an intercept not significantly different from zero). Such a shift in the weightings of the head and eye velocity signals in low gain animals is not consistent with the view that the flocculus is the site of modifiable elements subserving VOR gain changes. We suggest that such changes in the floccular output are more probably a secondary consequence of it receiving a modified input. However, since the signals carried by the flocculus changed less than the VOR, flocculus Purkinje cell discharges would be responsible for some of the observed reduction in the VOR gain.

509 PURKINJE CELL ACTIVITY IN THE MONKEY FLOCCULUS DURING SMOOTH PURSUIT EYE MOVEMENTS. Hiroharu Noda and David A. Suzuki*. Brain Research Institute, Depts. Physiol. Anat., Sch. Med., UCLA, Los Angeles, CA 90024.

The activity of Purkinje cells in the flocculus was studied in alert monkeys that were tracking a small, colored spot moving in straight lines at various orientations on a tangent screen. Target movement was either as a sine wave or as a composite of sinusoids of different frequencies; the latter was employed in order to eliminate the predictability of a pure sine wave. Activity in Purkinje cells of the flocculus was studied in association with the three paradigms of smooth pursuit in darkness, tracking in the presence of a black and white random dot background, and fixation during movement of the random dot background.

During smooth pursuit eye movements, modulation of simple spike activity was observed in Purkinje cells. In a portion of this population, this modulation was clearly related to eye movement velocity, but in many cells, peak activity did not occur in phase with maximum velocity. A phase difference of about 45° was typically observed between peak activity and the point of peak velocity. Interestingly, a 45° phase shift was also commonly observed in Purkinje cells classified as position coded, indicating that the output of these Purkinje cells in the flocculus may reflect the integration of eye position and velocity information.

In Purkinje cells exhibiting both smooth pursuit related discharge modulation and saccade related suppression of simple spike activity, suppression appeared to be relatively potent. Saccadic pauses were observed even during the high frequency, maximal Purkinje cell discharge associated with pursuit in the preferred direction. It appears that signals related to saccades reach the flocculus independent of eye position and/or velocity information and act to disinhibit cells upon which floccular Purkinje cell axons terminate.

In a small sub-population of the cells exhibiting smooth pursuit modulation, cellular activity was also correlated with background movement during fixation. A 180° phase difference was observed between unit activity associated with tracking and with background movement, indicating that information concerning retinal image motion also reaches the flocculus. To be noted, however, is the observation that in the majority of cells exhibiting smooth pursuit modulation, the modulation occurred regardless of the presence or absence of the background, implicating the primacy of eye movement signals to these cells in the flocculus. To different degrees, therefore, oculomotor and visual information aid the flocculus in its role in the control of eye movements. (Supported by NIH Grant EY01051)

510 VOR GAIN CHANGES PRODUCED BY TARGET ROTATION WITHOUT HEAD MOVEMENT IN GOLDFISH. J.O.Schairer*& M.V.L.Bennett (SPON: R.Babb). Dept. Neurosci., Albert Einstein Coll. of Med., Bronx, N.Y.10461.

Goldfish can be trained to change the gain of their vestibulo-ocular reflex (VOR) over the course of a few hours (Neurosci. Abs. 3:157, 1977). Training the gain to increase towards two consists of sinusoidally rotating a restrained goldfish in the center of a cylindrical, vertically striped drum which is rotated equal and opposite to the fish. In order to assess the effect of visual stimulation alone on VOR gain, the striped drum was rotated sinusoidally with an amplitude of 20 deg and a period of 8 sec around stationary fish. The VOR gain was measured by rotating the fish at the same period and amplitude in the dark. Fish were not rotated in the light during the experiment. Nine fish underwent this "stripes only" treatment for times varying from two hours to six hours. In every fish the gain increased. The mean initial gain (±S.D.) was 0.67 ±0.13 and the mean final gain was 0.99 ±0.14. For comparison six fish that were trained for two hours to increase their gain towards two, increased their gains from 0.77 ±0.05 to 1.40 ±0.07. Four fish that were run for two hours in the stripes only condition increased their gain from 0.72 ±0.08 to 0.95 ±0.09. The peak amplitude of the slip (the difference between the drum velocity and slow phase eye velocity with respect to a non-rotating frame of reference) in both conditions dropped: from 10.1 ±0.9 deg/sec to 6.3 ±2.2 deg/sec for the fish which underwent the stripes only condition and from 12.3 ±2.5 deg/sec to 3.8 ±2.2 deg/sec for the fish trained towards two. Thus the greater effectiveness of training towards two does not appear to be simply due to greater slip.

An optokinetic stimulus drives goldfish vestibular neurons in such a way that a stationary visual scene improves their response to long constant velocity rotations (Dichgans et al., Br. Res. 18:319, 1973). Thus when the striped drum is rotated around a fish, it sees slip in one direction while its vestibular neurons are responding as if it were rotating in the other direction. This pairing of slip and activity of vestibular neurons is what occurs when the animal is being trained towards two with rotation. The similarity may explain why the VOR increases with the visual stimulus alone.

This work was supported by NIH grant 5T32 GM 7288.

511 THALAMIC UNITS DETECTING ABSOLUTE SPACE POSITION OF VISUAL TARGETS. John D. Schlag and Madeleine Schlag-Rey. Dept. Anat. and BRI, Sch. Med., UCLA, Los Angeles, CA 90024.

Saccade neurons of the cat's thalamic internal medullary lamina (IML) fire before and during eye movements in specific directions. The same IML neurons can also respond phasically (On and Off) and/or tonically to the presentation of visual stimuli. Visual stimuli are effective only when they are so placed that, should a targeting saccade occur, it would be oriented in the unit's preferred direction for producing saccadic bursts. These results, already reported, imply that visual responses correspond to the detection of targets for orienting eye movements. If there is a spatial coding in these responses, is it exclusively based on retinal coordinates?

Microelectrode recordings were made in the IML of alert cats with the head fixed and facing a tangent screen on which dim light patterns were projected. Stimulus and electrooculographic (EOG) coordinates were continuously monitored. Tested with 1-deg. targets on which fixation was conditioned, the EOG measurements were consistent within a ± 1-deg. range. Maps of receptive fields were determined for individual cells. While the animals fixated in various directions, stimuli were systematically presented at sites on the screen corresponding to equivalent positions within the receptive field. For most IML neurons tested in this manner, On-responses were absent or much weaker when stimuli were offered in the hemispace opposite to the side of receptive field location. The effect of absolute spatial location was even clearer for tonically activated units. Those with a small central receptive field fired continuously only when the animal fixated a stationary target or tracked a moving target within a circumscribed area of the screen. Thus, the adequate stimulus location was defined in terms of head-body coordinates during fixation. Units with a large receptive field started to discharge profusely when stimuli reached, moved in, or appeared in an hemispace, whether the target was fixated or not. The competence of this type of cell was limited to a half visual space around the animal, in which the cells hardly ever failed to notice visual events. In most cases, the frequency of firing increased gradually as the stimulus moved far away from the center of the screen. Thus some IML neurons receive information on the orientation of the eyeball but this information appears at the cell output only in presence of a visual target. This interpretation is supported by the finding, in the IML, of pure fixation units and eye position units which could provide the adequate inputs to explain the properties described. The data provide a basis for models assuming spatial visual coding referred to non-retinal systems of coordinates (Supported by USPHS grant NS-04955).

512 FIRING CHARACTERISTICS OF UNITS IN CAT SUPERIOR COLLICULUS DURING TRACKING. Madeleine Schlag-Rey and Carol K. Peck. Dept. Anat. and BRI, Sch. Med., UCLA, Los Angeles, CA 90024.

In alert cats, cells of the superficial and intermediate layers of the superior colliculus (SC) respond profusely to moving visual targets over a wide range of velocities (see Peck and Schlag-Rey's abstract, this meeting). Some cells are activated only when something moves in the visual environment. We have tried to determine the parameters responsible for this activity during eye tracking, the head being fixed.

The moving target was a dim, blue 1.5°-circle projected on a tangent screen. Stimulus velocities ranged from 3 to 400°/sec. Tests were run with single ramps, sinusoidal and sawtooth movements with various amplitudes and directions. The sawtooth motion was effective in inducing the cats to bring their gaze exactly on the linear path of the moving target. Stimulus and electrooculographic (EOG) coordinates were continuously recorded. Calibration adjustments afforded EOG measurements consistent within ± 1 deg. The cats were not reinforced for tracking but prior conditioning had given them an incentive to explore their visual environment.

All cells analyzed had a preferred direction. 1. When the cats were fixating a point on the path of the target and the target was moving in the preferred direction, the firing rate gradually increased as the target approached the point of fixation of the gaze. At some distance past this point, while the target moved away, the firing rate started to decrease. This was shown by synchronizing rasters of unit activity on the time when the target met the eyes. This pattern of response was condensed over a shorter time interval as stimulus velocities were higher, suggesting a rather constant relationship with the target position in the receptive field. 2. During pursuit episodes in the preferred direction, when eye velocity matched target velocity, the rate of discharge remained constant; some cells fired intensively whereas others were silenced. 3. When the cats approximated a smooth pursuit by a succession of small catching-up eye movements, velocity errors of opposite signs were associated with increased activity (eyes lagging) or decreased activity (eyes catching up). These changes followed the eye movements. They could be due to changes in the virtual movement of the target or to a proprioceptive input interacting with the visual input. However, predictions of unit activity based solely on the effect of virtual movement are contradicted by results obtained when the target moved in the null direction. These observations support the hypothesis that visual activity of SC neurons is better understood in terms of the information needed for tracking than perception of movement (Supported by USPHS grant 04955).

513 INNERVATION OF EXTRINSIC OCULAR MUSCLES. Marjorie D. Shaw* and Keith Alley. Case Western Reserve University, Cleveland, Ohio 44106.

The oculomotor system provides an excellent model for the study of developing motor systems in mammals. As a basis for such an analysis, we are charting the basic innervation patterns of the extrinsic ocular muscles. Neonatal and adult rabbits are being used to follow the development of motoneuron pools, and adult cats and rabbits to localize the primary sensory neurons.

Horseradish peroxidase injected into specific eye muscles was retrogradely transported to cells in the brainstem and trigeminal ganglion. Even at birth, the motoneurons of the rabbit oculomotor nucleus are segregated into distinct subpopulations, similar to those described in cat and monkey. Injections of tritiated amino acids into motor nuclei have been shown to label motor terminals intensely, and are being used to confirm such projections.

The peroxidase-filled cells of the trigeminal ganglion are presumed to represent the cell bodies of primary sensory neurons to the eye muscles. No peroxidase labelled cells could be detected in the mesencephalic nucleus after eye muscle injections, in contrast to previous reports in the literature, even though labelled cells were numerous after injection into the masseter. To document our presumption that the ganglion cells supply sensory receptors to the eye muscles, we are injecting tritiated amino-acids into these neurons, to label their endings via fast axonal transport. (Supported by NIH grants NS 12781, NS 00147 and NSF fellowship.)

514 IDENTIFICATION AND LOCALIZATION OF MOTONEURONES INNERVATING THE CAT RETRACTOR BULBI MUSCLE. Robert F. Spencer. Dept. of Anat., Med. Coll. Virginia, Richmond, VA 23298.

The retractor bulbi (RB) muscle is an extraocular muscle which is divided into four slips that are closely associated with the lateral rectus (LR) and medial rectus (MR) muscles. In the present study, motoneurones innervating the four slips of the cat RB muscle have been identified by retrograde intraaxonal transport of horseradish peroxidase (HRP).

Following injections of HRP into all four slips of the RB muscle, motoneurones were found distributed throughout the rostral-caudal extent of the ipsilateral abducens nucleus, overlapping the distribution of LR motoneurones and abducens internuclear neurones. The RB motoneurones accounted for approximately 30% of the total number of neurones in the abducens nucleus. HRP-labelled motoneurones were also found in the ipsilateral accessory abducens nucleus, situated considerably ventral and lateral to the abducens nucleus, and in the ipsilateral oculomotor nucleus, overlapping the distribution of MR motoneurones. Injections of HRP into individual slips of the RB muscle also resulted in labelled motoneurones in the abducens, accessory abducens, and oculomotor nuclei, though in fewer numbers than when all four slips were injected. There was no apparent topographical organization of neurones innervating individual slips within any of these nuclei. Light microscopic examination of semithin sections revealed the RB motoneurones to be circular to pyriform in shape, ranging from 32 - 52 μm in mean diameter. Examination of ultrathin sections by electron microscopy showed these motoneurones to be cytologically similar to LR and MR motoneurones.

The results indicate that RB motoneurones comprise a more homogeneous population of neurones than LR or MR motoneurones. This is consistent with the reported physiological homogeneity of RB motor units, in contrast to the heterogeneity of LR motor units. The results also indicate that a single muscle may be innervated by neurones located in more than one motor nucleus. This finding suggests that functional differences may exist between motoneurones in different motor nuclei that innervate the same muscle.

Supported by A.D. Williams Foundation Grant 3558(561) and U.S.P.H.S. Research Grant EY 02191.

515 OCULAR MOTOR ABNORMALITIES IN TRAINED MONKEYS WITH FLOCCULAR LESIONS. David S. Zee, Atsumi Yamazaki* and Gundez Gucer. Dept. Neurol., Johns Hopkins University, Baltimore, Md. 21205.

Eye movements were recorded (search coil technique) in three trained juvenile macaques with bilateral lesions of the cerebellar flocculi and paraflocculi. One monkey with histologically verified total flocculectomy showed: 1) decreased smooth pursuit (gain (eye vel/targ vel) = 0.65), 2) decreased ability to cancel the vestibulo-ocular reflex (VOR) using fixation of a small target moving with the head (gain (eye vel/head vel) = 0.4), 3) inability to hold eccentric gaze (gaze-paretic nystagmus) with a time constant of centripetal drift of 2 secs, 4) increased VOR gain in darkness (35%), 5) impaired suppression of the VOR when rotating with an optokinetic (OK) drum fixed to the chair (gain = 0.95; preop = 0.35), 6) abnormal OK nystagmus with a slow rise to maximum eye velocity (time constant $>$ 15 secs; preop $<$ 7 secs) and decreased steady state gain (eye vel/drum vel = .85), however, the time constant of OK after nystagmus in darkness was unchanged, 7) downward beating vertical nystagmus, 8) rebound nystagmus in primary position after prolonged eccentric gaze and 9) post-saccadic drift (glissades). These abnormalities partially recovered in several months.

A second monkey with a presumed complete ablation showed similar but less marked abnormalities. A third monkey had an asymmetrical partial lesion and showed a transient ipsilateral deficit in smooth pursuit, OK nystagmus, cancellation of the vestibulo-ocular reflex and holding eccentric gaze.

These results directly implicate the flocculi (and possibly paraflocculi) in a variety of retinal image stabilizing reflexes. Smooth pursuit and cancellation of the VOR using fixation were comparably affected by flocculus ablation although other cerebellar structures must also be important since the defects were partial. OK nystagmus was also impaired, the dynamic more than the steady state response. This finding may reflect a loss of the normal smooth pursuit contribution to the intact monkey's nystagmus response to OK stimulation. During rotation with a chair fixed OK drum, suppression of the VOR was markedly impaired. This finding correlates with reports of impaired suppression of caloric-induced nystagmus in flocculectomized monkeys. Gaze holding was also impaired and suggests the flocculus acts to increase the time constant of the brain stem neural integrator that holds positions of gaze. Downbeat and rebound nystagmus as well as abnormal pursuit and gaze paretic nystagmus are features of the human ocular motor cerebellar syndrome which suggests that floccular damage may contribute to its production.

FEEDING AND DRINKING

516 THE EFFECTS OF KNIFE CUTS ON EATING ELICITED BY NORADRENERGIC STIMULATION OF THE HYPOTHALAMUS. Paul F. Aravich*, Anthony Sclafani, and Sarah F. Leibowitz. Dept. Psychol., Brooklyn Coll. of CUNY, Brooklyn, N.Y. 11210 and Rockefeller Univ., New York, N.Y. 10021.

The present experiment examines the interrelationship between three hypothalamic manipulations known to increase feeding: first, norepinephrine (NE) injections into the paraventricular nucleus (PVN) which elicit feeding in satiated rats; second, PVN injections of the presynaptically-acting tricyclic antidepressant protriptyline which elicit feeding through activation of endogenous NE stores; and third, bilateral parasagittal knife cuts between the medial and lateral hypothalamus which cause hyperphagia and obesity.

Male rats received either large parasagittal knife cuts rostro-lateral to the PVN or sham knife cut surgery. Following recovery a second surgery was performed on all animals during which a unilateral drug cannula aimed at the PVN was implanted. Possible disruption of efferent fibers mediating the noradrenergic feeding response was assessed by examining the effectiveness of postsynaptic receptor stimulation through l-norepinephrine bitartrate injections (20, 40, and 80 nmoles in .5 microliters of saline). Disruption to afferent noradrenergic fibers mediating this feeding response was appraised by examining the effectiveness of the presynaptically-acting drug protriptyline hydrochloride (100 nmoles in .5 microliters of water).

The results of the experiment indicate that, while the knife cuts increased daily food intake, they did not block NE or protriptyline induced eating. Histological examination revealed that the drug cannulae for all animals were in the region of the PVN. The knife cuts, on the other hand, extended rostro-caudally from the anterior preoptic region through most of the mamillary region. They were 4 to 5mm in height and extended dorsally above the cannula tips and ventrally to the base of the brain. In the medio-lateral plane the cuts were located just medial or just lateral to the fornix.

These findings appear to demonstrate that the NE-elicited feeding response can be disassociated from the hyperphagia syndrome produced by parasagittal hypothalamic knife cuts. Furthermore, it would appear that neither the afferents or efferents responsible for NE-elicited eating have been transected by these particular cuts. To further explicate the fiber systems responsible for noradrenergic feeding, an additional study designed to examine the effects of coronal knife cuts anterior and posterior to the paraventricular region is being conducted.

517 EFFECTS OF CERVICAL VAGUS NERVE STIMULATION ON LPA-LH-MFB SINGLE UNIT ACTIVITY. F.C. Barone, M.J. Wayner, U.H. Aguilar-Baturoni and R. Guevara-Aguilar. Brain Research Laboratory, Syracuse University, Syracuse, New York, USA and Departamento de Fisiologia, Facultad de Medicina, U.N.A.M., Mexico.

The whole left cervical vagus nerve was stimulated in female rats. Data collected from those animals which displayed a constant bradycardia effect at a stable threshold were used for analysis. Recordings were made from neurons in both the ipsilateral and contralateral hemispheres during vagus nerve stimulation. The polarity of rectangular pulses used to stimulate the vagus nerve during brain cell recording was the opposite to that which elicited the largest bradycardia response at the lowest threshold. Following a 3 minute baseline period of data collection on a unit under study, a train of pulses 0.5 m seconds in duration, was applied to the vagus nerve for a period of 4 seconds. Each subsequent 4 second pulse train was separated by at least 3 minutes. Both frequency, 10 to 100 Hz, and voltage, 1 to 20 volts, of the vagus nerve stimulation were varied in a random manner for units under study. The graphical output in spikes per second were used to evaluate the effects of vagal nerve stimulation on central neural activity. Frequency response relationships were established for most of the lateral preoptic-lateral hypothalamic-medial forebrain bundle (LPA-LH-MFB) neurons which were affected by vagus nerve stimulation. Four types of neuronal responses were observed due to vagus nerve stimulation. Cells studied would exhibit increases or decreases in discharge rate as stimulation frequency was increased, or increases or decreases in discharge rate as stimulation frequency was decreased. In all cases, when increasing or decreasing the stimulation frequency increased or decreased the unit discharge rate, increasing the stimulation voltage facilitated further the same effect. Eighty-two percent of the cells studied in the LPA-LH-MFB area exhibited significantly altered discharge rates due to vagus nerve stimulation. Of these 33 percent increased discharge rate when vagus stimulation frequency was increased (22 percent) or decreased (11 percent). Sixty seven percent of the cells affected decreased discharge rate when vagus stimulation frequency was increased (44 percent) or decreased (22 percent). None of the cells sampled from some other areas of the brain responded significantly to vagus nerve stimulation and indicated a somewhat specific effect due to vagus nerve stimulation in this area.

518 THE EFFECT OF HYPOPHYSECTOMY (HYPOX) AND ADRENALECTOMY (ADX) ON DIURNAL FEEDING AND DRINKING RHYTHMS IN THE RAT. Larry L. Bellinger, Fred E. Williams* and Lee L. Bernardis. Dept. Physiol., Baylor Coll. of Dentistry, Dallas, TX 75246 and Dept. Surg. and Path., S.U.N.Y., Buffalo, N.Y. 14215.

Hypox alone has been shown (Bellinger and Mendel, Can. Fed. Biol. Soc., in press) to alter the normal diurnal feeding rhythm of rats. Corticosterone rhythms also appear to be closely aligned to feeding patterns in the rat (Bellinger et al, Neuroendocrin. 22:216. 1976). This study was undertaken to first, confirm our earlier findings that Hypox alone would alter the diurnal feeding rhythms of rats and secondly, to observe if the modified patterns could be attributed to a loss of corticosterone rhythm. Hypox (n=14), Adx (n=16) and non operated control (CON) (n=14) male Sprague-Dawley rats of the same age were purchased from Simonsen Lab., Gilroy, Ca. The animals were housed individually under a L:D of 12:12. The animals were allowed 13 days to adjust to their new environment. At the end of this period the amount of food and water consumed during the light phase and dark phase was recorded on each rat for six days. No one was allowed in the animal room except to measure food and water consumption. There was a significant difference in the % of food the groups consumed during the dark and light phases over the six day period [H(2)= 21.2; P<0.001]. The Hypox rats ate a significantly lower % (z= 5.2; P<0.001) of their food at night compared to the CON (77.2 $\pm$ 1.5% vs 87.0 $\pm$ 0.6%). The % of food the Adx rats consumed during the dark (86.9 $\pm$ 1.1%) did not differ from the CON but did differ (z=4.9; P<0.001) from the Hypox rats. The Hypox rats showed a large day to day variation in the % of food consumed during the light and dark phases (χ^2=172.9; P<0.001) while the CON showed little variation (χ^2=30.4; P>.99). There was also a significant difference in the % of water the groups consumed during the dark and light phases [H(2)=52.8; P<0.001]. The Hypox group drank a lower % (z=5.1; P<0.001) of their water at night compared to the CON (74.2 $\pm$ 2.3% vs 86.3 $\pm$ 1.5%). The Adx rats consumed a larger % (91.3 $\pm$ 1.4) of their water at night than either the CON (z=4.3; P<0.001) or the Hypox rats (z=6.6; P<0.001). These data show that Hypox can somewhat alter the normal diurnal food and water intake rhythms in rats. However, it should be noted that the Hypox rats still consume a large % of their food during the dark phase and thus appear to be still strongly influence by the photoperiod. The data further reveal that Adx does not cause an alteration of the normal food intake rhythm but does appear to slightly enhance the % of water the animals will consume during the dark phase.

519 EFFECT OF EPI- AND SUBTHALAMIC LESIONS ON SOMATIC AND ENDOCRINE PARAMETERS IN WEANLING AND MATURE RATS. Lee L. Bernardis and Larry L. Bellinger, Depts. Surg. and Path., SUNY at Buffalo, Buffalo, N.Y. 14215, and Dept. Physiol, Baylor Coll. Dent., Dallas, Texas 75246.

Weanling rats received bilateral electrolytic lesions in the zona incerta (ZIN); sham-operated animals served as controls. The animals were maintained for 30 days postoperatively and trunk blood was collected at sacrifice. Two experiments were performed several months apart. The data show that ponderal and linear growth, food and water intake and water/food intake ratio and pituitary weights were significantly reduced in the ZIN-lesioned rats. Body composition and circulating glucose, growth hormone and insulin were normal, however. The findings are surprising since previous reports indicate that ZIN lesions in mature rats do not cause alterations in food intake and body weight. This is possibly so since in the present studies the rats were in a state of rapid growth and thus more sensitive to the disruptive effects of the lesions. The above changes-growth retardation in the face of normal body composition and growth hormone levels-are similar to those reported for the weanling rat dorsomedial syndrome and point to an extrapituitary origin of the alterations. The changes observed may be related to the mesencephalic reticular formation that could bring to bear its influence on the subthalamus, into which it extends as the ZIN.

Weanling and mature (220 gm) rats received bilateral electrolytic lesions in the medial habenula (HAB); Sham-operated rats served as controls. The animals were maintained for 43 postoperative days and trunk blood collected at sacrifice. Both weanling and mature rats showed no significant changes in any of the parameters except for a slight reduction of linear growth in the weanling rats and of water intake in the mature rats. The lack of profound changes in the HAB-lesioned rats is surprising since this area has been described as exerting an influence in thyroid control and of playing a role in thermoregulation as well as serving as an important link between olfactory sensor information with motor areas of the brain. HAB lesions of long standing have also been reported to bring about obesity. The HAB are linked to the brain via the fasciculus retroflexus of Meynert and could thus come under the influence of the forebrain reticular formation. This has been described as a pathway through which primitive aspects of brain function are influenced by the viscera.

NIH-HD 03331 and NSF-PCM 76-8[illegible]81.

520 SHORT AND LONG TERM EFFECTS OF KAINIC ACID INJECTIONS INTO THE LATERAL HYPOTHALAMUS. Michael Britt* and Darryl B. Neill* (SPON: David Freides). Department of Psychology, Emory University, Atlanta, GA 30322.

Electrolytic lesions of the lateral hypothalamus (LH) have indicated the importance of LH structures in the control of food and water intake. However, damage to the monoaminergic fibers passing through the LH duplicates many of the effects of LH electrolytic lesions. This experiment assessed the effects of LH injections of kainic acid, which appears to damage neuronal somata while leaving fibers of passage intact.

Rats receiving kainic acid injections (0.4 µl/0.2 µl; pH4) were compared with those receiving:electrolytic lesions (2 mA, 15-20 s), saline (0.2 µl), lactic acid (0.2 µl; pH4), or sham surgery. The latter three groups showed no significant internal differences and will be collectively referred to as controls.

Postoperatively, both kainic-injected (KI) and lesioned (EL) rats were aphagic (food intake $\leq$ 2 g/day) for equivalent periods. Hypophagia, however, was much shorter for KI than EL rats. The duration of adipsia ($\leq$ 4 ml/day) was shorter for KI than EL rats. Controls showed no aphagia or adipsia. Whereas EL rats were hunched and inattentive, KI rats retained normal posture and were hyperactive to many stimuli. Locomotor activity on the 2nd postoperative day was severely depressed in EL rats but only moderately depressed in KI rats. By the 4th postoperative day, KI and control rats were equally mobile and both more active than EL rats. Neither KI nor EL rats increased food intake above baseline levels following intraperitoneal (IP) administration of 2-deoxy-D-glucose (750 mg/kg), nor did either group show normal increases in water intake in response to IP injections of hypertonic saline (2 M).

Spectrophotofluorometric measurement of neocortical and striatal norepinephrine, dopamine, and serotonin content showed no differences among control and KI groups, indicating no damage to monoaminergic fibers of passage. Electrolytic lesioning depleted monoamines. Examination of nissl-stained sections revealed kainic-induced cell loss apparently limited to the LH. (Supported by NIH 5-R01-DA01701-02 and NIDA 1-F31-DA05015-01)

521 ADULTLIKE DRINKING BEHAVIOR IN SIX AND TEN DAY OLD RATS. John P. Bruno* (Spon. Elliott M. Blass) Dept. Psychology, Johns Hopkins Univ., Baltimore, MD 21218.

The demonstration of alternate patterns of ingestion in suckling rats would be of interest. It would permit direct analyses of the ontogeny of adultlike feeding and drinking behaviors. Accordingly, the capacity of infant rats to ingest in a manner other than suckling was investigated.

Pups were individually placed on a paper towel that was saturated with either milk, water, or hypertonic saline (1M). Rats were tested at 35°C. This high temperature is necessary for the elicitation of the consummatory response. Six and ten day old pups were randomly assigned to one of the following conditions: 1) nondeprived (pups remained with the mother until tested); 2) nondeprived + NaCl (1M, 2% BW, s.c.); 3) 4 hr. deprived (pups were removed from the mother for four hours prior to testing); and 4) 4 hr. deprived + NaCl (1M, 2% BW, s.c.). Subjects from each treatment condition were allowed to consume either milk, water, or hypertonic saline during a 20 minute test.

There were four major findings. First, nondeprived and deprived pups consumed little of any fluid: milk intake: 1-2% BW, water and saline intake: .5-1% BW. Second, acute cellular dehydration increased intake of all fluids. Third, acute cellular dehydration differentially affected water intake, increasing it to 6-7% BW. In fact, dehydrated rats drank more water than milk. This is a reversal of the preference exhibited by both nondeprived and deprived pups. The response to dehydration was selective, since dehydrated subjects still consumed only very small amounts (1-2% BW) of the saline solution. This selectivity is important as it is a major defining characteristic of adult drinking behavior. Finally, there were no age-related differences in the responses of six and ten day old pups.

These findings demonstrate that adultlike drinking behavior can be elicited by cellular dehydration in rats as young as six days of age. This demonstrated ability now permits a detailed investigation of the earliest forms of ingestive behavior leading to an appreciation of how they become expressed under natural conditions.

522 BLOCK OF CHOLINERGIC INDUCED THIRST AFTER OBSTRUCTION OF ANTERIOR VENTRAL THIRD VENTRICLE OR PERIVENTRICULAR PREOPTIC ABLATION. James Buggy, Dept. Physiol., Sch. Med., Univ. S. Carolina, Columbia, SC 29208.

A cholinergic coded circuit in the limbic system mediating thirst behavior has been postulated in the rat since cholinergic brain stimulation at a variety of limbic sites induces water intake. Drinking normally observed after cholinergic stimulation is blocked by atropine pretreatment at any of several circuit sites yet ablation of these same sites is without effect. This result and the fact that many positive sites are located near the cerebral ventricles suggests that the injected chemicals may have acted at one or more distant sites after spread through cerebral ventricles. To assess this hypothesis, drinking after lateral preoptic or lateral ventricle injection of carbachol was measured before and after placement of cold cream plugs obstructing cerebral ventricles. Plugs obstructing the anterior ventral portion of the third ventricle at the preoptic level nearly abolished drinking while plugs that did not prevent cerebrospinal fluid circulation through this region did not alter the drinking response.

Since the ventricular obstruction experiment suggested that cholinergic induced thirst involves activation of neurons near the anterior ventral third ventricle, the effect of preoptic-anterior hypothalamic periventricular ablation was next determined. Drinking responses to lateral preoptic injections of carbachol were monitored before and after the periventricular ablation or a sham ablation. Post lesion drinking tests were delayed until ad lib water intake recovered to normal after transient adipsia. Drinking after carbachol injection was virtually abolished after periventricular preoptic ablation but unchanged after the sham lesion procedure. The failure of drinking after cholinergic stimulation when cerebrospinal fluid circulation through anterior-ventral third ventricle is impaired or when surrounding tissue is ablated suggests that cholinergic stimulation arouses drinking through an action on the preoptic periventricular region.

It is interesting to note that plug and lesion experiments implicate the same region in angiotensin induced thirst. However, studies with pharmacological blockers show that the cholinergic blocker atropine does not affect angiotensin thirst while angiotensin antagonists or the dopamine blocker haloperidol blocks angiotensin but not cholinergic thirst. Moreover, angiotensin results in water plus NaCl solution intake while carbachol increases water intake only. Thus, while angiotensin and carbachol may both act in the same region, they apparently involve separate systems. Supported by Iowa Medical Research Council and Univ. S. Carolina funds.

523 CHANGES IN SERUM CORTICOSTERONE, FREE FATTY ACIDS, AND GLUCOSE ASSOCIATED WITH EATING, CONDITIONED CUES, AND VENTROMEDIAL HYPOTHALAMIC LESIONS. Gary D. Coover, Stephen L. Welle* and Robert P. Hart*. Dept. Psych. Northern Illinois Univ., DeKalb, IL 60115.

Male hooded rats were fed once daily for 14 days with 1-hr access to food mash (40% ground Wayne Lab Blox) and water. Feeding times each morning varied between 9 a.m. and 1 p.m. Feeding was always associated with the stimuli (CS) of the experimenter entering the animal quarters for the first time that day (Exp. 1) or placement of the rat's cage into a sound-attenuating chamber (Exp. 2). The CS-feeding interval varied each day, between 0 and 10 min. On the 15th day the rats were decapitated either before (Time 0) or after 10, 20, or 40 min of feeding or CS presentation. Blood serum was analyzed fluorometrically for corticosterone and colorimetrically for free fatty acid (FFA) and glucose concentration.

Both experiments confirmed (Coover, Sutton, & Heybach, J.c.p.p., 1977, 91, 716-726) that normal rats exhibit a decline in corticosterone level when they are fed, from a baseline of over 20 µg/100 ml (at Time 0) to less than 10 µg/100 ml after 20 min, and that corticosterone level exhibits a conditioned decline of equivalent rate for the first 10 min. However, the rats did not exhibit conditioned changes in serum FFA or glucose. During feeding, glucose levels increased from a baseline of about 100 mg/100 ml to levels well above those found in ad lib fed rats by 10 min, and then declined toward ad lib levels by 20 min. FFA levels fell by about 50% during feeding in the first 10 min.

In Exp. 2, 39 of the 77 rats received bilateral electrolytic lesions of the ventromedial hypothalamus (VMH) 9 days before the initiation of the feeding-conditioning regimen. The VMH rats exhibited body wt. gain and eating pattern and preference signs of the VMH syndrome during the first 8 postsurgical days, but they did not, by the end of the conditioning phase, weigh more, or eat more vigorously, than the controls. Their baseline (Time 0) serum concentrations of corticosterone, FFA, and glucose did not differ from control values, and their FFA responses to feeding and CS presentation were normal. However, their corticosterone response to feeding was very small, levels remaining elevated at over twice those of the controls at 20 min, and they did not exhibit a conditioned decline. Also, serum glucose declined more between 10 and 20 min after the initiation of feeding. Most important of all, the VMH rats did exhibit a decline in glucose level after 20 min of CS presentation. The results confirm recent findings of metabolic imbalance in VMH rats and suggestions (e.g. Powley, Psych. Rev., 1977, 84, 89-126) that VMH rats have abnormal conditioned responses to food cues.

524 EQUIVALENCE OF INTRAVENTRICULAR p-CHLOROPHENYLALANINE AND PHENYLALANINE IN PRODUCING HYPERPHAGIA AND OBESITY IN RATS. Donald V. Coscina, Juri V. Daniel*, Peter Li* and Jerry J. Warsh. Sects. of Biopsychol. and Neurochem., Clarke Inst. Psychiat., and Depts. of Psychol., Psychiat., and Pharmacol., Univ. Toronto, Toronto, CAN.

Recent work from Hoebel's lab (Science 192: 382, 1976) shows that intraventricular (i.v.) infusions of p-chlorophenylalanine (pCPA) can induce transient hyperphagia and bodyweight (BW) gain in rats. Since the time course of this effect was shown to parallel that of maximal depletions in brain serotonin (5-hydroxytryptamine or 5HT), it was suggested that central 5HT neurons might normally serve to inhibit feeding. Problematic to this interpretation are many previous reports in which depletions of rat brain 5HT by midbrain raphe lesions fail to elicit overeating or BW gain. Since control infusions of a pCPA congener were not studied despite the large quantities (2-4 mg) of drug required to generate hyperphagia, a non-specificity of this pCPA action remains to be discounted. Accordingly, we compared the efficacy of i.v. pCPA to that of its parent amino acid, phenylalanine (PA), in modifying ad lib food intake. For all experiments, single-housed adult (225-283 g) female Wistar rats were used. Under Nembutal anesthesia, separate groups of 4 rats received stereotaxically-placed i.v. infusions of 2, 3, or 4 mg d,l-pCPA methyl ester HCl or 2, 3, or 4 mg d,l-PA methyl ester HCl in 20 µl saline (10 µl/lateral ventricle infused 1 µl/min; infusion needle left in situ 5 min after each infusion). Compared to additional groups of normal or saline-infused controls, daily intake of Teklad 4%-fat pellets and attendant BW gain reliably increased in both magnitude and duration as a function of dosage. However, this effect was equivalent for pCPA and PA (see table). Assays of endogenous forebrain 5HT, norepinephrine (NE) and dopamine (DA) in these and additional rats sacrificed at the peak of their hyperphagia showed no reliable depletion of any amine irrespective of drug injected or time at which animals were sacrificed. Therefore, we find no evidence that the transient "obesifying" effect of central pCPA infusion relates specifically to brain 5HT depletion.

Group	Dose	$\bar{X}$ daily eating above controls	$\bar{X}$ daily BW gain above controls	Days post-injection
pCPA	4 mg	16.2 g	.9 g	7-36
	3 mg	11.0 g	.6 g	2-31
	2 mg	9.0 g	.2 g	1-12
PA	4 mg	16.2 g	1.2 g	1-40
	3 mg	10.0 g	.9 g	1-28
	2 mg	10.0 g	.4 g	1-10

525 ANOREXIA AND BODY WEIGHT LOSS CAUSED BY INTRAVENTICULAR GLYCEROL INFUSIONS. John D. Davis and David Wirtshafter, Dept. Psychol., Univ. of Illinois, Chicago, Ill. 60680.

Food and water intake and body weight were measured in rats before, during and after 7 days of continuous infusion of glycerol into the lateral or third ventricle of the brain. Unincumbered infusion was achieved by means of a subcutaneously implanted Alzet osmotic pump which was connected to a ventricular cannula by a polyethylene tube. The pump delivered fluid to the ventricular space at a rate of 1 µl/hr. During the first day of glycerol infusion at a rate of 54 µg/hr into the lateral ventricle food and water intake and body weight were significantly reduced. After the initial anorexia food and water intake recovered toward normal levels, but body weight gain was inhibited during the period of glycerol infusion. At the termination of the infusion period food and water intake continued to increase and the animals recovered their normal rate of body weight gain of about 2.5 gm/day. Infusion of glycerol into the third ventricle at a rate of 27 µg/hr completely abolished food and water intake for a period of 3 days after which food and water intake began to recover. This infusion rate led to a loss of 130 gm in body weight during the 7 days of glycerol infusion when compared to the body weights of rats receiving intraventricular normal saline at a rate of 1 µl/hr. Infusion of glycerol at rates of 13 and 7 µg/hr inhibited food intake in a dose related fashion, and led to body weight losses of 100 gm and 70 gm respectively during the 7 day infusion period. When glycerol was infused at a rate of 7 µg/hr into the third ventricle or at a rate of 54 µg/hr into the lateral ventricle food and water intake during the light phase of the light-dark cycle were unaffected. Inhibition of ingestion occured only during the dark phase of the cycle. This differential effect of glycerol on the diurnal feeding pattern of the rat, and the fact that the food to water intake ratio was normal at these infusion rates indicates that glycerol induced anorexia was not due to a toxic effect of glycerol. Rather, these results together with those previously reported by Wirtshafter and Davis (Science 198:1271, 1977), suggest that glycerol plays a regulatory role in the feedback system responsible for the control of food intake and body weight.

Supported in part by NSF Grant BMS 75-17091.

526 DEPLETION OF CENTRAL CATECHOLAMINES BY CHEMICAL LESIONS THAT CAUSE OBESITY. Ralph Dawson, Jr.,*Michael Callahan,*and Joan F. Lorden (SPON: Lawrence Mays). Dept. Psychol. and Neurosci. Program, Univ. Alabama in Birmingham, Birmingham, AL 35294

In the mouse a single injection of goldthioglucose (GTG) can produce both severe obesity and disturbances in anterior pituitary function. The obesity syndrome which follows GTG injections is associated with hypothalamic damage; however, the precise anatomical substrate for the syndrome has not been established. Several investigators have proposed that the hypothalamic monoamine systems may be involved. Damage to monoamine-containing neurons could account for the altered secretion of prolactin and growth hormone in GTG-injected mice. In addition the catecholamines (CA) have been implicated in the elicitation and suppression of feeding behavior. A loss of CA fluorescence in the median eminence of GTG-treated mice has been reported; however, there have been no quantitative studies of regional levels of CA's following GTG injection.

In order to study the relationship of central CA's to GTG-induced obesity, we injected 50 female and 67 male C57Bl/6J mice 60-90 days of age with GTG (.8 mg/g body weight). An additional 23 females and 34 males received injections of physiological saline. The mortality rate for females receiving GTG was 37% and for males, 40%. After a 6 wk observation period during which the mice were weighed every 4 days, all mice were sacrificed by decapitation. The brains were dissected into hypothalamic and telencephalic sections and the pituitaries were removed from the base of the skull. The sections were analyzed for norepinephrine (NE) and dopamine (DA).

Substantial weight gain was observed in both male and female mice injected with GTG; however, 18% of the females and 30% of the males failed to show a weight gain which exceeded the range of the saline-injected mice of the same sex. No significant differences between GTG and saline-injected mice were observed in telencephalic NE or DA or hypothalamic DA. Hypothalamic NE was slightly reduced and a significant sex x injection interaction was obtained. Hypothalamic NE was reduced further in females than males. The correlation between hypothalamic NE and body weight in the GTG groups was not significant.

A large decrease in pituitary DA was observed in the GTG-injected mice of both sexes when compared with saline-injected controls. Furthermore, a significant negative correlation ($r = -.5$, $p < .001$) was obtained between pituitary DA levels and body weight change. These changes in CA levels may account for the adiposity of the GTG mouse. Recent evidence suggests that the development of obesity in the GTG mouse is pituitary-dependent.

527 HYPOSALIVATION IN RATS FOLLOWING VENTRAL MEDIAL HYPOTHALAMIC LESIONS. F.W. Flynn, D.L. Schirer and J.C. Mitchell*. Dept. of Psychology, Kansas State University, Manhattan, Ks., 66506.

Salivation was recorded for 6 normal and 6 rats with ventro-medial hypothalamic lesions. Salivation was quantified by inserting a cotton-tipped applicator into the rat's mouth for one minute. The difference in the pre-insertion weight and the post-insertion weight of the applicator reflected the amount of salivation. Measures were taken twice daily for 9 days during ad libitum food availability. Hypothalamic lesions resulted in the usual dramatic weight gain but were associated with a 50% reduction in salivation compared to normal animals (mean salivation measurement: normals = 11.7 mg; VMH = 5.6 mg).

On the 10th post operative day, animals were placed on a six hour food deprivation schedule (0900-1300 hours). Measures were taken for 20 days 1/2 hour into the deprivation schedule and immediately prior to the reintroduction of food into the food bin.

As in the ad libitum period, the VMH animals continued to display a statistically reliable lowered salivation (normals = 13.26 mg; VMH = 5.82 mg).

These results are contrasted with those of Rozkowska and Fonberg (1973) who found VMH animals to be hypersalivatory compared to normal animals. However, in their study, the animal's salivation was examined in a classical conditioning paradigm. Both findings are consistent with the view that VMH animals are hyperresponsive to appetive cues; both those that signal food, and those that signal an absence of food. Rather than a unidirectional effect, there is reduced salivation to the absence of food and heightened salivation to food.

Following these baseline measures, animals were given prostigmine, a parasympathomimetic agent that produces salivation. Animals were given subcutaneous injections of .10 mg/kg. On three test days salivation in VMH animals equalled normals.

In order to test both normal and VMH animal's responsiveness to natural food or taste stimuli, lemon juice, vanilla-glucose solution and wintergreen were introduced into the animal's mouth and salivation measured. The response to these stimuli will be discussed.

528 CALCITONIN AND FEEDING. William J. Freed, Mark J. Perlow, John Scott Carman*, and Richard Jed Wyatt. Laboratory of Clinical Psychopharmacology, SMR, IRP, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

Plasma calcitonin (CT) concentrations increase following a meal, and the administration of the gastrointestional hormones glucagon, gastrin, pancreozymin, and cholecystokinin. A series of experiments was performed to test the hypothesis that this feeding-induced increase in CT secretion plays a role in reducing subsequent food intake. In rats, a single s.c. injection of synthetic salmon calcitonin was found to inhibit 24-hr food intake (up to 58%, $p<0.005$) in proportion to the dose (12.5-50 U/kg). The decreased food intake was accompanied by a pronounced diuresis and increase in drinking--not observed in food deprived animals. In rhesus monkeys, a single injection of CT (30 U/kg) greatly suppressed feeding for 3 days and drinking for one day (both $p<0.01$). A retrospective study of humans demonstrated a 2% reduction in body weight ($p=0.03$) during the 12-36 hr following a single s.c. injection of CT (2 U/kg).

To test whether these effects were due to a direct action on the brain, CT was administered intraventricularly to rats. On a dosage basis these injections were approximately 75-fold more effective than s.c. injections in reducing feeding; amounts as small as 43 ng (0.2 U) significantly suppressed 24-hr food intake ($p<0.02$). Other experiments in 23½-hr food-deprived rats indicated that CT (12.5 U/kg, s.c.) effectively suppressed feeding only when administered several hours prior to 30 min of access to food; injections 1 hr before feeding were ineffective.

Thus CT reduces feeding, apparently through a direct action on the brain. Because this action does not seem to be manifest immediately, it is suggested that post-prandial secretion of CT acts on the brain to inhibit the ingestion of subsequent meals. Thus, when a meal is not eaten, the absence of the normally concomitant CT secretion may serve to enhance feeding during subsequent meals.

529 BOMBESIN SUPPRESSES FOOD INTAKE IN THE RAT. J. Gibbs, D.J. Fauser*, E.A. Rowe*, B.J. Rolls*, E.T. Rolls* and S. Maddison*. Dept. Psychiatry, Cornell Univ. Medical College and E.W. Bourne Laboratory, The New York Hospital, White Plains, N.Y. 10605, and Dept. of Experimental Psychology, Univ. of Oxford, Oxford, UK.

Bombesin (BBS) is a tetradecapeptide isolated from the skin of the frog Bombina bombina. BBS-like activity, as determined by radioimmunoassay, has been found in mammalian gut. Since BBS has several of the visceral gut actions of cholecystokinin (CCK), we tested BBS to determine if it also possessed the satiety action of CCK.

Eleven male Sprague Dawley rats were injected intraperitoneally with synthetic BBS (courtesy of R. de Castiglione, Farmitalia, Milan) or the synthetic C-terminal octapeptide of CCK (CCK-8, courtesy of M. Ondetti, Squibb Institute, Princeton) immediately before presentation of a balanced liquid food (25% EC 116, GIBCO) during the light phase following a 3-hr food deprivation. Drinking water was always available ad-libitum.

Both peptides produced a marked, dose-related suppression of food intake. CCK-8 was 5-10 times as potent as BBS, on a molar basis. Maximal suppression by each peptide occurred 15 to 30 minutes following injection. The lowest dose of BBS tested (2 μg/kg) produced a 15% suppression ($p<0.01$). The D50 was 8 μg/kg. BBS also suppressed consumption of solid food (n=20) and operant responding for pellets (CRF schedule, n=5) over a similar dose range in black hooded Lister rats.

BBS did not appear to suppress food intake by causing illness: (1) Rats did not appear ill after BBS injections. They began eating eagerly when food was presented, but stopped eating sooner and they exhibited the characteristic sequence of behaviors seen during normal satiety. (2) Rats maintained normal body weight gain over a 6-month period despite frequent BBS administration. (3) BBS did not alter rectal temperatures under typical test conditions. (4) BBS did not affect water intake when it was injected after minimal water deprivation.

Since circulating BBS-like radioimmunoassay activity rises rapidly following a meal in humans (V. Erspamer, personal communication), we suggest that BBS may play a role as a satiety signal.

This study was supported by USPHS NIH grant AM17240, NIMH RSDA 5 KO2MH70874, and the Medical Research Council of Great Britain.

530 THE ORGANIZATION OF FEEDING IN THE DEVELOPING RAT. W. G. Hall. North Carolina Div. Mental Health, Raleigh, NC 27611.

Neural mechanisms subserving components of the feeding system are present from birth in the rat. Diet infusions (made through oral cannulas) stimulate mouthing, licking and ingestion in deprived infant rats tested in an incubator. In addition, exposure to the taste and smell of food produces a profound, but general, increase in motor activity.

In fact, pups were found to be capable of independent ingestion when placed in a warm test container where diet was spread on the entire surface of the floor. The diets tested were milk, 10% sucrose, and a semi-solid chow mash. In a ½-hr test, deprived pups of 3, 6 and 9 days of age consumed 4.4, 8.6, and 6.4% body weight respectively of the milk diet; 5.3, 12.3, and 7.1% body weight of 10% sucrose; and 2.4, 4.8 and 4.0% body weight of the semi-solid mash. At each age, and with each diet, there was an increase in intake from 1 to 7 and 7 to 22 hr deprivation. There can be little doubt that the infant rat is capable of a regulated form of independent feeding.

Yet, detailed behavioral observations made every 30 seconds during oral-infusion and independent-feeding tests revealed: 1) that while components of feeding were present in the neonate they were not organized in the same way as in the adult; and 2) that the pattern of ingestion changed from one age to the next. Taste, smell, and stimulation from swallowing the diet produced vigorous head probing and mouthing, locomotion, rolling, and tumbling in 3-day-old pups. Tongue protrusion was noted, but with little licking. Food also stimulated an activation in 6-day-old pups, but their activity was directed toward the floor and corners of the test container. Probing and mouthing of the floor was frantic, and there was obvious licking of the floor; but these behaviors were nondirected. At 9 days of age, diet did not generate a difuse activation. These pups predominately fed, showing mouthing and licking which were directed and focused. This channeling of activity into appropriate output patterns is documented by the finding that when diet was made available on just 1/10th of the area of the floor 3- and 6-day-old pups consumed only a fraction (18%) of the volume they had ingested when diet was freely available over the entire area. In contrast, 9-day-old pups consumed almost as much diet from the localized source (71%), demonstrating that they could direct their ingestive behavior.

Thus, at birth, components of feeding in the rat pup are not yet organized into coherent patterns. At the start, feeding is under the control of deprivation. Gustatory and olfactory stimulation can excite or arouse pups, facilitating or energizing responses, but in a nonspecific fashion. By 9 days of age, feeding has become organized into a directed and specific form of behavior.

531 ALTERATIONS IN FEEDING BEHAVIOR FOLLOWING DORSAL BRAINSTEM LESIONS IN RATS. Jeffrey M. Halperin*, Yaakov Stern*, Barbara Burt*, Robert Farrell*, Constantine Pavlides*, Solomon S. Steiner and Steven J. Ellman. Dept. Psych., CCNY, New York, N.Y. 10031.

The present study examined the role of dorsal brainstem (DBS) structures in the regulation of feeding behavior by placing bilateral DBS lesions in ten rats, housed individually on a L-12, D-12 schedule. Following at least 15 days of adaptation to a sweetened milk diet, body weight, the volume of milk ingested, and the number and temporal distribution of licks were recorded for the light and dark period of each day during a seven day pre-lesion baseline and at least 21 days following the lesion. The meal pattern records were scored for meal size and meal frequency. Response to quinine adulteration was also measured for each animal.

Six rats had lesions in the pontine central gray area. These lesions impinged upon the dorsal tegmental nucleus, the locus coeruleus, the sub-coeruleus, and the trigeminal motor nucleus. Four rats had lesions ventral or lateral to these structures leaving the dorsomedial pontine tegmentum intact. Those rats with lesions in the pontine central gray, but not in other DBS loci, significantly increased their food consumption as measured by volume and number of licks. The hyperphagia was characterized by a significant increase in the number of meals; meal size remained unaltered. Interestingly, the hyperphagic rats did not gain significantly more weight than controls. The non-hyperphagic animals showed no changes in meal size or frequency. Quinine adulteration did not differentially reduce feeding between groups.

This experiment is being replicated using a solid food diet. Data, histological and neurochemical analyses are in progress. The data will be discussed in terms of 1) the similarities and differences of solid and liquid food diets in response to DBS lesions, and 2) the similarities and differences between the DBS feeding syndrome and hypothalamic hyperphagia.

532 VENTROMEDIAL HYPOTHALAMIC DESTRUCTION IN NEONATAL RATS: EFFECTS UPON GROWTH AND CONSUMMATORY BEHAVIORS. David L. Hill*, C. Robert Almli, David M. Williams* (Spon: M. M. Patterson). Dept. Psychol., Ohio University, Athens, Ohio 45701.

Neonatal rats (albino, male and female) at one day of age (24 hours of age) sustained bilateral destruction of the ventromedial hypothalamus (VMH). Body weight and body length (nose-anus) were measured daily, and the Lee Index of relative obesity was calculated each 10 days. At adulthood the rats were tested for water intake during food deprivation, and intake of quinine adulterated food was measured. The rats were on a high fat (25% Crisco by weight with powdered rat mash) diet from 25 days of age through completion of the study at 200 days of age.

Following VMH damage at one day of age, the rat pups displayed slightly attenuated growth for approximately 10 days when body weights were approximately 90% of control. Following this, the rats grew at an accelerated rate and surpassed control body weights by 70-80 days of age. Female VMH rats continued to be heavier than controls than were males throughout the measurement period.

Body lengths of the VMH pups were within the control range for the first 50-60 days of age. Thereafter, the brain damaged rats displayed attenuated body length increases, and were slightly stunted in linear growth for the remainder of their lives.

Lee Index, a measure of relative obesity, most clearly separated the brain damaged from control rats, as elevated Lee Indices became apparent when the rats were 60-70 days of age. The VMH rats had elevated Lee Indices for the duration of the study, and VMH males and females were similarily elevated.

The VMH rats were not hyperphagic during any period of development, however, they did display finickiness as evidenced by decreased quinine intake, and water intake during food deprivation was decreased.

The present results demonstrate that neonatal VMH damage produces relative obesity and finickiness, and the development of obesity awaits puberty. Thus, when rats sustain VMH damage at 1, 10, 25, or 40 days of age, body growth is within the normal range until puberty at 50-70 days of age, when important endocrinological alteration results in the manifestation of relative obesity. Growth hormone and gonadal hormone alteration are likely candidates for the etiology of VMH lesion induced obesity.

533 SOME PROPERTIES OF THE CENTRAL AFFERENT SYSTEM THAT CONTROLS SWALLOWING IN CATS. Charles H. Hockman, Ananda Weerasuriya* and Detlef Bieger. Sch. Basic Med. Sci., and Dept. Physiol. and Biophysics, Univ. Illinois, Urbana, IL 61801.

The swallowing apparatus may be conceptualized as a central afferent system that decodes sensory information and, when appropriate, sends commands to a swallowing center where they are elaborated upon and organized so as to produce swallowing through an effector system by the sequential activation of relevant muscles. The afferent system, organized in and around the nucleus of the solitary tract (NTS) in the dorsal medulla, receives inputs from the superior laryngeal (SLN) and glossopharyngeal (GN) nerves. In cats anesthetized with urethane, swallowing was induced by electrical stimulation of the SLN or GN, and monitored by means of a xylocaine-coated balloon in the oropharynx. The frequency-response curve for SLN-induced reflex swallowing shows a distinct bell shape with an optimal frequency range between 25 and 45 Hz. The limiting frequency was about 100 Hz. In the production of reflex swallowing, a type of spatial summation could be demonstrated between both SLNs and between ipsi- and contralateral SLN and GN. The swallowing response could be facilitated when one nerve (SLN) was stimulated immediately after cessation of stimulation of another nerve (contralateral SLN or ipsilateral or contralateral GN). Both types of facilitatory interaction were observed between contralateral nerves after a mid-sagittal cut just behind the obex: the point at which primary visceral afferents cross over to terminate in the contralateral NTS. Neither type of interaction was seen when both SLNs were stimulated at values above the limiting frequency. With continuous stimulation of one nerve, the response rate decreased, which could be reversed by switching the stimulation to another ipsi- or contralateral nerve. The response decrement could also be reversed by stimulating in the brain stem in and around the contralateral solitary tract. By partial hemisections and punctate electrolytic lesions, it was established that the region of the NTS essential for the elicitation of reflex swallowing lies at least 2.0 mm in front of the rostralmost point of the dorsal median sulcus. These results suggest that (a) the stimulus response relationship reflects input-output properties of the central afferent system; (b) the facilitatory interaction between contralateral nerves occurs at the level of second or higher order neurons of the central afferent system; (c) the neural substrates responsible for the response decrement are situated in a localized region of the central afferent system; and (d) the essential component of the central afferent system is located in and around the anterior region of the medial NTS. (Supported by the State of Illinois Dept. of Mental Health and Developmental Disabilities.)

534 THE THRESHOLDS FOR HYPERGLYCEMIA AND FEEDING TO GLUCOPRIVATION PRODUCED BY INTRAVENOUS 2-DEOXY-D-GLUCOSE. Thomas M. Hyde* and Richard R. Miselis. Dept. of Anim. Biol., Sch. Vet. Med. and Inst. Neur. Sciences, Univ. of Penn., Philadelphia, PA 19104.

Rats prepared with chronic intravenous (i.v.) catheters were tested individually in their home cages either for the hyperglycemic reflex or feeding in response to i.v. infusions of 2-deoxy-D-glucose (2DG), a glucose analogue which causes glucoprivation. Infusions for the feeding study were given to 34 rats in an intermeal interval 1.5 to 3.0 hours after the last spontaneous meal. In a similar manner 37 rats were studied for their hyperglycemic response to i.v. 2DG given during an intermeal interval but without food available 2.0 hours before and for the duration of blood sampling. For both studies a remote infusion and blood sampling technique was used to avoid stress and interference with spontaneous behavior. The threshold dose for hyperglycemia was between 40 and 80 mg of 2DG/kg of body weight and a dose response relationship was present. The threshold for the feeding response was between 25 and 50 mg/kg and was evident as an increase in the percentage of rats responding to a particular dose over the percentage of rats responding to the control infusion of isotonic saline. The percentage of rats responding increased in a dose response fashion. At all doses tested, rats that responded ate a normal sized meal which did not vary with dose. The threshold for feeding is at least the same if not lower than the threshold for hyperglycemia indicating a possible role for the glucoprivic control in normal feeding. Using doses of 2DG which produce feeding without triggering the hyperglycemic reflex is a physiologically meaningful way to study the glucoprivic control of feeding. Location of a site of detection of glucoprivation operating physiologically in the control of normal feeding will depend upon using the same low doses.
Supported by RR 07083 and RR 546414

535 PLASMA ANGIOTENSIN II (AII) LEVELS AND THIRST. A. K. Johnson, J. F. E. Mann*, M. W. Houshk*, and D. Ganten*. Dept. Psychology, Univ. Iowa, Iowa City, IA 52242 USA, and Dept. Pharmacology, Univ. Heidelberg, Heidelberg, GFR.

Investigations of the hormonal basis of thirst leave no doubt that AII is a potent dipsogenic stimulus and that it acts on the brain to induce water intake. It is also clear that under normal conditions AII is not the sole stimulus for water intake and that to understand its role in the physiological control of drinking, the dynamics of the effector peptide, AII, must be characterized. We have determined plasma AII levels by radioimmunoassay following experimental treatments that induce thirst.

1) AII was infused intravenously into nephrectomized rats over a period of 60 min (n=30). Blood samples were taken at the end of the infusion. Results are given in the table:

Dose of AII (ng/kg/min)	1	25	50	100
Plasma AII (pg/ml)	121.6 ±13.4	187.9 ±21.6	394.6 ±42.9	843.0 ±86.9

2) Rats (n=28) were water deprived for 12, 24, and 48 hr. Plasma AII levels were 136.7 ± 8.6, 117.4 ± 16.1, and 399.6 ± 90.8 pg/ml, respectively (controls with ad lib water 50.2 ± 5.3).

3) Isoproterenol was injected subcutaneously (0.1 and 0.3 mg/kg) in rats (n=15). Plasma AII levels 60 min later were 619.2 ± 84.9 and 1001.0 ± 112.5, respectively, and in sham treated controls 71.5 ± 8.3.

4) The inferior vena cava was ligated in rats (n=48). One, 2, and 4 hr later plasma AII levels were significantly elevated to 881.6 ± 168.0, 749.0 ± 112.0, and 557.2 ± 101.7 pg/ml, as compared to the levels in sham operated controls (332.4 ± 30.2).

5) Subcutaneous injections of 20% polyethylene glycol (15 mg/kg) in rats (n=26) elevated plasma AII levels after 1, 2, 4, and 8 hr to 67.59 ± 11.75, 100.73 ± 17.93, 170.78 ± 18.21, and 349.21 ± 48.40 pg/ml. Saline injections (n=24) of the same volume, after the same time intervals, produced plasma AII levels of 41.3 ± 14.73, 27.04 ± 2.64, 47.04 ± 2.0, and 67.08 ± 7.49 pg/ml.

6) Rats (n=30) on a 1 hr daily feeding schedule with ad lib access to water were forced to eat a meal without access to water. One hour after access to the food, levels of plasma AII were elevated to 138.8 ± 11.45 pg/ml, whereas the level in control animals that had not eaten a dry meal was 91.5 ± 6.93 ($p < .01$).

The results of these experiments define the range of changes that occur in plasma AII levels after several thirst-inducing challenges and show that AII can act in some cases as a dipsogenic stimulus within the physiological range.

536 KAINIC ACID TOXICITY IN THE LATERAL HYPOTHALAMUS. K.M. Kantak, R.C. Cook*, F.C. Barone and M.J. Wayner. Brain Res. Lab., Syracuse Univ., Syracuse, NY 13210.

Bilateral infusions of kainic acid (KA, 3µg/0.5µl and 3µg/1.0µl) or 0.9% saline (Sal, 0.5µl and 1.0µl) into the posterior lateral hypothalamus were made. Within 30 min following KA infusions signs of autonomic and other effects were noted. These included exophthalamus, chromodacryorrhea, mydriasis, salivation, pulmonary edema, whole body tremors, tonic seizures and convulsions. The disturbances lasted for several hours post infusion and 50% of the animals died. The Sal infused animals did not show any signs of autonomic disturbance and had a 100% survival rate. Prior to and 11 days following the infusions animals were tested for salt arousal of drinking (1 hr test); post 24 hr water deprivation induced drinking (1 hr test); and post 24 hr food deprivation induced eating and drinking (3 hr test).

Following the infusions, KA treated animals (3µg/0.5µl) decreased water intake on post infusion days 1,2 and 3 relative to baseline and the Sal (0.5µl) control group. Food intake was significantly decreased in both groups on the first and second days post infusion. Thereafter food intakes were stable in both groups. Body weight in the KA treated animals decreased post infusion and returned to pre-operative body weight on day 9 post infusion but remained at a reduced level with respect to the Sal control group on post infusion days 1 through 11. Animals treated with 3µg/0.5µl KA failed to respond to salt arousal of drinking and post 24 hr food deprivation drinking. Following 24 hr water deprivation KA treated animals did respond but at an attenuated level with respect to the Sal control animals. There were no differences in post 24 hr food deprivation induced eating between groups.

There were no differences in post infusion food and water intakes, body weights, salt arousal of drinking, post 24 hr deprivation induced drinking, and post 24 hr food deprivation induced eating and drinking in animals treated with 3µg/1.0µl KA and 1.0 µl Sal.

These data indicate that the concentrations of KA were toxic in the lateral hypothalamus immediately following infusions. Of those animals which survived a concentration dependent effect on behavioral and physiological deficits was evident. That these effects are specific to the lateral hypothalamus remains to be determined.

537 EFFECT OF INTRAVENTRICULAR β-ENDORPHIN ON FOOD INTAKE IN RATS. Nancy J. Kenney, L. David McKay*, Stephen C. Woods*, and R. H. Williams*. Depts. of Psychology and Medicine, Univ. Washington, Seattle, WA 98195.

The recent discoveries that many endogenous peptides including cholecystokinin (CCK), somatostatin and β-endorphin are found in both brain and gut have made these compounds potential candidates for a role in energy balance regulation. Systemic administration of CCK or somatostatin previously have been reported to suppress food intake in food-deprived rats. On the other hand, central administration of somatostatin was found to have no effect on food intake in the rat or the baboon.

We examined the effect of low doses of β-endorphin, a morphine-like compound reported to produce analgesia and catatonia at high doses, on food intake of mildly deprived rats. Male Wistar rats were adapted to a feeding regimen on which they were deprived of food, but not water, for 6 hr during the light portion of the light-dark sequence. At the end of this period they were offered a liquid diet (50% sweetened condensed milk, 50% tap water) for ½ hr. At all other times the animals were maintained on dry pelleted lab chow. Animals were tested in blocks of three days consisting of a vehicle control day immediately before and after the β-endorphin test day. β-endorphin (200 ng/µl/rat) or isotonic saline (1 µl/rat) was injected into the lateral cerebral ventricle through a permanently implanted stainless steel cannula immediately prior to each test session.

Central administration of β-endorphin resulted in a significant and substantial increase of liquid-diet intake during the 30-min test session (average intake on control days 5.6 ± 1.0 ml vs. 10.8 ± 1.9 ml on the β-endorphin test day, $t(7)=3.0$, $p<.05$). The effect was limited to the 30-min test session with no treatment effects evident in the subsequent overnight food intakes. The effect appears to be centrally mediated; intraperitoneal injection of the same dose of the peptide had no effect on food intake under these conditions. Central injections of CCK (100 ng), substance P (200 ng) and neurotensin (100 ng) resulted in no change of food intake under identical conditions suggesting that the effect is specific to β-endorphin and not a general property of this class of peptides. These data, in conjunction with the finding that β-endorphin is endogenous to brain and gut, suggest the possibility that the peptide may play a role in the central regulation of energy balance.

Supported by funds from the Graduate School of Arts and Sciences (NJK), AM-17844 (SCW) and the Kroc Foundation (RHW).

538 NEUROLOGICAL SEPARATION OF FEEDING AND BEHAVIORAL ACTIVATION IN NEONATAL RATS. Carol L. Kornblith and W. G. Hall. Dept. of Psychology, U. North Carolina, Chapel Hill, NC 27514 and NC Div. of Mental Health, Raleigh, NC 27611.

When milk is delivered directly into the mouth of the infant rat via an intraoral cannula, significant ingestion occurs and is accompanied by a dramatic behavioral activation. The ingestion and activation occur when the pup is food deprived (22 hr) and tested in a warm, moist environment (33°C). Intact 3 day old pups allowed to ingest milk presented in discrete pulses consumed 60% of the injected diet (1.8% body weight) compared to 10% when nondeprived. The behavioral activation consisted of probing, mouthing, licking, swallowing, face grooming, rolling over, stretching, twitching, head waving and wall climbing (mean activity rating of 40). These results indicate the presence, at a very early age, of neuronal systems for both ingestive behavior and a primitive form of arousal. This activation may be an indication of one of the first reinforcers experienced by the infant rat.

As an initial step in describing the neurology of these responses, knife cuts were made at various levels of the neuraxis. Two day old pups were separated from their mothers, lightly anesthetized and given transverse knife cuts across the bottom half of the brain. The cuts were made at various anterior-posterior levels within a litter, ranging from the olfactory bulbs to the pons. Several hours before testing on the next day, the intraoral cannulas were implanted.

The 18 of 35 subjects with complete cuts could be divided into four groups. Both activity (ratings of 23-41) and intake (45-62% of injected diet) were normal in 4 of 5 subjects with cuts ranging from the anterior olfactory bulb to a line drawn through the middle of the caudate, the anterior commissure and in front of the supraoptic nucleus. Four subjects with cuts ranging from the supraoptic nucleus to a line running posterior to the thalamic reticular nucleus and behind the ventral supraoptic commissure were not activated (9-18) but had normal intake (37-78%). Six subjects with cuts extending from behind the ventral supraoptic commissure to in front of the substantia nigra were not activated (5-19.5) and had low intake (1-27%). The final group of 3 subjects had low activity ratings (5.5-21) and normal intake (37-41%). These cuts extended from the posterior substantia nigra through the interpeduncular nucleus.

Thus, the occurrence of behavioral activation seems to require an intact diencephalon, while cuts within the diencephalon decreased both activity and feeding. Interestingly, ingestion could still occur with cuts at least as far posterior as the interpeduncular nucleus.

539 VAGOTOMY FAILS TO BLOCK THE SATIATING EFFECT OF FOOD IN THE STOMACH. F. Scott Kraly and James Gibbs. Dept. Psychiatry, Cornell Med. Coll. and Bourne Laboratory, N.Y. Hospital, White Plains, N.Y. 10605

When rats eat after 3-hr food deprivation, pregastric food-contingent stimulation alone (during sham feeding) is not sufficient for normal satiety because meal size (MS) is abnormally large and the latency to rest (LR) in the behavioral sequence of satiety is abnormally long (Kraly, Carty & Smith, 1978). Thus, gastric and/or intestinal food-contingent stimulation is necessary to control normal MS and LR.

Combined food-contingent stimulation of pregastric and gastric sites is sufficient for normal MS and LR: Rats ($n=4$) equipped with a pyloric noose ate liquid food after 3-hr food deprivation. When the noose was open, ingested food accumulated in the stomach and entered the intestine in normal fashion. When the noose was open, rats ate a mean (± SE) MS of 4.2 ± 0.6 ml and displayed a postprandial satiety sequence with a mean LR of 11.1 ± 0.4 min. When the noose was closed, ingested food was trapped in the stomach and did not enter the intestine. When the noose was closed, rats ate a mean MS of 5.8 ± 1.9 ml ($p > .20$, open vs. closed noose) and displayed a postprandial satiety sequence with a mean LR of 10.9 ± 1.6 min ($p > .20$, open vs. closed noose). These results confirm the findings of Kraly and Smith (in press) and show that a satiety signal of gastric origin, when combined with pregastric stimulation by food, produces normal MS and LR.

Vagotomy does not block the ability of combined gastric and pregastric food-contingent stimulation to control MS and LR: Rats ($n=7$) with bilateral subdiaphragmatic vagotomy (with hepatic vagal branch intact), equipped with a pyloric noose, ate with the noose open or closed after 3-hr food deprivation. When the noose was open, vagotomized rats ate a mean MS of 4.4 ± 0.7 ml and displayed a postprandial satiety sequence with a mean LR of 10.6 ± 1.6 min. When the noose was closed, vagotomized rats ate a mean MS of 2.7 ± 0.8 ml ($p > .05$, open vs. closed noose) and displayed a postprandial satiety sequence with a mean LR of 16.8 ± 3.2 min ($p > .10$, open vs. closed noose). These results show that the satiety effect of gastric food-contingent stimulation is not dependent upon vagal mediation, and suggest that gastric distension is not the critical stimulus for satiety under these conditions.

540 THE BEHAVIORAL AND EEG RESPONSE TO KAINIC ACID INJECTIONS IN THE LATERAL HYPOTHALAMUS OF RATS. L. R. Leach*, I. Q. Whishaw* & B. Kolb (SPON: E. Davis). University of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4.

Bilateral electrolytic lesions of the lateral hypothalamus (LH) of rats cause transient aphagia, anorexia, behavioral depression, alterations in hippocampal and neocortical EEG, and chronic disturbances in water intake (Kolb and Whishaw, 1977). Electrolytic lesions destroy both axons passing through and neurons intrinsic to the LH. We used microinjections of the neurotoxin kainic acid (KA) to determine how destruction of neurons intrinsic to the LH might affect the ingestive and locomotor behavior and the EEG activity of rats. Rats with chronic EEG recording electrodes implanted in the hippocampus and neocortex, received bilateral .1 ug injections of KA (.5 to 4 ug/ul of CSF) in the LH. Following KA injection, a transient period of aphagia, adipsia, hypokinesia, poor postural support, ataxia, abnormal spike and slow wave activity in the hippocampus, and abnormal slow wave activity in the neocortex lasting 1 to 5 days was observed in all rats. The presence of abnormal hippocampal slow wave activity simultaneous with neocortical slow wave activity was associated with aphagia: all rats that exhibited both conditions of abnormal EEG activity were also aphagic. After 7 days the behavior deficits were not apparent, and after 15 days normal patterns of hippocampal and neocortical activity could be recorded from all rats. The effects of KA injections differed from previously reported effects of electrolytic LH lesions in the following ways: KA injections did not cause the chronic disruption of water intake regulation, the chronic decrease in frequency of atropine resistant hippocampal theta rhythm, or the appearance of atropine-sensitive hippocampal theta activity seen after electrolytic LH lesions. KA injections caused abnormal hippocampal spike and slow wave activity which electrolytic lesions did not. The results suggest that destruction of cells intrinsic to the LH contributes to the aphagia, behavioral depression, locomotor deficits and some transient abnormal EEG activity. However, chronic changes in water intake regulation and EEG activity observed only after electrolytic lesions are probably the result of damage to axons passing through the LH.

Kolb, B. and Whishaw, I. Q. Effects of brain lesions and atropine on hippocampal and neocortical electroencephalograms in the rat. Experimental Neurology, 56, 1-22, 1977.

541 THE EFFECT OF INTRACEREBROVENTRICULAR INFUSION OF SARALASIN ON DRINKING FOLLOWING CAVAL LIGATION, HYPERTONIC SALINE AND WATER-DEPRIVATION IN RATS. Maw-Chang Lee*, Terry N. Thrasher* and David J. Ramsay. Dept.Physiol., Sch.Med.,UCSF,San Francisco, CA 94143.

Ligation of the inferior vena cava in rats is known to be a potent dipsogenic stimulus (Fitzsimons,J.T.,Nature,204,479,1964). However, it is not clear to what extent the drinking is dependent on stimulation of the renin/angiotensin system. In the present series of experiments, the effect of central blockade of angiotensin with intracerebroventricular (IVT) infusion of saralasin(Sar^1-Ile^8-Angiotensin II) on drinking following caval ligation was investigated. Using similar experimental protocols, the effect of IVT administration of saralasin on drinking following hypertonic saline injection and 24-hour water deprivation was tested.

Inferior vena cavae of Sprague-Dawley rats of 260-360 gm were completely ligated above the renal veins under ether anesthesia. The rats then received an initial loading dose of 1 µl of either normal saline or saralasin (4 µg/µl) followed by infusions of the same solutions at 1.3 µl/h for 5 hours through chronically implanted lateral ventricular cannulae. Central administration of saralasin significantly reduced ($P<0.01$) the water intake during the 5 h after caval ligation from 1.47±0.38 ml/100 gm (n=6) in IVT saline controls to 0.28±0.18 ml/100 gm (n=8).

In a second group of 8 rats, the effect of saralasin IVT on drinking following 1.0 M NaCl (1% body weight, ip) was investigated. The saline was injected under light ether anesthesia 75 min following the commencement of IVT saralasin infusion. The rats receiving saralasin IVT drank 2.63±0.36 ml/100 gm (n=8) in the 90 min following hypertonic saline injection compared with 2.30±0.73 ml/100 gm (n=5) in IVT saline controls, intakes that were not significantly different.

Drinking following 24-hour water deprivation was measured in a third group of rats during IVT saralasin infusion. Access to water was allowed 75 min following the start of the IVT infusions of either saralasin or normal saline. Cumulative water intake following 10 min of water access was 1.96±0.31 and 1.19±0.36, and at 3h 3.30±0.26 and 2.38±0.46 ml/100 gm in rats receiving saralasin IVT (n=8) and saline IVT (n=7) respectively. Saralasin did not significantly affect either the latency or the total amount consumed.

These data show that the drinking following caval ligation drinking in rats is abolished by central blockade of the renin/angiotensin system. In contrast to this, drinking to hypertonic saline and to water deprivation was not affected by treatment with saralasin. It is concluded that drinking to caval ligation depends on the renin/angiotensin system, whereas drinking to hypertonic saline and dehydration does not.

Supported by USPHS Grant HL-18862.

542 SEROTONIN-NOREPINEPHRINE INTERACTION IN THE PARAVENTRICULAR NUCLEUS: ANTAGONISTIC EFFECTS ON FEEDING BEHAVIOR IN THE RAT. Sarah F. Leibowitz and Peter J. Papadakos*, Rockefeller Univ., New York, NY 10021.

The paraventricular nucleus (PVN) has been identified as the most sensitive brain area for the elicitation of feeding through direct α-noradrenergic stimulation. Studies described in this abstract provide evidence for an antagonistic action of serotonin in this region of the hypothalamus.

Brain-cannulated rats maintained on ad libitum lab chow pellets and water eat a meal of approximately 3 g after injection of norepinephrine (NE, 30 nmoles) into the PVN. Local injection of 5-hydroxytryptamine (5-HT, 13 nmoles in 0.02% ascorbic acid) immediately prior to NE consistently reduced this feeding response by 60-90%. This inhibition was dose-dependent and occurred reliably at doses as low as 0.5 nmole (88 ng free base). (Doses above 26 nmoles started to exhibit sedative properties and thus were not used.) This 5-HT effect was abolished, in a dose-related, competitive manner, by prior PVN injection of the 5-HT receptor antagonists methysergide (3-60 nmoles) and cinanserin (16-64 nmoles). It was unaffected, in contrast, by the dopamine antagonists haloperidol (1-14 nmoles) and pimozide (10-30 nmoles) and the cholinergic antagonist atropine (1.5-6 nmoles). Consistent with previous literature, preliminary studies with the phenothiazine fluphenazine (1.25-10 nmoles) and the β-adrenergic blocker alprenolol (100-400 nmoles) have indicated that these compounds may be effective in reversing 5-HT's receptor action in the PVN.

The 5-HT precursor, 5-hydroxytryptophan (5-HTP), produced the same inhibitory effect on NE-elicited eating (when injected 5-10 min prior to NE). It was approximately equipotent to 5-HT, with respect to both its threshold dose and its efficacy at maximum dose levels (10-20 nmoles). The inhibition produced by 5-HTP, in contrast to that of 5-HT, was prevented, in a dose-related fashion by PVN administration (30 min prior) of the nonspecific aromatic decarboxylase inhibitors Ro 4-4602 (1.35-135 nmoles) and MK 486 (2.4-40 nmoles). Similar to 5-HT, however, 5-HTP's action was blocked by the receptor antagonist methysergide.

These findings provide a preliminary basis for the hypothesis that NE and 5-HT interact, in an antagonistic fashion, in their effects on food intake. While the present evidence suggests that this antagonism may occur in the region of the PVN, further analyses will need to be conducted to establish the anatomical and pharmacological specificity of this phenomenon, its relation to noradrenergic stimulation versus a variety of other stimuli known to induce feeding, and its significance to brain serotonin mediation of anorectic drug action. (Research supported by NIH grant MH 22879, a Sloan Foundation Fellowship, and funds from Whitehall Foundation.)

543 SUPPRESSION OF FEEDING BEHAVIOR IN FOOD DEPRIVED MONKEYS BY LOCUS COERULEUS STIMULATION. K. Leverenz*, D.E. Redmond, Jr. and Y.H. Huang.

Norepinephrine has been found to have both stimulatory and suppressant effects on feeding behavior in non primate species. Lesions of the noradrenergic locus coeruleus (LC) have led to no consistent effects on food intake or body weight in rodents, but a recent study showed long-lasting hyperphagia, hyperdipsia, and weight gain in female monkeys after discrete bilateral LC lesions.[1] We have now studied the effects of intermittent electrical stimulation of the LC on feeding behavior in four adult female *Macaca artoides* implanted with LC electrodes.[2]

The monkeys were first acclimated to cable restraint and baseline feeding behaviors were studied under identical conditions to characterize feeding behavior during a 2 hour *ad lib* feeding period each day, after 22 hours with water but no food present. Total water intake, number of standard laboratory monkey biscuits grabbed, total biscuits wasted, total biscuits consumed, and the precise rate of biscuit grabbing were recorded. Biphasic square waves of electrical stimulation were delivered unilaterally to a bipolar locus coeruleus electrode at intensities from 0.15 to 0.65 mA, 0.5 msec pulse duration, 30-50 Hz frequency, for trains of 10 seconds each minute, with the parameters chosen to be below the threshold for typical observable behavioral effects of LC stimulation, previously described.[3]

Intermittent stimulation for 10 seconds/minute, beginning 5 minutes prior to and during the first 10 minutes of the feeding session had no effect on any overall measure of feeding or drinking for the entire 2 hour feeding session. No competing behaviors were elicited which would have prevented food grabbing, chewing, drinking, and swallowing, and all were sometimes observed to occur during stimulation. Stimulation did, however, produce a significant suppressant effect on spontaneous feeding behavior in 3 monkeys during the first ten minutes when intermittent LC stimulation was occurring at the beginning of each minute. The effect was reversed usually within 5 minutes of termination of stimulation, with a trend toward a post-stimulation increase in feeding. One monkey did not show this suppressant effect. Histological localization of the electrodes at the end of the study showed that the monkey without feeding suppression had an electrode placement anterior and outside the LC, while two of the other three electrodes were correctly located. One monkey's placement could not be verified. The specificity of these effects to LC-noradrenergic neurons require further study, but are consistent with the appetite suppressant effects of noradrenergic agonist drugs in humans and with other functions of the locus coeruleus we have proposed.

[1] *LifeSci* 20:1619,1977; [2] *BrRes* 100:157,1975; [3] *BrRes* 116:502,1976.

544 EFFECT OF ANTEROVENTRAL THIRD VENTRICLE (AV3V) LESIONS ON SCHEDULE-INDUCED POLYDIPSIA IN THE RAT. R. W. Lind and A. K. Johnson. Dept. Psychology, Univ. Iowa, Iowa City, IA 52242.

Periventricular lesions of the anteroventral third ventricle (AV3V) in the rat produce adipsia accompanied by a lack of compensating antidiuresis (Johnson & Buggy, Am. J. Physiol. 234: R122, 1978). In animals that recover from this acute adipsia, there are persistent deficits in drinking to angiotensin and hypertonic NaCl thirst challenges (Buggy & Johnson, Am. J. Physiol. 233:R44, 1977). To assess the effect of this lesion on secondary drinking (i.e., drinking not associated with a body water deficit) (Fitzsimons, Phys. Rev. 52:468, 1972), the AV3V was ablated in eight rats exhibiting schedule-induced polydipsia (Falk, Science 133:195, 1961) on a FT 60" schedule of food presentation. As compared with seven sham-operated controls, the lesioned rats drank significantly less ($p < .01$) in the polydipsia situation on the first day post-lesion. In animals (5 of 8) which were hypodipsic (<10 ml intake) in the home cage on the first night after surgery, a 74% decrease in schedule-induced drinking was observed. Controls showed no change in home-cage or schedule-induced drinking following surgery. This finding is consistent with the observation that prandial drinking, another form of secondary thirst induced by salivarectomy, does not enhance post-lesion water intake or recovery from adipsia. Thus, the AV3V lesion not only disrupts homeostatic mechanisms of thirst, but also reduces the animals' acceptance of water when water intake is under non-homeostatic control.

545 EFFECT OF 6-HYDROXYDOPAMINE LESIONS ON BODY WEIGHT IN GENETICALLY OBESE MICE. Joan F. Lorden. Dept. Psychol. and Neurosci. Program, Univ. Alabama in Birmingham, Birmingham, AL 35294.

Similarities between genetically transmitted obesity and hypothalamic lesion-induced obesity have suggested the possibility that the hypothalamus may be the locus of a genetic lesion responsible for obesity in obob and dbdb mice. In addition to obesity and concomitant metabolic changes, both obob and dbdb mice have numerous endocrine abnormalities, suggesting either anterior pituitary malfunction or a failure in hypothalamic control of the pituitary. No naturally-occurring hypothalamic lesions have been reported in either obob or dbdb mice; however, elevated levels of hypothalamic and telencephalic norepinephrine (NE) have been reported in both mutants when compared with lean littermate controls. Central catecholamines (CA) have been implicated in the control of food intake and body weight and are known to have an important function in the control of pituitary hormone release. To begin to assess the potential role of altered CA levels in the obesity syndromes of obob and dbdb mice, intraventricular infusions of 6-hydroxydopamine (6OHDA) were used to reduce CA levels in the brains of both mutants.

Female dbdb mice (C57Bl/Ks-db) and male and female obob mice (C57Bl/6J-ob) were obtained from the Jackson Laboratories, along with lean controls. Lesions were produced in both lean and obese mice by the infusion of 80 nmol (free base) of 6OHDA·HBr, dissolved in a .9% saline-.02% ascorbic acid vehicle. The 6OHDA was delivered in a 2 µl volume through a 30 gauge cannula, stereotaxically aimed at the third ventricle. Lean and obese control animals received 2 µl of the vehicle. All animals were weighed every four days. After 6 wk, all mice were sacrificed by decapitation and the brains assayed for NE and dopamine (DA). Blood samples were collected for determination of blood glucose.

The 6OHDA lesions had no significant effects on the body weights or blood glucose levels of lean mice of either strain. The lesions significantly reduced both body weight (-15%) and blood glucose (-24%) in dbdb mice when compared with vehicle-infused obese controls. Neither body weight nor blood glucose, however, was reduced to the levels of lean controls. In contrast, neither blood glucose nor body weight was significantly altered in obob mice of either sex by the 6OHDA infusion.

Assays of hypothalamic and telencephalic NE and DA showed that the 6OHDA infusions produced large decreases in the levels of both amines. The failure of the 6OHDA infusions to alter body weight in lean mice or obob mice was not due to a failure to produce a lesion. The results indicate that despite the similarities between the two syndromes, central monoamine systems play different roles in the development of obesity in these mutants.

546 DEPENDENCE OF THE CHOLECYSTOKININ SATIETY EFFECT ON VAGAL INNERVATION. Dennis N. Lorenz* and Steven A. Goldman* (SPON: Alan N. Epstein) Inst. Neurol. Sciences, U. Pa., Phila., Pa. 19104.

The intestinal hormone cholecystokinin has been implicated as a satiety signal specific for food intake (Smith, G.P. and Gibbs,J. Pharm. Biochem. and Behav. 3:135, 1975). Cholecystokinin must affect the brain either directly or indirectly to elicit satiety behavior, but its mechanism and site(s) of action are unknown. To investigate this mechanism, we tested the satiety effect of cholecystokinin in rats after selectively denervating portions of their viscera.

Holtzman albino male rats (300-450gm) underwent either a complete subdiaphragmatic bilateral vagotomy, including the hepatic branches, or section of the spinal cord between the 3rd and 4th thoracic vertebrae. All splanchnic afferents are believed to be located in the cord at this level. A third group of rats served as intact controls. The rats were tested daily after a 6hr food deprivation. Water was present at all times. At test time (1600hr), the rats were given Purina Pellets after an i.p. injection of either 1)cholecystokinin extract (20% pure); 2)the synthetically pure C-terminal octapeptide of CCK; or 3)the isotonic saline vehicle. Food was removed and weighed after the rats displayed satiety resting behavior.

The complete vagotomy group failed to suppress their food intake after the octapeptide and all but the largest dose of CCK (Table). All other groups suppressed their food intake. We conclude that some component of the subdiaphragmatic vagus is necessary for the cholecystokinin satiety mechanism, within our experimental paradigm; the splanchnic nerves are not required. The importance of the hepatic branches, as well as of the separate afferent and efferent projections of the vagi, in contributing to the cholecystokinin satiety effect, remain to be determined.

Food Consumed (Mean ± S.E.% of Saline Control)

Group	Complete Vagotomy	Complete Vagotomy	Cord Section	Controls	Controls
Horm.	Oct	CCK	Oct	Oct	CCK
N	(5)	(4)	(10)	(6)	(6)
20U/Kg	+12±9.7	-2±15.0	-53±12.0	-54±10.8	-44±7.1
40U/Kg	+10±9.6	+26±14.7	-54±12.7	-59±9.0	-53±18.1
80U/Kg	+8±8.6	+7±10.7	-70±14.5	-59±15.1	-81±5.3
160U/Kg	+8±4.8	-31±33.0	-	-83±10.3	-90±4.2
320U/Kg	-14±26.3	-	-	-	-
640U/Kg	+12±17.6	-	-	-	

Key:
+ = % increase
- = % decrease

Supported by NSF SMI77-12349 and NINCDS 03469.

547 SUBFORNICAL ORGAN: SITE OF PRESSOR AND DRINKING ACTIONS OF ACETYLCHOLINE. Michael L. Mangiapane and John B. Simpson, Depts. Physiol.-Biophys. & Psychol., Univ. Washington, Seattle, WA 98195

Iontophoretic and histochemical evidence indicates the presence of a dense cholinergic innervation of the subfornical organ (SFO), a circumventricular organ of the dorsal third ventricle. Simpson and Routtenberg have shown that the SFO is a sensitive site of dipsogenic action of acetylcholine (ACh; Brain Res., 1974) as well as of angiotensin II (A II; Science, 1973). The demonstration that SFO application of A II also elicits a pressor effect (Mangiapane and Simpson, Neurosci. Abstr., 1977) suggested that, like A II-induced drinking, drinking induced by SFO injection of cholinergic agonists also might be accompanied by a pressor effect. In unanesthetized, unrestrained rats prepared each with an SFO cannula and an aortic catheter, the SFO injection of carbachol induced within seconds a dose-dependent pressor effect which in all cases preceded onset of drinking. At 10^{-9} mols, the pressor response averaged 80 mm Hg, and decreased to 9 mm Hg at 10^{-13} mols. The pressor effect was not a consequence of drinking since it remained under chloralose anesthesia. Both responses were abolished by prior SFO injection of an equimolal quantity of atropine.

The effects of injection of ACh per se were next examined at three loci: SFO; adjacent ventral fornical commissure (VFC); and dorsal third ventricle (III V). Injection into SFO of 10^{-7}, 10^{-8}, and 10^{-9} mols of ACh elicited rapidly rising pressor responses of 37.1, 24.6, and 8.4 mm Hg, with concurrent intakes of 4.3, 1.0, and 0.5 ml H_2O, respectively. Injection of ACh at III V and VFC sites adjacent to SFO elicited significantly smaller pressor effects and provoked drinking only at the largest dose. At SFO, the mean latency for all doses, from offset of the 5 sec injection to onset of blood pressure increase, was 3.2±1.5 sec; indeed, 60% of responses commenced within 1 sec. Latencies to drinking were longer in all cases. At VFC and III V sites, latencies for both effects, if elicited, were significantly longer than at SFO. The extremely rapid onset of the SFO pressor effect suggested sympathetic participation in the response. Preliminary results indicate that the pressor effect of SFO ACh is sharply reduced following ip injection of 15 mg/kg phentolamine, whereas the drinking response is unaltered. These data: i)indicate the presence of a muscarinic cholinergic innervation of the SFO; ii)identify two functions of this innervation which are quite similar to those of SFO-injected A II; and iii)suggest that the ACh-induced pressor effect is at least partly sympathetically mediated.

Supported by HL 21799.

548 ONTOGENY OF DRINKING TO ANGIOTENSIN II IN THE SUCKLING RAT. L.J. Misantone, S.E. Natale*, and A.N. Epstein. Dept. Anat. Hahnemann Med. Coll. and Inst. Neurol. Sci, Univ. of Pa., Phila., Pa. 19102.

Angiotensin II (AII) has been shown to be a potent dipsogen in all adult species tested. This study examined the ontogeny of the water drinking response to AII in the rat. One hundred ng. of AII (in 1 µl of a vehicle of 50% distilled water and 50% India ink) was injected into the anterior right lateral ventricle of unanesthetized suckling rats. Littermate controls that were matched for weight with the AII treated rats received 1 µl of the vehicle alone. Responsiveness to AII was evaluated by a modification of Wirth and Epstein's technique (1976 Amer. J. Physiol. 230: 188-198), which uses weight gain during the test period as a measure of water ingested by consumatory responding. To avoid confusion of drinking responses with eating, only pups that gained weight in the nursing period preceding testing were used in this experiment. Successful injections were confirmed by decapitating the animals immediately after testing, making a coronal section through the injection site, and noting the presence or absence of ink in the anterior ventricles.

While drinking to AII appeared in several of the pups tested at early ages (0-3 days), the first consistent and statistically significant differences from control in response to AII were seen at 4 days of age ($\bar{x} \pm$ S.E.: AII = 85mg ± 14, n = 16 vs. control = 25mg ± 4, n = 12). At 5 days the weight gain due to water drinking after AII was double that at 4 days (AII = 177mg ± 65, n = 13 vs. control = 17mg ± 9, n = 12). Responsiveness to AII at 6 days of age did not differ from that at 5 days. When 3% NaCl was substituted for water in 5 day old rats, no significant differences in drinking occurred. When Esbilac, a synthetic bitches milk, was offered to 5 day old AII treated rats, they drank significantly more than controls (AII = 318mg ± 23, n = 8 vs. 45mg ± 14, n = 8) and this milk intake was significantly greater than the amount of water drunk by the 5 day olds described above. It does not seem likely that this intake of more milk than water indicates a role for AII in control of suckling behavior rather than as a primary dipsogen, because 1 day old rats treated with AII did not differ from controls in the amount of Esbilac drunk. Perhaps this apparent preference for milk over water in 5 day old rats treated with AII is related to the fact that milk, rather than water, is the substance with which the suckling rat fills its needs for both water and food. (Supported by NINCDS 03469 and MH 28782).

549 SUBFORNICAL ORGAN EFFERENT AND SUPRAOPTIC NUCLEUS AFFERENT CONNECTIONS AND THEIR IMPLICATIONS IN THE CONTROL OF WATER BALANCE. Richard R. Miselis, Robert E. Shapiro* and Peter J. Hand. Dept. of Animal Biol., Sch. Vet. Med. and Inst. Neur. Sci., Univ. of Penn., Philadelphia, PA, 19104.

Angiotensin II (AII) is known to initiate physiological and behavioral responses which participate in homeostatic maintenance of body water balance. The subfornical organ (SFO), a circumventricular organ of the brain, is a receptor site for the dipsogenic action of angiotensin II (AII) (Simpson and Routtenberg, Sci. 181:1172, 1973). The efferent projections of the SFO are of interest since they may provide the necessary initial connections in a neuronal circuitry mediating the drinking response or other AII induced effects. An investigation of the afferent connectivity of the supra optic nuclei (SON), the brain's primary site of anti-diuretic hormone (ADH) production, might help illuminate the mechanisms of control of this final common path in the conservation of body water balance. To study these connections we applied the axoplasmic tracing techniques of anterograde autoradiography and retrograde horseradish peroxidase (HRP) histochemistry. As reported previously (Neurosci Abst. 3:484, 1977), the SFO projects to the nucleus medianus, the organum vasculosum of the lamina terminalis (OVLT) and to the SON bilaterally. The SFO projection to the SON was confirmed by injections of HRP into the SON and subsequent observation of labelled bipolar neurons 10-18µ in width in the SFO (Anat. Rec. 190:538, 1978). There are afferents to the SON from the OVLT and NM as well. These connections involve the SON in a circuit with structures known to be involved with drinking behavior. We now report additional forebrain projections to the SON from the circularis and paraventricular nuclei of the hypothalamus. These nuclei are moderately labelled with HRP reaction product granules suggesting light projections. The medial and triangularis nuclei of the septum, the nucleus of the diagonal band, the thalamic periventricular and parataenial nuclei also have retrogradely labelled neurons following HRP injections into the SON. However, the septal and thalamic nuclei, but not the hypothalamic nuclei, are also labelled when the HRP injections miss the SON and involve only the adjacent ventral lateral hypothalamus. The anatomical links demonstrated in these studies may subserve the physiological function of AII in the central control of water balance.

Supported by RR 546414, RR07083 and NS 06716-11.

550 NALOXONE INHIBITION OF OVEREATING IN GENETICALLY OBESE MICE C57BL/6J ob/ob. Beatriz Moisset* and David L. Margules. Psychol. Dept., Temple University. Philadelphia, PA 19122.

The genetically obese mouse ob/ob shows a syndrome of marked hyperphagia, obesity, mild hyperglycemia and hyperinsulinemia. This syndrome is caused by a single autosomal recessive gene (Coleman and Hummel, Diabetologia 9: 287, 1973). Obese mice present pituitary levels of ACTH 14 times higher than lean controls at 16 weeks of age (Edwardson and Hough, J. Endocr. 65: 99, 1975). ACTH and β-endorphin are released concomitantly in the rat (Guillemin et al. Science 197: 1367, 1977) suggesting that the obese mouse may have abnormally high levels of β-endorphin in the pituitary. Intrahypothalamic injections of β-endorphin cause an increase in food intake in the rat (Grandison and Guidotti, Neuropharmacology 16: 533, 1977) suggesting that some forms of hyperphagia may be due to excessive production of β-endorphin. If this is so then the opiate receptor blocker, naloxone, would be expected to reduce food intake more markedly in obese mice than in lean controls. Food intake during the first hour after 20 hours of food deprivation was measured following injections of saline, 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 mg/kg s.c. of naloxone hydrochloride in C57BL/6J ob/ob and their lean littermates. Obese mice ate more than the lean littermates after saline injection (1.54 ±0.07g and 1.10±0.05g, respectively). Food intake was selectively depressed in ob/ob mice with doses of naloxone as low as 0.25 mg/kg. Lean mice also showed a reduction in food intake but this was significantly smaller at all doses, except for the lowest one. The present experiment suggests that β-endorphin may be involved in the regulation of food intake and that hyperphagia in the genetically obese mouse may be associated with excessive β-endorphin.

Supported by Grants to David L. Margules from the Alcohol, Drug Abuse and Mental Health Administration (MH-19438) and from The National Science Foundation (BNS77-22630).

551 COMPARISON OF NOREPINEPHRINE AND OTHER SELECTED APPETITE STIMULANTS ON CIRCADIAN REGULATION OF FEEDING IN RATS. W.R. Pfister*, M.T. Lowy*, G.K.W. Yim. Dept. Pharmacol. and Toxicol. Purdue Univ., West Lafayette, IN 47907.

We have recently reported that chlordimeform (CDM), librium, clonidine, and cyproheptadine increase food intake in non-food deprived rats (NFD) (Yim et al., Fed. Proc. 37(3): 860, 1977). Increased consumption of milk induced by norepinephrine (NE) (Margules et al., Science 178: 640, 1972) and of dry food induced by theophylline (Sakata et al., Eur. J. Pharmacol. 19: 318, 1972) has been attributed to their ability to produce a phase shift in circadian feeding rhythm. This possibility was further explored by examining the effects of these agents on dry food intake when they were administered during the day vs at night. In NFD rats, NE (20 ug/5 ul; i.v.c.), clonidine (0.025 mg/Kg, i.p.), librium (40 mg/Kg, i.p.), CDM (10 mg/Kg, i.p.), theophylline (20 mg/Kg, i.p.), and cyproheptadine (10 mg/Kg, i.p.) increased daytime 3 hr food intake by 176, 293, 657, 570, 458, and 312 percent (control = 2.8 ± 0.5 g/Kg). With the exception of the 20% decrease observed with clonidine, 24 hr intakes were unaffected. In food deprived (FD) rats, the appetite stimulants increased 3 hr daytime food intake by 15-50 percent, except librium which decreased intake by 44 percent (control = 16.3 ± 2.5). In NFD rats tested at night, the same doses of NE, clonidine, CDM, theophylline, and cyproheptadine increased 3 hr food intake by 27-47 percent (controls = 7.3 ± 0.1 g/Kg). These results suggest that the increased daytime feeding induced by these agents (excepting librium) is not a result of their ability to produce a phase shift in the circadian feeding rhythm of the rat. Supported in part by grants from NIH (NS 12077, 5-R01CA17482-09 and EPA (R-803965).

552 THE ROLE OF SOMATOSTATIN (SRIF) IN THE CONTROL OF FEEDING. PART II: EFFECT ON FOOD INTAKE AS A FUNCTION OF METABOLIC CONDITION AND VISCERAL INNERVATION. M. Rezek, V. Havlicek, Sheu-Lun Lee* and H. Friesen*, Dept. of Physiology, U. of Manitoba, Winnipeg, Manitoba, Canada.

In Part I (Rezek et al., Federation Proceedings, 1978) we reported the differential effect of SRIF on food intake (FI) as a function of the dose and infusion site. Intraperitoneal infusions of 50ug and 100ug were strongly anorexigenic while the same doses of SRIF passed as a bolus through the hepatic-portal (HP) circulation produced a brief dose-dependent stimulation of FI. Present experiments examined the mechanism of this differential effect of SRIF on FI. It was found that HP infusions of SRIF retained their stimulating effect when administered during the period of nocturnal overeating or following cyclical food deprivation although the increases of the amount eaten were smaller than in the free-feeding animals or during the diurnal feeding period. On the other hand, stimulation of FI by HP bolus infusions of SRIF was reduced or eliminated in vagotomized animals, in reversibly diabetic animals (mannoheptulose-250 mg/kg) or following the infusion of endocrinologically less active analog of SRIF (Ala^8-SRIF). These results suggest that both the neural and endocrine components are necessary for the expression of this response. In contrast to the stimulating effect of HP bolus infusions of SRIF, the prolonged 3 hr SRIF infusions (300ug, 100ug/hr) produced a significant ($p < 0.05$) and long lasting suppression of FI without producing general behavorial depressions. This effect was preserved following repeated prolonged infusions of SRIF performed on alternate days or during the period of nocturnal insulin-dependent overeating. On the other hand, our preliminary results with the HP infusion of anti-SRIF serum (with binding capacity in excess of estimated endogenous content of SRIF) showed that following a temporary suppression (1st postinfusion hour) these infusions slightly facilitated FI as indicated by elevation of total daily intakes (11%).

Supported by MRC Canada.

553 GLUCOSE FAILS TO SUPPRESS 2-DEOXYGLUCOSE-INDUCED FEEDING WHEN INFUSED AFTER THE ONSET OF GLUCOPRIVATION. R.C. Ritter and M. A. Miller*. Dept. Veterinary and Comparative Anatomy, Pharmacology and Physiology, Coll. Vet. Med., WSU, Pullman, WA 99164.

Feeding in response to 2-deoxy-d-glucose (2DG) or insulin is presumed to be a response to ongoing cerebral glucoprivation. If ongoing glucoprivation is the adequate stimulus for 2DG- and insulin-induced feeding, then one might predict that (1) feeding should persist as long as glucoprivation persists and (2) the stimulus to eat should subside when glucoprivation abates. However, recent work from our lab (Am. J. Physiol. 1978) has shown that rats eating in response to 2DG or insulin stop eating within 2 hrs after injection, when other signs of glucoprivation are near maximal. Furthermore, if food is withheld for 6 to 8 hrs, until physiological signs of glucoprivation have disappeared, rats still eat significantly more than they do under control conditions. We have hypothesized that 2DG or insulin-induced feeding triggered by some persistent biochemical change which outlasts glucoprivation itself and which is not immediately reversed simply by restoring glucose availability postglucoprivically. If this hypothesis is correct, it should be possible to suppress 2DG-induced feeding by infusing glucose concurrently with 2DG but not by infusing glucose after 2DG-induced glucoprivation had been allowed to occur. Adult male rats implanted with chronic intracardiac catheters were infused with 2DG (200 mg/kg) or equiosmolal saline at 8 AM. When food was immediately available 2DG caused a significant increase in food intake (5.7 ± 0.5 g) over saline control conditions during a 2-hr test. If food was withheld for 7 hrs post 2DG, the rats still ate significantly more (5.6 ± 0.7 g) than under control conditions. Infusions of 4 cc of 12.5% glucose given over 15 mins at 5 min, 1, 2 and 3 hrs post 2DG suppressed feeding (1.4 ± .6 g) when food was returned 7 hrs post 2DG. However, when identical glucose infusions were made 4, 5, 6 and 7 hrs post 2DG, feeding in response to 2DG was not suppressed (4.7 ± 0.6 g). Our data show that (1) 2DG-induced feeding may occur after other signs of cerebral glucoprivation have vanished, (2) 2DG-induced feeding can be prevented by exogenous glucose only if glucose is infused in such a way that may compete with 2DG and prevent glucoprivation from occurring, and (3) glucose infusions made after exposure to and recovery from glucoprivation are insufficient to suppress the stimulus to eat. These data support the suggestion that 2DG-induced feeding is mediated by a biochemical change which persists after restoration of glucose availability.

554 ABLATION OF PERIVENTRICULAR TISSUE SURROUNDING THE ANTEROVENTRAL THIRD VENTRICLE (AV3V) BLOCKS DRINKING TO CAVAL LIGATION BUT NOT RENIN RELEASE. E.E. Shrager and A.K. Johnson (SPON: M.Z.M. Ibrahim). Dept. Psychology, Univ. Iowa, Iowa City, IA. 52242.

Lesions of AV3V periventricular tissue result in acute adipsia without aphagia; animals which regain ad lib water intake demonstrate chronic deficits in the drinking response to exogenous angiotensin II and/or hypertonic saline (Johnson & Buggy, Am. J. Physiol. 234:R122, 1978; Buggy & Johnson, Am. J. Physiol. 233:R44, 1977). Further, these animals do not become hypertensive or increase water intake following the Grollman procedure for 1-kidney hypertension (Buggy, Fink, Johnson & Brody, Circ. Res. 40:I-110, 1977).

Animals which have recovered from the acute effects of AV3V lesions drink normally in response to 24hr water deprivation but fail to increase water intake in the 4hr following vena caval ligation.

Determinations of plasma renin concentration (PRC) prior to and following water deprivation indicate that AV3V lesioned rats have significantly elevated basal PRC's despite concurrent hypernatremia. Nevertheless, following 24hr water deprivation, lesioned animals show further increases in renin release comparable to those demonstrated by sham-lesioned animals.

These results suggest that the failure of AV3V lesioned animals to develop renal hypertension and concommitant increased water intake is mediated not by a failure of renin release but rather, an inability to respond to increased circulating levels of angiotensin II.

555 CHOLECYSTOKININ ACTS AT A VAGALLY INNERVATED ABDOMINAL SITE TO ELICIT SATIETY. Gerard P. Smith and Brian J. Cushin*. Dept. Psychiatry, Cornell Univ. Med. College and E.W. Bourne Behavioral Res. Lab., The New York Hospital, White Plains, N.Y. 10605.

The intestinal hormone cholecystokinin (CCK) and its synthetic C-terminal octapeptide (CCK-8) have a potent satiety effect (Gibbs et al, 1973). The site where CCK acts to elicit this effect is unknown. Since the size and peptide nature of CCK and CCK-8 make it improbable that circulating CCK or CCK-8 acts directly on brain sites protected by the blood brain barrier, we have investigated the possibility that CCK-8 acts at a peripheral site innervated by the abdominal vagus. After overnight food deprivation, three groups of rats (intact n=8, bilateral vagotomized n=8 and unilateral vagotomized n=8) were administered CCK-8 (10, 20, 40, 80, 160 and 640 $U\text{-}kg^{-1}$, i.p.) 15 minutes before a test meal of 25% E.C. 116 liquid diet. The threshold dose for significant inhibition of food intake was 640 $U\text{-}kg^{-1}$ in bilateral vagotomized rats, 40 $U\text{-}kg^{-1}$ in unilateral vagotomized rats and 10 $U\text{-}kg^{-1}$ in intact rats. Since bilateral vagotomy (hepatic branch preserved) abolished the satiety effect of doses of CCK-8 < 640 $U\text{-}kg^{-1}$, we conclude that under these conditions low doses of CCK-8 act to elicit satiety at an extra-hepatic abdominal site innervated by the vagus nerve.

Supported by NIH Grants MH15455, AM17240 and MH00149.

556 ANTICIPATION OF 24 HR FEEDING SCHEDULES IN RATS WITH LESIONS OF THE SUPRACHIASMATIC NUCLEUS.
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Anticipatory wheel running in response to restricted feeding schedules was studied in rats with lesions of the suprachiasmatic nucleus (SCN) and in sham operated controls. Despite the absence of circadian periodicity in free feeding conditions, rats with SCN lesions anticipated restricted access to food at 24-hour intervals in the presence of a light-dark cycle and in constant light. Neither rats with SCN lesions, nor controls were able to anticipate feedings at 18-hour intervals. Adrenalectomy did not prevent anticipatory activity to a 24-hour feeding schedule in either group. These results suggest that circadian oscillators outside SCN can be entrained by restricted feeding schedules or, alternatively, that anticipatory activity is based on a clock which operates on the hourglass principle; i.e., a clock which requires daily resetting.

(Supported by NIH Grants GM21728 and MH 11218)

557 INTRAHYPOTHALAMIC INJECTIONS OF KAINIC ACID PRODUCE DEFICITS IN FEEDING AND DRINKING DURING ACUTE HOMEOSTATIC IMBALANCES.
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Large electrolytic lesions of the far lateral hypothalamic (LH) area of rats produce aphagia and adipsia. Even when voluntary ingestive behaviors return, after several weeks or longer, rats sustaining such lesions do not eat or drink normally in response to acute treatments producing severe glucoprivation or dehydration. It had been traditional to interpret these observations as indicating the presence of feeding and drinking centers within the ventral diencephalon. However, it is now known that LH lesions interrupt dopamine-containing neurons ascending from the mesencephalon to the forebrain, and that specific destruction of those neurons by intracranial administration of the neurotoxin 6-hydroxydopamine reproduces the severe initial impairments, gradual recovery of function, and residual deficits that characterize animals with LH lesions. These and other recent findings have shifted attention away from the hypothalamus in discussion of the central control of feeding and drinking, and suggested that LH lesions are effective because they disrupt nonspecific activational components of hunger and thirst (and other sensations) that are prerequisites for motivated consummatory behavior. In order to further examine this hypothesis we have studied the behavior of rats given intrahypothalamic injections of kainic acid, a rigid analogue of glutamate that appears to destroy neurons with cell bodies near the injection site while sparing fibers of passage. Kainic acid was dissolved in 0.9% NaCl (1.14 µg/.57 µl) and administered over 60 sec to 10 rats. Controls received an equivalent injection of vehicle. Rats given kainic acid showed a brief aphagia and adipsia (2-3 days) after which voluntary ingestive behaviors resumed. Nevertheless, feeding and drinking in response to acute regulatory challenges was greatly impaired in most animals. Of 10 rats given kainic acid that were tested, the rapid drinking response to acute cellular dehydration (1 M NaCl, 5 ml, i.p.) was found to be abolished in 4 animals and impaired in 5 others, the gradual drinking response to acute hypovolemia (30% PEG, 5 ml, s.c.) was abolished in 6 animals and impaired in 1 other, and the feeding response to acute glucoprivation (2-deoxyglucose, 750 mg/kg, i.p.) was abolished in all 10 animals. Control rats responded normally to these treatments. The concentration of dopamine and norepinephrine in whole brain was not significantly affected by kainic acid. The fact that functional deficits were obtained in the absence of damage to central catecholaminergic fibers suggests that cells in the LH area may indeed play a role in the control of ingestive behaviors.
(Supported, in part, by NIMH grants MH25140, MH20620, and MH00058.)

558 THE EFFECT OF THE METABOLITE GLYCEROL ON FOOD INTAKE PATTERNS AND BODY WEIGHT IN RATS.
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Levels of glycerol are correlated with adipose tissue mass and cell size. Glycerol has thus been proposed as a humoral signal to the brain representing the state of fat storage (Bray, 1976).

This study measured the effects of the infusion of near physiological doses of glycerol (12 and 24 mg/day) on body weight and 24 hour food intake patterns in six-month-old male Sprague Dawley rats. Using a computerized continuous data collection system, 24 hour feeding patterns (meal size, number, interval and duration) of 6 rats were monitored. Rats were initially adapted to the feeding chamber and to the powdered lab chow diet. Baseline food intakes and weight data were collected for 5 days on a 12 hour light/dark cycle. Rats were then implanted with Alzet Mini-pumps which delivered a continuous subcutaneous infusion of a glycerol solution at 1 µl/hour for 170 hours.

There were no changes in body weights over the 7 day infusion period. Total food intake during the light phase was decreased in a dose related fashion, while dark phase intake showed compensatory increases. The decrease in light phase intake was accomplished primarily by decreased meal frequency while meal size remained unchanged. These data suggest that the metabolic effect of glycerol interacts with circadian patterns of feeding in rats.

Other investigators have reported (Wirtshafter & Davis, 1977) that reductions in body weight following injections of glycerol are a function of both dose and time of administration. Additionally, (Kraly & Smith, 1978) have reported the magnitude of the satiety effect of cholecystokinin is also dependent on the time of administration relative to the light/dark cycle. These studies, in conjunction with the present study, suggest that the controls of food intake are altered with changes in photo period. Thus continuous analysis of 24 hour meal patterns may show a unique sensitivity to pharmacological and metabolic manipulations of feeding.

(Research supported in part by NSF grant BNS 76-09957 and New York State Health Research Council grant 1202).

559 SEPARATE OSCILLATORS GOVERN ULTRADIAN ACTIVITY, FEEDING, AND DRINKING RHYTHMS AFTER SUPRACHIASMATIC NUCLEUS LESIONS.
Walter N. Tapp*, David C. Bird*, and Frank A. Holloway. Dept. Psychiatry and Behavioral Sciences, Univ. of Okla. Health Sciences Center, Oklahoma City, Ok 73190.

The suprachiasmatic nucleus of hypothalamus (SCN) appears to be a key element in the control of circadian rhythms in mammals. The presence of circa 8 h and circa 12 h rhythms in the locomotor activity spectrum of SCN-lesioned hamsters (B. Rusak, J. Comp. Physiol., 118:145-146, 1977) has been suggested to reflect mutual coupling of self-sustaining oscillators outside the SCN. It is not known if other functions exhibit such ultradian rhythms after SCN lesions or if such rhythms would be synchronized with activity.

Rats with bilateral, radiofrequency SCN lesions (n=10) were compared with unoperated controls (n=6) on activity, feeding, and drinking patterns. Both groups were maintained in constant dim red illumination. Control rats free-ran with periods ranging from 23.4 h - 28.7 h. In some controls the free-running period of activity, feeding, and drinking were different reflecting internal desynchrony.

Spectrum analysis revealed circa 8 h or circa 12 h (but not circa 24 h) components in the activity rhythms of most SCN lesioned rats. Food and water intake displayed similar circa 8 h or circa 12 h rhythms. The ultradian periods of feeding, drinking, and activity components were not always the same within individual lesioned rats. Ultradian feeding, drinking, and activity rhythms generally were not phase-coordinated.

The presence of circa 8 h and circa 12 h rhythms of feeding, drinking, and activity in SCN rats may reflect mutual coupling of oscillators outside the SCN. But the present results suggest that separate oscillators govern each function, interacting only weakly.

560 EFFECT OF THIRD VENTRICULAR INFUSIONS OF HYPERTONIC SOLUTIONS ON THIRST IN THE DOG. Terry N. Thrasher*, Richard G. Jones* and David J. Ramsay (SPON: I. Reid). Dept. Physiol., Sch. Med., University of California at San Francisco, CA 94143

A controversy exists with regard to the mechanism by which an increase in the effective osmotic pressure of extracellular fluid stimulates thirst. The osmometric hypothesis of thirst is based on Verney's (Proc. Roy. Soc. Lond. Ser. B, 135, 25-106, 1947) suggestion that increases in extracellular fluid osmolality cause withdrawal of water from sensitive receptor cells within the hypothalamus and lead to vasopressin release. Alternatively, Andersson and colleagues(Am.Scientist, 59, 408-415, 1971) have proposed the existence of receptors sensitive to the concentration of sodium in cerebrospinal fluid (CSF). As a test of the Andersson mechanism we infused 5 conscious dogs with hypertonic equiosmolar (1.7 Osm/L) solutions of NaCl, sucrose, glucose, urea or isotonic NaCl iv and measured the change in CSF sodium conc. at the thirst threshold or arbitrarily, after 45 min. Hypertonic NaCl or sucrose, which do not penetrate cell membranes, stimulated thirst and increased CSF sodium conc. by 1.6±0.3 and 1.2±0.3 mEq/L respectively. Hypertonic glucose or urea, which do penetrate cell membranes, did not stimulate thirst but did increase CSF sodium conc. by 1.4± 0.4 and 2.6±0.5 mEq/L respectively. Isotonic NaCl did not stimulate thirst or alter CSF sodium conc. significantly. Since all hypertonic solutions elevated CSF sodium conc., but only non-penetrating solutes stimulated thirst, these data indicate that an increase in CSF sodium conc. is not sufficient to stimulate drinking.

As a more direct test of the sodium receptor mechanism, we decided to increase the CSF osmolality by intracerebroventricular infusions of different solutes. Six dogs were prepared with chronic third ventricular cannulae. Each was infused with hypertonic, equiosmolar (0.2 Osm/L) solutions of NaCl, sucrose or glucose in artificial dog CSF for 45 min at 17 µL/min. The volume of water drunk during the infusion was measured and compared to the volume drunk during infusion of artificial CSF alone. Infusion of artificial CSF produced insignificant drinking. Infusion of hypertonic solutions containing NaCl or sucrose stimulated significant drinking amounting to 369±139 and 113±56 ml respectively. However, infusions of hypertonic glucose did not produce significant drinking. The only solutes which stimulated drinking were those which do not penetrate cell membranes and thus cause osmotic withdrawal of water. Furthermore, sucrose infused centrally initiated drinking without increasing CSF sodium conc. Therefore, we conclude that in the dog, thirst induced by increased extracellular fluid osmolality is mediated by an osmoreceptor mechanism.

Supported by USPHS grant AM-06704.

561 RAPHE NEURON FIRING RATE AND SEROTONIN RELEASE VERSUS SEROTONIN BIOSYNTHESIS AS MODULATORS OF FOOD INTAKE IN THE RAT. Susan B. Weinberger* and Arnold J. Mandell. Dept. Psychiatry, Sch. Med., UCSD, La Jolla, CA 92093

In the search for understanding the role of the serotonergic neurotransmitter system in appetite regulation, earlier studies by several investigators have suggested a reciprocal relationship between food intake and brain serotonin levels. However, interpretation of such reports is made more complex by our recent finding that graded doses of tryptophan that more than doubled brain serotonin synthesis in the rat failed to alter food consumption. These data, along with other studies in our laboratory involving both quantitative microhistofluorescent examination of intracellular and extracellular single raphe cell body serotonin levels and regional brain patterns of tryptophan, serotonin, and 5-hydroxyindoleacetic acid (5-HIAA), have led us to the hypothesis that information encoded by firing rate and serotonin release, rather than by serotonin biosynthesis, may be the prepotent serotonergic factor in the modulation of appetite. Using a number of agents active on the serotonin system we have begun evaluating this hypothesis by studying the relative contributions of these variables to the control of food intake.

We have found that the serotonin uptake blocker fluoxetine produces a dose-dependent decrease in food consumption. Similarly chlorimipramine (CMI), which arrests raphe cell discharge and induces an increase in tryptophan hydroxylase activity, reduces food intake in rats food-deprived for 24 hours. However, CMI's dose-dependent reduction of appetitional behavior is partially reversed by tryptophan, which increases serotonin synthesis, when both drugs are administered simultaneously. Changes in patterns of the whole brain levels of tryptophan, serotonin, and 5-HIAA induced by these various drugs and combinations, along with the appetitional behavior data, suggest two possible mechanisms of serotonin's modulatory action on appetite: 1) the serotonin cells may act from outside the primary appetite informational circuit to modulate transmission along this pathway by altering the synthesis of serotonin, or 2) some serotonin cells may themselves be contained within the primary pathway for the neuronal representation of appetitional behavior and may modulate appetite by variations in neuronal discharge rate and neurotransmitter release without an increase in serotonin synthesis.

This work is supported by DA-00265-07

562 THE EFFECTS OF COPULATION ON FEEDING AND RELATED PROCESSES IN THE MALE. Freya A. Weizenbaum. Dept. of Psych., Va Polytech. Inst. & State University, Blacksburg, VA 24061.

Feeding behavior, body weight and carcass composition were monitored in males that copulated regularly (Group Sexual Activity [SA]) and in males that were not copulating (Group Sexual Rest [SR]). Sexually inexperienced males were screened for sexual vigor. The 12 males that were selected were divided into two groups, one group (Group SA) mated twice each week (1 ejaculation/test). The other 6 males comprised the second group (Group SR), which did not mate during the following 6 weeks. At the end of the 6 week period, body weight and carcass composition were compared. Group SA males had lower body weights and fat levels than the Group SR males. Examination of food intake patterns of the 2 groups revealed that food intake was depressed in Group SR males during the 24 hours following copulation. Since Group SR males were not permitted access to food during the periods when Group SA males were mating, but did not show a decrease in intake on those days, these findings indicate that the copulatory situation caused the change in feeding behavior body fat levels, and body weight.

In the second study the effect of the social component of the sexual situation was assessed. The overall procedure was the same as in the first study with one modification. Individual SR males were yoked to SA males, so that while each SA male was mated his yoked SR male was paired with an ovariectomized female. Both SR and SA males decreased food intake during the 24 hours after pairing with a female. These results suggest that the decrease in feeding was related to social aspects of the copulatory situation. However there were also indications that the effects of pairing a male with an estrous female were different from pairing with an ovariectomized rat. The SR males tended to show less of a decrement in feeding behavior than did SA males. The results of these two experiments suggest that social interactions can alter regulatory processes in the male. Other have shown that testosterone, (Science 189: 1104, 1975) a hormone which is known to alter food intake in males (J. Comp. Physiol. Psych. 90: 18, 1976), and testosterone-sensitive tissues (Physiol. Behav. 9: 401, 1972), are increased following exposure to females. The present studies extend the previous experiments concerned with the effect of the female on reproductive functions in the male because the present studies indicate that non-reproductive, testosterone-sensitive processes are also altered by exposure to female conspecifics.

563 REINSTATEMENT OF SPECIFIC COMPONENTS OF SUCKLING BEHAVIOR BY METHYSERGIDE IN YOUNG RATS. Christina L. Williams*, W. G. Hall, and Jay S. Rosenblatt* (SPON: B. R. Komisaruk). Inst. Anim. Behav., Rutgers Univ., Newark, N. J. and N. Carolina Dept. Mental Health, Raleigh, N. C.

Previous studies have demonstrated that suckling behavior of infant rats is not an unmodifiable reflex but that many of its components develop and change prior to weaning. Before 15 days of age pups will continuously suckle regardless of the amount of food in their stomachs, and thus cannot directly control their intake. After 15 days of age, pups will initiate suckling only after food deprivation and thus can control their food intake.

We have recently found that pharmacological manipulations of the serotonin system can alter the initiation component of suckling. For example, treatment with the putative 5-HT receptor blocker methysergide reinstates suckling in nondeprived pups older than 15 days. If suckling were a unitary behavioral system, then this pharmacological manipulation should similarly reinstate other components of suckling commonly displayed by pups younger than 15 days of age.

In order to test the effects of methysergide on the components of suckling involved in milk intake, we utilized an intraoral cannula which opens onto the back of the pup's tongue. With this technique, diet can be delivered to a pup while it is suckling from the nipples of its anesthetized dam. Saline- and methysergide-treated (20 mg/kg), 20-day-old littermates were tested in this manner.

We found that methysergide altered only some of the behavior patterns associated with suckling for milk. For example, all the drug-treated pups, but none of the saline controls, displayed the dramatic extensor reflex during milk ingestion which is normally shown only by pups younger than 15 days of age. Similarly, at the termination of milk intake, only the drug-treated animals could be induced to reattach by placing them at a nipple, a behavior normally shown only by pups less than 15 days of age. We found, however, that this dose of methysergide did not alter the pups' control of milk intake. Although pups younger than 15 days cannot control their intake, the drug-treated pups, like the saline-treated pups controlled their food intake, i.e. consumed moderate amounts of diet before leaving the nipple (Methy: 6.4± 0.6% body wt.; Sal: 6.8± 0.3%, n=8).

Thus, these data suggest that the neurochemical systems which control the initiation of suckling and the ingestive reflex may be different from those which control food intake and the termination of suckling or may be more refractory to manipulation by methysergide.

564 A Comparison of Ventral Noradrenergic Bundle and Ventromedial Hypothalamic Lesion-Induced Hyperphagia and Obesity. Robert M. Zacharko* and Thomas B. Wishart. Dept. Psych., University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

Gold (1970) reported that lesions restricted to the ventromedial nucleus (VMH) were ineffective in producing hyperphagia and obesity in rats. Gold argued that hyperphagia is produced only if the lesion extends beyond the VMH itself, and suggested a role for the ascending noradrenergic bundle (VNB) in mediating "satiety". Ahlskog (1973) subsequently reported that damage to the VNB with the catecholamine neurotoxin, 6-hydroxydopamine, produced hyperphagia and obesity in rats. In the present study we compared the ingestive behaviors of VMH and VNB animals under both drug and control conditions. Male rats were given lesions confined to the VMH (1 mA, 12 sec) or the VNB at the level of the oculomotor nerve (.8 mA, 5 sec). Immediately after surgery, rats with lesions of the VMH consume food so rapidly that gagging, retching and (sometimes) death ensue. If water, but not food, is available these animals will drink to the point of water intoxication death (Wishart and Walls, 1975). In marked contrast, VNB rats were observed to consume significantly less food and water than did controls during the first 3 hours after recovery from the anesthetic. However both VMH and VNB rats were hyperphagic and gained more weight than did controls during the first 10 days after surgery. VNB rats were hyperphagic only during the dark cycle whereas VMH rats overate during both light and dark phases. When these rats were switched to a 25% corn oil diet mixed with mash, the hyperphagic tendency of the VMH and VNB rats was maintained, but again VNB rats were hyperphagic only during the dark phase. The three groups of animals made equivalent adjustments to the alteration in caloric density of the diet. At the end of this phase the body weights of VNB, VMH and control rats were 474, 458 and 418 grams respectively. During the first 3 hours after d-amphetamine (2 mg/kg), VNB animals did not reduce food intake as much as controls while VMH rats reduced their intakes below that of controls. In rats with VMH lesions, feeding was suppressed significantly below that of either VNB or intact rats during the subsequent 21 hour interval. Under fenfluramine (2 mg/kg) all three groups consumed equivalent amounts during both the initial 3 and subsequent 21 hour periods.

Thus, while both VMH and VNB lesions eventually result in obesity, the onset of hyperphagia occurs sooner in VMH rats, suggesting that the etiologies of the syndromes are different. Further, d-amphetamine differentially affects the two preparations indicating a catecholaminergic alteration only in VNB rats. Serotonergic systems however appear functionally intact in both VMH and VNB animals.

INVERTEBRATE NEUROBIOLOGY

565 BURSTING IN APLYSIA CELL R15 IS MAINTAINED BY THE TEMPORAL PROPERTIES OF MEMBRANE CONDUCTANCE, NOT ITS NEGATIVE RESISTANCE. William B. Adams and Irwin B. Levitan. Friedrich Miescher-Institut, P.O.Box 273, CH-4002 Basel, Switzerland.

Steady-state current-voltage curves of the cell membrane of R15 reveal a region of negative slope resistance between -20 and -50 mV which, it has been suggested, produces an instability that maintains bursting activity. This hypothesis presupposes that the cell can actually "see" the negative resistance during normal activity, i.e. that a small depolarization increases the inward current, thereby further depolarizing the cell. We have found that this is not the case. Current-voltage curves measured at short times following application of a voltage-clamp have positive slope resistances over most of the operating range of the cell, except during times just preceding a burst. Our data suggest a mechanism for burst production that resembles the classical description of action potential generation: A conductance which allows an inward current flow is activated by depolarization and subsequently inactivated as a result of the depolarization produced during the burst. The conductance differs from the fast sodium conductance in that it is partially activated at the resting potential, is orders of magnitude slower and is not blocked by tetrodotoxin. The largest inward (negative) currents are found near the midpoint of the burst, as expected from the large values of dv/dt observed between action potentials. As the clamp is left on, the currents become less negative with little change in the shape of the i-v curve, indicating inactivation of the inward current. During the interburst, hyperpolarizing clamps elicit inward currents which do not change with time, suggesting that the inward conductance is inactivated. Depolarizing clamps at mid-interburst elicit large outward currents which then become more negative as the inward current turns on. The negative resistance that appears near the end of the interburst may accelerate the onset of depolarization, thus enhancing activation of the inward current.

566 BIOCHEMICAL AND PHYSIOLOGIC HOMOLOGY BETWEEN THE APLYSIA EGG-LAYING HORMONE AND A FACTOR FROM THE ATRIAL GLAND. S. Arch, T. Smock, W. Frost, and S. Naughton*(SPON: G. F. Gwilliam). Biological Laboratories, Reed College, Portland, OR 97202

The atrial gland is a specialized structure associated with the rostral portion of the Aplysia reproductive tract. Upon microscopic examination it appears to have a secretory function. Previous attention to this gland has not resulted in assignment of a specific role in reproduction. We have found that the gland contains a water-soluble substance that will cause egg laying when injected into mature animals. The activity of the substance is sensitive to enzymatic proteolysis. Heretofore, the only substance known to evoke egg laying in this animal was the egg-laying hormone (ELH) of the bag cell neuroendocrine organs associated with the central nervous system. Since ELH is also proteinaceous, the possibility that the atrial gland factor (AGF) and ELH are related molecules was investigated. Our biochemical studies show AGF to be stable to boiling for 10 min, to be insensitive to exposure to 1% sodium dodecyl sulfate, 6M urea, and 0.35% Triton X-100, and to be precipitable from neutral aqueous solution by addition of $(NH_4)_2SO_4$ to 40% final concentration. Gel filtration chromatography[2] shows the substance to have a molecular weight in the range 5000-8000. Gel isoelectric focusing of atrial gland homogenates shows the active substance to be located at two positions (pH 7.4 and pH 9.3). Thus, these investigations have shown AGF to have properties identical to those of ELH, with the exception that the neutral band of activity on isoelectric focusing is not found in the bag cells. Our physiologic studies have revealed identity of action of the two substances. Gel filtration purified samples containing egg-laying activity from both bag cell and atrial gland preparations stimulate the Aplysia heart in vitro, cause stereotypic egg-laying behavior, and markedly raise the threshold for elicitation of feeding activity. Moreover, extracts of the atrial gland cause clear and long-lasting changes in the electrical activity of at least 5 putative motor neurons in the buccal ganglion of Aplysia. This last result is consistent with the findings by D. Stuart (Caltech Ann. Report, Biology, p. 102, 1977) when ELH-containing preparations are applied to the same ganglion. In sum, the close correspondence between the molecular characteristics and physiologic actions of AGF and ELH suggest a striking homology between these substances derived from two distinct tissue types. Until further study reveals the physiologic regulation of AGF secretion the unique role assigned to the bag cell neurons in the control of egg laying must be considered to be in doubt.

567 INSIGHTS INTO THE MORPHOLOGICAL BASIS OF PRESYNAPTIC FACILITATION IN THE GILL-WITHDRAWAL REFLEX OF APLYSIA: ANALYSIS OF THE FINE STRUCTURE OF A MODULATORY SYNAPSE. C.H. Bailey,* E.B. Thompson, M. Chen,* and R. Hawkins. Division of Neurobiology and Behav., Depts. of Physiol. & Anatomy, Columbia Univ., College of P&S, New York, N.Y. 10032.

Hawkins et al. (1976,1977) have identified a group of neurons (L28, L29) in the abdominal ganglion of Aplysia which produce presynaptic facilitation of the EPSPs at synapses between the sensory and motor neurons of the gill-withdrawal reflex. It has been postulated that these facilitatory neurons may be serotonergic. The structure of the synapses made by these facilitating neurons on sensory neurons is interesting to study because the facilitators may produce their actions on transmitter release via a serotonin-stimulated second messenger (cAMP) that triggers a voltage sensitive Ca^{++} conductance (Klein and Kandel, 1978).

Whereas the structure of synapses mediating conventional synaptic potentials is well understood, little is known about the morphology of modulating synapses. In order to analyze the ultrastructure of both the conventional and the modulatory synapses of facilitatory neurons, we have injected L29 with horseradish peroxidase (HRP) to identify its neurites and synapses. L29 both makes and receives conventional synapses, which have active zones typical of Aplysia neurons. The most frequent vesicle type in the presynaptic terminals of L29 is relatively electron lucent and has a mean long axis of 68 nm ± 20 nm S.D. A subpopulation of vesicles (mean long axis 114 nm ± 29 nm S.D.) contains a small inner core of variable density. The morphology of the vesicles in L29 terminals is similar to that of vesicles in terminals of a serotonergic neuron (GCN) in Aplysia described by Schwartz et al. (1978). Both the serotonergic vesicles of the GCN and the vesicles of the facilitators resemble vesicles found by Bailey et al. (1976,1978) in terminals which synapse onto HRP-labeled sensory neuron processes.

The exact relationship of these facilitating synapses to the terminals of the sensory neurons can be further studied with double label experiments (Thompson et al., 1976, 1978) in which HRP is injected into the sensory neuron and ^3H-serotonin into the facilitator. These experiments would also provide a further test of the serotonergic nature of the facilitator.

568 THE SALIVARY BURSTER OF LIMAX MAXIMUS: A PRESUMPTIVE SENSORY-MOTOR NEURON. Barbara Beltz* and Alan Gelperin. Dept. Biology, Princeton University, Princeton, New Jersey 08540.

The salivary burster (SB) is an autoactive motor neuron which innervates the musculature of the salivary duct (Prior and Gelperin, J. Comp. Physiol. 114: 217-232, 1977). Feedback from salivary duct stretch modulates SB activity. Experiments reported here indicate that the SB itself is stretch sensitive. Sensory input from stretch sensitive processes is believed to modulate motor output at different branches of the same cell.

Microliter injections of saline are delivered into the salivary duct via a cannula placed in the lumen of the duct. These injections expand the duct less than the maximal amount of duct expansion measured in vivo. Salivary nerve activity is monitored while the duct is stretched by intralumenal saline injections.

In a preparation consisting of cerebral and buccal ganglia plus salivary nerves and ducts, a single stretch of the duct elicits a burst of activity from the SB with a fixed latency. This response persists during treatments which block chemical synapses in this preparation (.006 x Ca^{++}, 5.25 x Mg^{++} saline, cooling to 0°C).

When the salivary nerve and duct are severed from the buccal ganglion, so that the SB axon is separated from its cell body, the SB axon can still burst or fire tonically. In this isolated duct and nerve preparation, an injection of saline into the duct can modulate SB activity. The SB response to duct stretch in an isolated duct-nerve preparation is also unaffected by the treatments which block chemical synaptic transmission.

Another indication that the SB itself has the capacity to respond to duct stretch is the observation that in some preparations, in response to duct stretch, a single afferent spike is elicited in the SB before each SB efferent burst. This afferent spike phenomenon demonstrates that the SB does possess a region peripherally which is sensitive to duct stretch, and which can spike.

The stretch reflex which we have characterized is thought to be mediated by a single cell. We propose, therefore, that the SB is a neuron with both sensory and motor capabilities.

(Supported by NSF grant BNS 76-18792 and NIH training grant 5 T01 MH13445.)

569 ANATOMY OF A COCKROACH EQUILIBRIUM RECEPTOR. Lisa P. Bennett and H. Bernard Hartman. Dept. Biol. Sci. Texas Tech University Lubbock, Texas 79409.

Insects are thought to lack a mechanism for directly detecting spatial orientation. Instead, light cues, proprioception, and differential limb loading provide them with an indirect means for deriving positional information. Recently, Fraser (Nature 268:523, 1977) has shown by behavioral means that the cerci of Periplaneta americana function in equilibrium reception. Physiologic experiments in our lab indicate that two rows of pendulous sensilla found on each cercus of the burrowing cockroach, Arenivaga, provide equilibrium information.

The plumb bob-like shape of the modified sensilla, tricholiths, is ideally suited for position reception. Tricholiths are composed of a large dense sphere located distally on a slender shaft, the shaft being elliptical in cross section. The sensilla, keeping a constant relationship with respect to the gravitational force vector, are deflected upon the insect's movement away from its primary orientation. Only one buttress hinges each sensillum, a condition which allows nearly universal movement of the tricholith. However, direction of movement is restricted by the eccentric placement of the shaft in its cuticular cup. Each row of tricholiths can only be deflected, then, in a direction away from the midline of the cercus.

The tricholith inserts into an innervated socket, and the movement of the sensillum elicits a neural response in the receptor cell. Four interneurons are responsive to equilibrium information, each interneuron being driven by afferents from a specific row of tricholiths. Polar plots of the interneuron responses mimic the quadrants of movement for the rows of tricholiths.

Supported by National Science Foundation grant #BNS77-22283 and National Aeronautics and Space Administration grant #NSG-7435.

570 INTEGRATIVE FUNCTIONS OF PRIMARY SENSORY NEURONS IN THE BUCCAL GANGLIA OF THE OPISTHOBRANCH NAVANAX INERMIS. M. V. L. Bennett, D. C. Spray, M. E. Spira* D. H. Hall*, Div. of Cellular Neurobiology, Dept. Neuroscience, Einstein College of Medicine, Bronx, NY

Primary mechanosensory neurons of Navanax buccal ganglia innervate widely separate pharyngeal fields and make excitatory and inhibitory synapses on other sensory neurons and on buccal motoneurons controlling pharyngeal expansion. During high rates of discharge evoked tactilely or by electrical stimulation impulses may fail to invade the soma. Failures may occur at different distances from the soma in different branches. Studies with intracellular Lucifer Yellow CH or cobalt sulfide show that single sensory neurons can have one or several processes with branch points several cell diameters away which provides a number of possible sites of impulse failure. When failures occur impulses may or may not propagate between branches and summation of partial spikes can relieve block of propagation. Thus some information processing is possible within the single cells. The effects will depend on location of synaptic outputs and will also be modulated by synaptic inputs. Sensory PSPs are blocked by high Mg^{++}; IPSPs are inverted by Cl^- injection. Thus, chemical mediation is indicated. The buccal region innervated by a sensory neuron may have an excitatory or an inhibitory region neighboring or surrounding. Inhibitory fields generally are larger. Synaptic interactions between sensory neurons may mediate prolonged sensory firing observed after brief stimulation. Interplay of excitation and inhibition within the sensory pool may be responsible for patterning of motor activity. In any case the sensory neurons combine the functions of primary sensory elements and integrative interneurons. DCS is a McKnight Scholar.

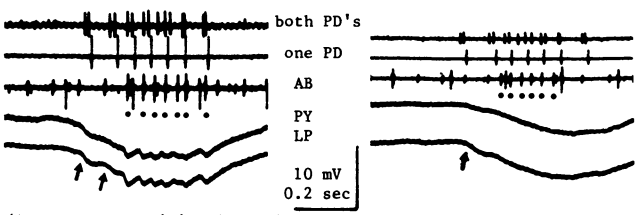
571 DISTINGUISHING TWO TYPES OF INHIBITORY SYNAPSES FROM PACEMAKER NEURONS IN THE PYLORIC SYSTEM OF THE LOBSTER STOMATOGASTRIC GANGLION. M. Bidaut*, D.F. Russell and D.K. Hartline (SPON: T.H. Bullock). Biology Dept. B-022, UCSD, La Jolla, CA 92093.

The pyloric rhythm is driven by 3 synchronously bursting neurons, the single AB interneuron and the two PD motorneurons, all of which make inhibitory synapses with the set of "pyloric follower" motorneurons. D.V. Gassie and D.K. Hartline (in preparation) have shown that ipsp's from the AB neuron have a rapid time course, rising sharply and being of brief duration, whereas ipsp's from PD neurons have a much slower time course, rising slowly and having a rounded waveform. We report, additionally, that picrotoxin in low concentrations (e.g. 2-5 x 10^{-7} M) rapidly blocks the fast-rise ipsp's from AB without stopping the rounded ipsp's from PD's. Microelectrodes were inserted in PY and LP "pyloric follower" neurons while monitoring PD firing from the pyloric dilator nerves and AB firng from the stomatogastric nerve. In Fig. 1, the first two ipsp's (arrows) were purely from doublets of PD spikes, since the AB did not fire. The subsequent AB spikes (dots) evoked 1:1 fast-rise ipsp's. Under picrotoxin, the summed inhibition of a pyloric follower neuron became very smooth, even when the neuron was polarized to various levels of membrane potential. In Fig. 2, the first doublet of PD spikes evoked a discrete ipsp (arrow), but the subsequent AB spikes (dots) no longer evoked fast-rise ipsp's (nerve records in Fig. 2 are at lower gain).

A parsimonious working hypothesis is that the fast-rise ipsp's from AB would be due to (fast) Cl^- channels associated with a picrotoxin-sensitive receptor, whereas the rounded ipsp's from PD's would be due to (slow) K^+ channels associated with a picrotoxin-insensitive receptor. Supported by NIH NS13138 to D.K. Hartline.

Fig. 1 - Control

Fig. 2 - Picrotoxin (5×10^{-7} M)



(Spontaneous activity in an isolated stomatogastric ganglion preparation.)

572 INKING IN APLYSIA: QUANTITATIVE ANALYSIS OF THE IONIC MECHANISMS UNDERLYING THE SELECTIVE SENSITIVITY TO LONG DURATION STIMULI. J. Byrne, Department of Physiology, School of Medicine, University of Pittsburgh, Pittsburgh, Pa., 15261.

Many neurons have unique biophysical characteristics which contribute to their integrative action and firing behavior. But given such a high degree of differentiation, the question arises as to what extent a cells specialized biophysical features quantitatively contribute to the features of the behavior which that cell mediates. This question can be examined using inking behavior in Aplysia as a simple test system.

The release of ink occurs selectively to long lasting stimuli since the initial synaptic input to the ink gland motor neurons is ineffective in firing the cells (Shapiro et al., this volumne). As a result there is a several second silent period or pause before the cells fire in an accelerating burst of spikes which causes the release of ink. Voltage clamp experiments were performed to determine the quantitative extent to which the motor neuron ionic conductances account for the firing pattern and thus the features of the behavior. Using pharmacologic separation techniques four voltage dependent ionic currents have been analyzed including: inward Na^+ and Ca^{++}, outward fast transient K^+ and delayed K^+ currents. Equilibrium potentials, steady state activation and inactivation and the respective voltage-dependent time constants were analyzed using standard single and double pulse techniques. The features of the voltage-dependent conductances appear qualitatively similar to comparable currents examined in other molluscan neurons. Leakage, capacitance and synaptic currents were also analyzed. The total membrane current for a single cell was formulated in terms of a modified Hodgkin-Huxley (1952) model with lst order activation and inactivation kinetics. The model also incorporates the features of the electrotonic coupling between the 3 ink gland motor neurons. A computer simulation revealed the cells selective response to long lasting stimuli is due to the fast transient K^+ current shunting the initial excitatory input. The high resting potential (-75mV) of the ink motor neurons insures the steady state inactivation of the fast K^+ current is essentially removed at the resting level. Inactivation of the fast transient K^+ current by the synaptic depolarization and a late increase in synaptic input contribute to further depolarizing the cells leading to the generation of an accelerating burst discharge which in turn causes the release of ink. The results indicate the feasibility of quantitatively relating the features of individual ionic conductance mechanisms in individual neurons to the features of the behavior which those cells mediate. Supported by NIH grants NS13511 and NS00200.

573 MODULATION OF THRESHOLD IN THE ESCAPE BEHAVIOR OF THE COCKROACH PERIPLANETA AMERICANA. Jeffrey M. Camhi and Susan Volman*. Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853.

The escape behavior of the cockroach Periplaneta americana is known to be evoked by gentle wind currents which excite filiform receptor hairs on the cerci (Camhi and Tom, 1978). The threshold for escape behavior was determined by directing controlled wind puffs at the cerci of restrained cockroaches which were free to make normal leg movements. The behavior consisted of anywhere from one rapid step to a prolonged running sequence, and generally resembled the escape behavior of unrestrained cockroaches (Camhi and Tom, 1978). As tested during, or within one second following, a bout of slow walking (stepping frequency 2-4/sec) the threshold was 1-5 millimeters/sec. This threshold generally did not increase during a period of up to four hours provided stimuli were presented no more often than once per two minutes, with several rest periods of 1/4-1/2 hr. This low threshold appears to be responsible for the fact that cockroaches escaped successfully from the predatory strike of a toad (Bufo marinus) by detecting the low-speed wind currents made by the toad's strike (Camhi, Tom and Volman, 1978).

Under certain conditions, the cockroach's threshold for wind-excitation of escape behavior was altered. Threshold was reversibly elevated when the insect groomed its antennae, when ambient illumination was reduced, and during prolonged periods when the insect did not walk. Threshold was also elevated, apparently irreversibly, when the cuticle was incised. The larger the incision, the higher the threshold. It was possible to record from the giant interneurons (GI's) of each connective with hook electrodes by making only a minimal ventral dissection. Thus the responsiveness of the GI's to wind could be tested during changes in behavioral threshold. The modulation of responsiveness of individual GI's could be studied by relying upon the known directional sensitivities of each of the GI's (Westin et al., 1977).

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574 LEARNED MODIFICATION OF A MOLLUSCAN FEEDING RESPONSE PRODUCED IN THE ISOLATED CNS. Joseph Jin Chang and Alan Gelperin. Dept. of Biology, Princeton University, Princeton, N. J. 08540.

The terrestrial slug Limax maximus can show one trial food avoidance learning lasting three weeks (Gelperin, 1975, Science 189: 567). We have now produced an analogous form of learning in the isolated brain.

The preparation consists of the lips and central ganglia arranged so that chemical stimuli can be applied to the lips selectively. Adequate lip chemostimulation results in production of the feeding motor program (FMP), a stereotyped pattern of rhythmic, coordinated motoneuron activity recorded from buccal nerves (Gelperin, Chang and Reingold, 1978, J. Neurobiol., in press). The stimulus delivery system is sufficiently quantitative that successive 30 sec. stimuli delivered at 30 min. intervals elicit reproducible bouts of FMP.

A naive preparation of lips and CNS will give reliable bouts of FMP when given lip chemostimulation with standard extracts of attractive food plants such as potato, mushroom or carrot. Repellent plant secondary substances such as quinine, colchicine and nicotine sometimes elicit bouts of FMP, but also can terminate ongoing FMP set up by chemostimulation with attractive food extracts.

We have attempted to modify the responsiveness of naive preparations by pairing a positive chemical stimulus such as potato, carrot or mushroom extract with stimulation using a plant secondary substance such as quinine, colchicine or nicotine. During training the positive chemostimulus is closely followed by the negative chemostimulus for 2-4 cycles of pairing. During the subsequent testing two positive chemostimuli (the one used during training and a new one) are given alternately at 30 min. intervals. Responsiveness is scored in terms of number of bites, bite frequency and firing frequency of identified motoneurons during a bout of FMP.

We have been able by this procedure to produce selective depression of the in vitro preparation's responsiveness to an initially positive chemostimulus while maintaining its responsiveness to a second positive chemostimulus. Responsiveness to each of the three positive chemostimuli has been suppressed in different preparations. The response decrement lasts 6-10 hours. Of 43 preparations tested, 19 showed the learned suppression to a greater or lesser degree. Of these 19 preparations, 8 showed very clear and repeated suppression of response to the first positive chemostimulus while responding normally to a second positive chemostimulus. We have never observed suppression of responsiveness to the second chemostimulus while responsiveness to the first was maintained.

Supported by NSF Grant BNS 76-18792.

575 SIMULTANEOUS RECORDING OF ACTIVITY OF MOST (ALL?) OF THE LARGE NEURONS IN THE BARNACLE SUPRAESOPHAGEAL GANGLION. L.B. Cohen, A. Grinvald* and S. Lesher*, Dept. of Physiol., Yale Univ. Sch. of Med., New Haven, CT. 06510.

Changes in absorption of neurons stained with merocyanine-rhodanine type dyes were used to monitor action potential activity in ganglia from Balanus nubilus. A square array of 100 photodetectors (20th Century Electronics Ltd.), each 1.4x1.4mm, was positioned in the image plane of a 32X objective. The output of each detector was amplified and fed to a computer which sampled each channel every 0.6 msec during a recording period of 0.9 seconds. The images of the large cell bodies (40-100 μm diameter) often superimposed on several (2-6) detectors.

Stimulation of the ipsilateral connective via a suction electrode led to spikes in 9-15 neurons. About half as many neurons were active in response to turning off a light illuminating the median ocellus. This preliminary experiment illustrates the use of an optical method to locate neurons involved in behavioral responses. The signal-to-noise ratios obtained in these experiments were relatively large and therefore we feel that if other large neurons had been active, their activity would have been detected. This conclusion is, however, based on the untested assumptions that all cell bodies stain equally and that the action potential size was large in all neurons.

Yet another possible difficulty is pharmacological effects of the dyes. Suction electrode recordings from the connective and antennular nerve were used to monitor neurons that respond to turning off ocellar illumination. Since this off-response is polysynaptic, this pharmacological test was more stringent than those we had used previously and several dyes did have irreversible effects. However, using a concentration of 0.5 mg/ml, the sulfobutyl derivative of dye XVII led to reduced pharmacological action (but acceptable signal-to-noise ratios) and in some experiments the off responses before and after staining were similar. We plan to screen additional dyes trying to minimize pharmacological effects while maximizing signal size.

Since the barnacle ganglion has only 30-40 large neurons, the 100 detectors were relatively underutilized. We think that a recording with 100 detectors should be able to monitor activity from 100-200 individual neurons without ambiguity.

Supported by grant number NS 08437 from the National Institute of Neurological and Communicative Diseases and Stroke and a Muscular Dystrophy Association fellowship to A.G.

576 SYNAPTIC TYPES IN THE ABDOMINAL GANGLION OF APLYSIA CALIFORNICA, WITH SPECIAL REFERENCE TO SYNAPTIC-LIKE CONTACTS ON GLIAL CELLS. M. Colonnier, J.P. Tremblay and H. McLennan*. Dept. Anat., Laval Univ., Québec, P.Q., G1K 7P4 and Dept. Physiol., U.B.C., Vancouver, B.C., V6T 1W5.

The abdominal ganlion of Aplysia californica has been observed in preparations fixed by immersion in buffered formaldehyde and glutaraldehyde, either directly or after a survival of a few hours in artificial sea-water. In the neuropile, synaptic vesicles are of several sizes, may be round, oval or flat, and either clear or filled with different finds of dense material. The population of vesicles within a single profile may consist either of a homogeneous group of similar vesicles, or of various mixtures of 2 or 3 kinds of vesicles, in a continuous spectrum which has defied attempts at a rigorous classification. In profiles with mixtures of clear and dense-core vesicles, it is often only the clear vesicles which agglomerate towards the differentiated membranes. In such cases the dense-core vesicles lie as a peripheral halo around the clear vesicles. In other cases, both types of vesicles touch the presynaptic membrane. The differentiated membranes are characterized by a slight increase in density and by being regularly parallel to each other. Presynaptic densities are sometimes quite prominent but specialized dense cytoplasmic opacities have never been seen bordering the postsynaptic membrane. The interlemmal opacities vary considerably in density.

Four different types of zones can be defined on the basis of the number and type of vesicle-containing-profiles. 1) Some regions are formed mainly of medium to small neuronal processes, with very few vesicle-containing-profiles. 2) Others are made up of small processes, running in parallel and forming many contacts with each other. This type of zone is often surrounded and infiltrated by the processes of glial cells, forming glomeruli of different sizes. 3) The third kind of zone is still more densely filled with synaptic profiles contacting neuronal processes of all sizes. It most closely resembles mammalian neuropile. 4) The fourth type consists of islands of closely packed profiles containing large dense-core vesicles and only rarely forming synaptic contacts.

Synaptic-like contacts, characterized by the agglomeration of vesicles towards differentiated membranes, are present on glial cell bodies and processes in the neuropile. They are only rarely seen on the somata but in one case at least, three have seen on a single cross-section of a single soma.

In the subcapsular sinus, contacts are also found on small profiles identical to the many glial processes forming the loose meshwork typical of the region. Synapses are not present on the neuronal cell bodies. Supported by the M.R.C. of Canada.

577 FUNCTION OF MOLLUSCAN SALIVARY NEURONS DURING FEEDING. Jonathan Copeland* and Alan Gelperin (SPON: M. A. Lampert). Department of Biology, Princeton University, Princeton, New Jersey 08540.

The salivary burster (SB) is an autoactive motoneuron which initiates contraction of the salivary duct of the giant garden slug Limax maximus (Prior and Gelperin, J. Comp. Physiol. 114: 217-232). We have investigated the function of 5 other large buccal and cerebral ganglion neurons which each send a process into the salivary nerve of Limax. These neurons are the bilateral salivary neuron (BSN) and salivary neurons 1-3 (SN1, SN2, SN3), all found in each buccal ganglion, and the metacerebral giant cell (MGC), found in each cerebral ganglion. These neurons affect either salivary duct or salivary gland and are co-activated with motoneurons to buccal muscles during chemically elicited feeding in vitro.

BSN is a bursting neuron with processes in both ipsilateral and contralateral salivary nerves. Its burst rate is always slower than that of SB. Extracellularly recorded potentials indicate an effect of BSN on the salivary duct. Intracellular recordings from salivary gland cells indicate that BSN also innervates the salivary gland. Both EPSPs and action potentials occur in gland cells in response to BSN activation. BSN activity is increased during a feeding bout. As judged by extracellular recordings of BSN activity, stimulation of the serotonergic MGC inhibits BSN at low frequencies (1 spike/sec) and excites BSN at higher frequencies (10 spikes/sec).

SN1, SN2, and SN3 are large buccal neurons which also have an effect on the salivary duct. Both SN1 and SN2 receive EPSPs simultaneously with the firing of SB. These EPSPs rarely trigger action potentials. SN1 and SN2 produce a few action potentials during in vitro feeding. They receive unitary EPSPs from MGC. SN3, another slow bursting neuron, also has an effect on the salivary duct. SN3 is not affected by MGC stimulation.

Some of the salivary neurons are active in non-feeding periods. All are modulated during feeding. We have measured the amount of saliva secreted during non-feeding and feeding periods. Saliva is continuously secreted into the salivary duct during non-feeding periods. During feeding, however, the amount of saliva delivered to the salivary duct is greatly enhanced.

(Supported by NIH fellowship 5 F32 NS05067-02, the Spencer Foundation, and NSF grant BNS 76-18792.)

578 THE ROLE OF SEROTONIN IN PHASE SHIFTING THE CIRCADIAN RHYTHM OF THE *APLYSIA* EYE. G. Corrent* and A. Eskin. Dept. Biol., Rice Univ., Houston, Tx 77001. D. J. McAdoo. Marine Biomed. Inst., Galveston, Tx 77550.

In studying the reception of temporal information by the circadian pacemaker (CP) in the isolated eye, we discovered that a putative neurotransmitter, serotonin (5-HT), can phase shift the circadian rhythm of spontaneous optic nerve impulses (CAPs) from the eye. 6 hr. treatments of 5-HT (10^{-5}M) produced 3.1 ± .4 hr. (N=13) advance phase shifts when the treatment was administered late in the subjective day. At some other phases, 5-HT produced delay phase shifts. Phase shifts and inhibition of the CAPs were produced by concentrations of 5-HT as low as 10^{-7}M. Other putative transmitter substances, dopamine and acetylcholine, do not cause phase shifts in the rhythm at phases where 5-HT is effective. However, bufotenine (10^{-5}M), a 5-HT analogue, produced advance phase shifts when administered to the eye during the late subjective day. 5-HT is acting either directly on the cells housing the CP or on cells electronically coupled to it, since solutions capable of inhibiting secretion (HiMg-200mM, LoCa-.1mM) did not block the 5-HT phase shifts.

If 5-HT performs a neurotransmitter role in the eye, 5-HT must be present in the eye with some portion of it in a releasable state. The eye contains a substantial amount of 5-HT, 50ng 5-HT/mg retinal protein, as measured by gas chromatography-mass spectrometry. Depolarizing solutions with reuptake of transmitter inhibited (raised potassium, lowered sodium) caused from 30-48% decreases in the amount of 5-HT in eyes.

These results suggest that 5-HT is involved in the transmission of phase shifting information within the eye. Release of a transmitter substance does not appear to be necessary for phase shifting the isolated eye by light (Eskin, J. Neurobiol. 8: 273, 1977). 5-HT may be involved in mediating phase shifting in two other ways: extraocular entrainment of the eye by red light (Block et al., J. Comp. Physiol. 89: 237, 1974) and mutual coupling of the CP's in the two eyes (Hudson, Lickey, Soc. Neurosci. Abst. 3: 179, 1977). 5-HT is the first transmitter substance shown to phase shift circadian rhythms. As such, it is a natural marker that can be used for localization work and physiological studies.

579 PREY CAPTURE STRIKES OF THE PRAYING MANTIS: MOVEMENT AND ELECTROMYOGRAPHIC ANALYSES. Brian J. Corrette* (SPON: E.A. Maynard). Dept. Biology, Univ. of Oregon, Eugene, OR 97403.

Prey capture strikes of the praying mantis (Tenodera aridifolia sinensis) were analysed using several techniques of high speed photography. Although too rapid to be steered visually once released, strikes are not stereotyped. Movements of the coxa, femur (including trochanter), and tibia of the prothoracic legs during a strike can be divided into two phases termed the approach and the sweep. During the approach, the foreleg is promoted proximal to the prey by rotation about the articulation of the coxa in approximate synchrony with extension of the tibia. Variable extension or flexion of the femur also occurs during this time. Movements of the approach are variable in initial position, duration, and velocity, but the position of the coxa and femur at the end of the approach is highly correlated with pre-strike location of the prey relative to the prothorax. During the sweep, a rapid extension of the femur occurs simultaneous with a rapid flexion of the tibia, and the prey is grasped between spined surfaces of the femur and tibia. Promotion of the foreleg is slowed during the sweep by a rapid increase in rotational inertia due to extension of the femur. The velocity of sweep movements is variable, but their form and duration are relatively constant.

Proprioception from hairplates located proximal to the articulation of the coxa is important for coordination of the movements of the approach and the sweep. Removing these hairplates or reducing their stimulation by limiting movement of the coxa produces long duration strikes, and the sweep occurs at positions of the coxa and femur that are independent of prey location.

Myograms were recorded from muscles of the foreleg during high speed photography of strikes. Detailed analysis of these myograms is difficult as all muscles examined have a complicated innervation of slow and fast axons which often fire synchronously and at frequencies as high as 350 Hz, obscuring identification of individual potentials. During a strike, muscles are activated by loosely patterned bursts of slow followed by fast potentials. Overlap of agonist and antagonist bursts occurs during promotion of the coxa and rapid extension of the femur. Simultaneous onset of fast potentials in the extensor of the femur and the flexor of the tibia occurs during the sweep. Timing between fast activity of the promotor of the coxa and that of the sweep is variable and related to prey location.

Supported by NSF Grant BNS 75-00463.

580 FACILITATION AT THE NEUROMUSCULAR JUNCTION OF PHASIC AND TONIC AXONS INDEPENDENT OF FIBER TYPE IN LOBSTER MUSCLE. Walter J. Costello and Fred Lang, Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Mass. 02543.

To gain perspective on the interaction between different types of nerve and different types of muscle, we studied the neuromuscular system of the closer muscles in the dimorphic claws of the lobster Homarus americanus. The closer muscle is innervated by two motor neurons - a phasic and a tonic. Additionally, it is composed of two types of fibers. In the cutter claw, the closer muscle consists of ≥ 60% fast fibers (2-4μm sarcomere length) and ≤ 40% slow fibers (>6μm sarcomere length). The closer muscle of the crusher claw contains only slow fibers (>6μm sarcomere length). With two populations of muscle fiber present, and two types of axon as well, the mutual effects of one upon the other could be examined among the various combinations of innervation. The degree of facilitation (Fe) was measured as a ratio of the height of the excitatory postsynaptic potential (EPSP) in the muscle fiber at 10 Hz stimulation to that at 1 Hz stimulation.

All possible combinations of innervation by the two motor neurons onto the fiber types were found except for one. The tonic axon was never seen to innervate fast fibers without the presence of the phasic axon also. The Fe of the synapses from both axons was inversely proportional to the height of the EPSP at 1 Hz stimulation. The Fe of an axon's synapse was highest when the EPSP elicited in the fiber at 1 Hz stimulation was at its lowest value. The Fe of the synapses was apparently independent of the type of muscle and the presence or absence of the other axon. The phasic axon formed synapses which facilitated poorly in both claws, on both fast and slow muscle. There was no significant difference ($p = 0.10$) between the Fe values of these synapses in the cutter or crusher closer muscles (ave Fe = 2.4 and 2.0, respectively). The tonic axon formed synapses with moderate-to-high degrees of facilitation in each claw (ave Fe = 6.4, cutter; 5.7, crusher). Likewise this was not significantly different ($p = 0.10$). From the evidence it appears that the muscle type is exerting little or no influence on the program of the neurons to specify the facilitatory characteristics of their synapses.

(Supported by grants from NIH-NINCDS and Muscular Dystrophy Association of America)

581 THE CRAYFISH THORACIC PHASIC FLEXORS: DIFFERENCES IN HOMOLOGOUS MOTONEURONS. R. L. Crabtree* and W. H. Evoy (SPON: S. W. Fox). Laboratory for Quantitative Biology, Univ. of Miami, Coral Gables, FL 33124

The deep flexor muscles of the crayfish are a complex series of four thoracic muscles which lie along the dorsal surface of the skeletal endophragmal shelf. The posterior portion of the deep flexors joins the phasic abdominal flexors via an inscription; the muscles contract during the rapid abdominal flexions of swimming and escape behaviors.

Ultrastructural studies revealed the deep flexors to be a homogenous population of typical crustacean phasic muscle fibers. Each fiber is innervated by three axons which reach the musculature through the ganglionic third roots of thoracic ganglia 1-3. Electrophysiological recordings of potential changes in the muscle fibers during stimulation of the third roots indicate that three synaptic potentials are present. One potential rapidly antifacilitates, even at stimulation frequencies of 1 Hz; one moderately facilitates; and the last is inhibitory. These results suggest that the thoracic muscles are innervated by neurons that are homologous to those found in the abdomen which innervate the phasic abdominal flexors: the motor giant, a non-giant excitor, and an inhibitor.

To examine the morphological characteristics of the thoracic motor neurons, the cells were filled intracellularly with cobalt sulfide, using the backfilling technique. In general, cells nearly identical to those previously found in the abdomen were located, further substantiating the homologous nature of the thoracic neurons. However, thoracic fast flexor motoneurons varied from those in the abdomen in: numbers, size, structure of the motor giant, and location of a posterior group of somata.

Fewer phasic flexor motoneurons per hemiganglion are found in the thorax. The first ganglion contains only three. Numbers increase in the two posterior ganglia. Soma size of the thoracic neurons is approximately 50% of corresponding abdominal neurons. These reductions may be related to the reduced musculature in the thorax.

No group of somata is located in the posterior ipsilateral portion of the ganglion. This group, found in the abdomen, appears to have shifted to a medial, posterior location in the thoracic ganglia.

Finally, the motor giant appears to have specialized branches in the ganglionic neuropile. These arborizations may be areas of synaptic contact to fibers contralateral to the motor giant axon, since the thoracic nerve cord connectives are split.

Supported by NSF Grant BNS 21721

582 NEURAL CORRELATES OF AN ASSOCIATIVE BEHAVIORAL CHANGE IN HERMISSENDA CRASSICORNIS. T. Crow and D. Alkon*. Sec. on Neural Systems, Lab. of Biophys., NINCDS, NIH, MBL, Woods Hole, MA 02543.

The response of the nudibranch mollusk Hermissenda crassicornis to a light stimulus can be modified by stimulation of two sensory pathways, the visual and statocyst (primitive vestibular). This long-term behavioral modification, consisting of an increase in the animal's response latency to enter an illuminated area, is dependent on the association of light and rotational stimuli. The behavioral modification is now correlated with neural changes within these two sensory pathways.

Replications of previous behavioral results (Crow and Alkon, 1977) showed significant overall differences between an experimental group and two control groups ($p<.05$) that were trained in an automated apparatus for three consecutive days and then tested at the end of the training period. The experimental group received 50 trials each day of light (30 seconds) paired with rotation (30 seconds). Control groups received either 50 trials of light and rotation on independent random schedules or 50 trials of rotation presented randomly. The latency to enter the light was measured automatically for all groups at the termination of the third day of behavioral training. The experimental group latencies to enter the light were significantly longer than the latencies of the random control group ($p<.05$) and random rotation group ($p<.015$), while the respective control groups were not significantly different from each other.

Intracellular recordings were made from photoreceptors and statocyst hair cells in the isolated nervous systems of experimental and control animals following training and behavioral testing. Significant overall differences in the spontaneous firing frequencies of dark adapted Type B photoreceptors were found for experimental and control groups ($p<.01$). Type B photoreceptor activity was significantly elevated following training for the experimental group as compared to the random light and rotation control group ($p<.01$) and random rotation group ($p<.01$) while the two control groups were not significantly different from each other. Average firing frequency of statocyst hair cells following dark adaptation was less than 1/second for experimental animals. An overall (across experimental and control groups) significant positive correlation was found between the response latencies to enter the light after training and Type B photoreceptor activity in the isolated nervous systems ($p<.05$). This increase in Type B impulse activity may be related to long-lasting depolarization of Type B photoreceptors (Alkon and Grossman, 1977) and long-lasting inhibition of Type A photoreceptors during a light response (Alkon, 1976) found after stimulation of the isolated nervous system with light and rotation.

583 CENTRAL AND PERIPHERAL CONTROL OF COCKROACH GIANT INTERNEURONS DURING WALKING. Darryl L. Daley. Program in Neural and Behavioral Biology, University of Illinois, Urbana, IL 61801.

Within the ventral nerve cord (VNC) of the American cockroach, the giant interneurons (GI's) are morphologically divisible into dorsal and ventral groups of three each, and one smaller neuron associated with the ventral group. Control of the excitability of the dorsal and ventral groups has been investigated intracellularly in deafferented preparations and during tethered walking. Dorsal GI's are excited during walking while ventral ones are inhibited (Daley, Neurosci. Abst. 3:174, 1977). The origin of the descending excitation was investigated by first monitoring the activity of a GI found to be active during walking and then looking at its activity following a VNC cut anterior to the recording site. The results of these experiments suggest that the major source of walking-activated excitatory input is from thoracic nervous centers. During walking the mean firing frequency of all dorsal GI's increases as the speed of walking increases, suggesting that descending excitation may arise specifically from locomotor centers. To test whether the descending excitation resulted from sensory input concomitant with walking, the thoracic ganglia were deafferented. After deafferentation, the dorsal GI's were still excited during periods of leg motoneuron activity, clearly showing that the excitation originates in the thoracic locomotor centers, not from sensory input.

Walking-activated inhibitory input to ventral GI's was investigated by comparing sound responsiveness of individual GI's in animals which were resting to that of the same GI's during walking. This inhibition could result from either central descending pathways or peripheral sensory receptors activated during walking. To separate these alternatives, deafferentation and VNC lesioning experiments were performed. Deafferentation of thoracic and abdominal ganglia (only the cercal nerves were intact) guaranteed sensory input associated with walking was eliminated. Yet during leg motoneuron activity sound responsiveness of ventral GI's was reduced, demonstrating walking-activated central inhibition. To test for peripheral inhibition, central pathways were cut anterior to the recording site. The sound responsiveness of ventral GI's in tethered animals with these cuts was again reduced during walking, thereby establishing walking-activated peripheral inhibition.

Thus, the morphological division of GI's into dorsal and ventral groups appears to have a physiological correlate. While the primary source of excitatory input activating dorsal GI's during walking arises from a central source, ventral GI's receive inhibitory input from both central and peripheral sources.

This research was supported in part by NIH grant NS12142 to F. Delcomyn and HEW PHS training grant GM-1076 to the author.

584 INDUCTION OF BURSTING IN NORMALLY SILENT APLYSIA CELLS. Peter F. Drake* and Steven N. Treistman (SPON: Donald Keefer). Department of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

Cyclic nucleotides may mediate the longlasting effects of hormones and synaptic stimulation in the abdominal bursting cell R15 of Aplysia (Treistman and Levitan, Nature 261: 62, 1976; PNAS 73: 4689, 1976). This conclusion was reached through the use of phosphodiesterase inhibitors and other drugs which affect cyclic nucleotide metabolism. The question to which we addressed ourselves was this: Do phosphodiesterase inhibitors enhance conductances already known to be present in bursting cells or do they induce conductance changes in cells which normally do not show such conductances, as in non-bursting cells? The phosphodiesterase inhibitor, IBMX (10^{-4} M) induces synchronous bursting in the two silent metacerebal giant cells of Aplysia. Two classical techniques were used to detect and to block the involvement of a presynaptic cell: 1) hyperpolarization of the MCG cells, to uncover underlying postsynaptic potentials, and 2) perfusion with a modified solution of reduced Ca++ -- high Mg++ artificial sea water shown previously to block central chemical synapses (Weiss, Cohen and Kupfermann, Brain Research 99: 381, 1975). Both results implicate a postsynaptic locus in the induction of bursting. A dynamic current-voltage curve was also generated during the IBMX induced bursting. The result shows an increased anomalous rectification as a function of incubation time in ASW-IBMX.

Other drugs affecting cyclic nucleotide metabolism were utilized and their possible Ca++ ion artifacts investigated. These results will also be discussed. (This work was supported by NSF grant BMS 77-01548).

585 BAG CELL HORMONE ACTS DIRECTLY ON OVOTESTIS OF APLYSIA CALIFORNICA IN VITRO: BIOASSAY FOR RELATING RELEASE TO ELECTROPHYSIOLOGY. F.E. Dudek, K. Burke*, B. Soutar*, S.S. Tobe*
Erindale College and Department of Zoology, University of Toronto

The bag cells of Aplysia fire a characteristic train of action potentials to release a peptide hormone which triggers egg-laying. It has been hypothesized that the neurohormone acts directly on the ovotestis to initiate egg release. We have found that extracts of the parietovisceral ganglion (PVG) with the bag cells increase the rate of egg release from pieces of ovotestis in vitro at physiological concentrations and in a dose-dependent manner. Extracts of the genital or pleural ganglia do not increase egg release above control levels in artificial sea water (ASW), thus indicating that macromolecules associated with pigmented neurons do not cause egg release. Furthermore, microdissection experiments indicate that egg release activity is located primarily in the clusters of bag cell somata and the nearby connective tissue sheath. The number of eggs released by ovotestis in vitro for a particular dilution of PVG extract or in ASW alone increases as a function of the number of days since egg-laying; this is consistent with previous in vivo experiments. Therefore, hormone-induced egg release is a function of concentration of bag cell hormone and egg-laying history.

A. californica has a seasonal rhythm of egg-laying; more eggs are laid in fall than winter. Consistent with these in vivo observations, both PVG-induced and spontaneous (in ASW only) egg release from fragments of ovotestis in vitro are higher in fall than winter. However, the relative effectiveness of PVG extract at increasing egg release is similar during fall and winter, thus providing in vitro physiological evidence that egg-laying hormone is still present in the bag cells during winter, but eggs are not released from the ovotestis.

The logarithmic relation between concentration of PVG extract and relative egg release provides an improved bioassay for bag cell hormone. Preliminary experiments with the in vitro assay have confirmed that electrically-evoked afterdischarges of the bag cells cause secretion of egg-laying hormone in vitro. Future studies are aimed at understanding the relationship between temporal pattern of bag cell spikes during afterdischarge and rate of hormone secretion.

Supported by the National Research Council of Canada and the Connaught Foundation.

586 ROLE OF INTERNEURON II IN LONG-TERM SENSITIZATION OF SIPHON WITHDRAWAL IN APLYSIA CALIFORNICA. Lewis Eberly and Harold Pinsker.
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Aplysia show spontaneous, stereotyped contractions of the organs of the mantle cavity (gill, siphon and mantle shelf) at irregular intervals. A similar patterned behavior can also be triggered by a water jet stimulus to the siphon (Kupfermann and Kandel, 1969).

This behavior is mediated by a network of interneurons in the abdominal ganglion called Interneuron II (INT II) (Byrne and Koester, 1978). In freely-behaving Aplysia, occurrence of the behavior (INT II response) is associated with a burst of activity recorded by cuff electrodes on the siphon nerve which innervates siphon skin and musculature (Pinsker, Cobbs and Kanz, 1976). Plastic changes in latency of triggered INT II activity can contribute to short-term habituation and sensitization of the siphon withdrawal reflex (Kanz, Cobbs and Pinsker, 1978). We now report that long-term sensitization training modifies the probability of triggering short-latency INT II responses.

Long-term sensitization of siphon withdrawal (Pinsker, Henning, Carew and Kandel, 1973) is produced by a series of electric shocks delivered to the anterior mantle region over a 4-day period, and is associated with a dramatic and long-lasting increase in response duration. Associated with this behavioral change is a significant increase (from 60% to 85%) in the probability of triggering a short-latency (less than 5 sec) INT II response. Also, sensitized animals show a significant decrease in the latency of triggered INT II responses with repeated siphon stimuli, whereas controls show either no change or an increase. The greater frequency of occurrence and reduced latencies of triggered INT IIs in sensitized animals is presumed to play a role in the behavioral changes associated with long-term sensitization.

Triggered INT II responses play a significant role in siphon withdrawal for several reasons. (1) Spontaneous INT II responses lead to a nearly maximum siphon contraction. (2) About 60% of moderate intensity stimuli to the siphon trigger short-latency INT II responses. (3) Triggered INT II responses typically occur with latencies less than 3 sec. (4) Duration of siphon withdrawal is significantly longer when a triggered INT II response occurs than when it does not. Therefore, the modulation of excitability of the INT II network which occurs during long-term sensitization may represent a correlate of behavioral plasticity.

This work was supported by BNS 76-17480.

587 SEROTONIN, CYCLIC AMP, AND THE MODULATION OF EVOKED TRANSMITTER RELEASE AT THE CRAYFISH NEUROMUSCULAR JUNCTION. J. J. Enyeart* and S. D. Erulkar. Dept. of Pharmacol., Sch. of Med., Univ. of Pennsylvania, Philadelphia, PA 19174.

In recent years evidence has accumulated suggesting that serotonin (5-HT) functions as a neurohormone in crustacea. When applied to the crayfish neuromuscular junction it produces separate pre- and postsynaptic effects. These include a marked increase in evoked transmitter release from excitatory nerve terminals (Dudel, Arch. Exp. Path. Pharmak., 249:518, 1965), as well as increases in muscle cAMP content and force of contraction (Battelle et al, Neuro. Abs., 2:774, 1976). Our experiments, performed on the abductor muscle of the dactyl in the first walking leg of the crayfish, were designed to determine whether the presynaptic modulatory action of 5-HT might be effected through a cAMP-dependent mechanism. Conventional intra- and extracellular recording techniques were employed to monitor responses elicited by stimulation of the excitatory motor nerve with a suction electrode.

The presence of a phosphodiesterase (PDE) inhibitor, i.e. Squibb 20,009 or papaverine (both 10^{-4} to 2×10^{-4} M), produced after a 30-60 minute delay a gradual increase in the amplitude of the excitatory postsynaptic potential (EPSP). This growth continued until a single stimulus induced contraction of the muscle fiber and dislodgement of the electrode. At this time the EPSP was frequently 50-100 times the control response. Statistical analysis indicated that this increase represented an enhanced quantal content. Results of similar experiments using focal extracellular recording techniques confirmed this finding.

In other experiments, the PDE resistant 8-benzylthio derivative of cAMP (4×10^{-4} to 1×10^{-3} M) produced 3-5 fold increases in the EPSP amplitudes. Neither cAMP nor its dibutyrl analogue had any effect at concentrations as high as 1×10^{-3} M.

A relationship between the cAMP and 5-HT induced effects was indicated. Alone, 5-HT (1×10^{-6} M) elicited a 3-5 fold increase in evoked transmitter release. Replacing this solution with one containing both 5-HT and a PDE inhibitor (5×10^{-5} M) produced a further 2-3 fold increase. By themselves, the PDE inhibitors at this concentration caused only a 0-0.3 fold increase in the response.

Focal extracellular recordings showed no change in the biphasic nerve terminal potential in the presence of 5-HT or PDE inhibitors. Neither was any increase in miniature end plate potential frequency or amplitude detected.

These findings indicate that procedures expected to increase levels of cAMP in crayfish motor nerve terminals also enhance evoked transmitter release. Furthermore, 5-HT may function by activating a presynaptic 5-HT sensitive adenylate cyclase. Evidence of a similar system in Aplysia has been reported (Shimahara et al, Brain Res., 127:168, 1977).

588 SYNAPTIC INTEGRATION BY CRAYFISH WALKING LEG MOTOR NEURONS W. H. Evoy and R. L. Crabtree*. Laboratory for Quantitative Biology, Univ. of Miami, Coral Gables, FL 33124

Input pathways and summation properties of identified motor neurons to the fifth walking leg have been investigated by intracellular recording and cobalt staining in the thoracic ganglia. Synaptic inputs to the anterior depressor of the coxopodite and the extensor of the meropodite of the leg are found in the fifth thoracic ganglion.

Proprioceptive inputs that originate in the MC_2 organ of the meropodite-carpopodite (MC) joint of the fifth leg were activated by electrical stimulation of the sensory branch. Intracellular recordings in motor neuron processes of that segment showed post-synaptic potentials with constant latency components in response to single stimuli. Both excitatory and inhibitory responses have been found.

Central nervous inputs to the walking leg motor neurons revealed by stimulation of fibers in the anterior nerve cord evoke repeatable changes in motor output that appear to be mediated by other interneurons. Intracellular recording and signal-averaging show a lack of constant-latency responses in the motor neurons in response to the stimuli, although depolarizations lacking discrete increments of potential are correlated with changes in spike frequency. Additional findings on interneuronal pathways in the thoracic chain will be presented.

Evidence for a discrete cellular or emergent network oscillatory system for the approximately one second cycle of forward walking is thus far lacking. Locomotor and postural behaviors appear to depend on complex interaction of interneuronal, motor, and sensory components.

Supported by NSF Grant BNS 21721

589 SYNAPTIC ORGANIZATION IN THE CORPORA PEDUNCULATA OF THE HORSESHOE CRAB (Limulus polyphemus). Wolf H. Fahrenbach* (SPON: D. S. Rushmer). Oregon Regional Primate Research Center, Beaverton, OR 97005.

The large, hemispherical mass of the Limulus corpora pedunculata consists of two highly branched lobes connected to the protocerebrum by narrow stalks. About 10^4 afferent fibers, belonging to 5 types, enter through the stalks and make diverse, profuse, and often reciprocal contacts with several million Kenyon (intrinsic) cells and one another. The Kenyon cell axonal arborizations converge on a few hundred efferent dendrites. The five afferent fiber types can be characterized as follows: Type A - forms club-shaped core of glomeruli and circumglomerular annuli, contains small flat vesicles; presumptive inhibitory input; Type B - terminates with bushy endings in glomeruli, contains clear round vesicles, is presynaptic to Kenyon cells and to Type A terminals; presumptive excitatory input; Type C - terminates on other afferents, in glomeruli, and rarely on Kenyon cell bodies, contains angular (neurosecretory) granules; postulated to impart circadian rhythm; Type D - terminates on Kenyon cell somata and initial neurite segment (but not in glomeruli), contains dense-cored vesicles; Type E - terminates in peduncles on other afferents and Kenyon cell telodendria, contains dense vesicles. The C, D, and E afferents have reciprocal synaptic connections with Kenyon cell axon terminals. Glomeruli receive three afferent inputs of presumptive inhibitory, excitatory, and neuromodulatory nature. Numerical and directional details of the circuitry in the corpora pedunculata have been extracted by a combination of light and electron microscopy, serial sectioning, silver staining, and stereology. Kenyon cells, increasing in number up to about 1×10^8 in the adult, show minor variations in their dendritic pattern and have only one rare variant cell type. Interactions between them occur primarily at their axonal boutons as they crowd around efferent fibers. The latter have large receptive fields, some of their large somata are located within the confines of the corpora pedunculata, and they receive a highly convergent input from Kenyon cells. The corpora pedunculata appear to process primarily the voluminous chemosensory input from the appendages, an assumption that is supported by the major connections of the organ. (Supported by Grants EY00392, RR00163, and RR05694 from the National Institutes of Health.)

590 MECHANICAL AND ELECTRICAL ACTIVITY FROM THE PARASITIC FLUKE, SCHISTOSOMA MANSONI. R.H. Fetterer, Jr.*, R.A. Pax*, and J.L. Bennett. Departments of Zoology and Pharmacology, Michigan State University, East Lansing, Michigan 48824.

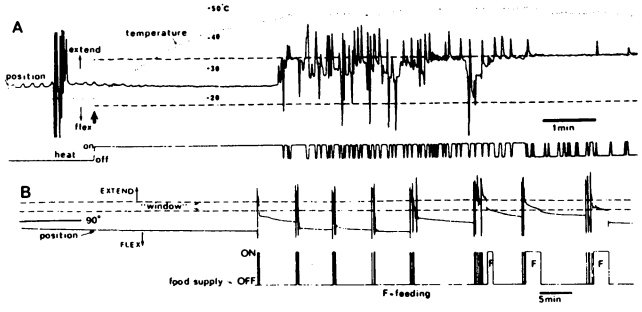
Parastaltic contractions and other muscular movements which appear to function in propulsion of the parasite through the mesenteric veins of its mammalian host can be observed in the blood fluke Schistosoma mansoni. Recordings of mechanical and surface electrical activity from the musculature of male parasites shows them capable of generating spontaneous contractions accompanied by large amplitude surface electrical activity. Isolated portions of the worm are capable of contractile activity. Putative neurotransmitters such as dopamine (10^{-4}M) and acetylcholine (10^{-4}M) inhibit spontaneous contractions and decrease the resting tension of the muscle. 5-Hydroxytryptamine (10^{-6}M) increases the rate of spontaneous contractions.

Upon penetration of the ventral tegument of an isolated male schistosome with a microelectrode a potential difference (-31.5±2.5 mV) can be recorded. Histological studies utilizing iontophoretic injection of horseradish peroxidase through the recording electrode indicate that the potential difference that is recorded exists across the tegumental membrane of the animal. Ion substitution experiments show that external potassium concentration has the greatest influence on both the tegumental membrane potential and the resting tension of the musculature. Spontaneous depolarizations of the membrane can be recorded and they appear to be correlated with contractions. Drugs such as carbachol and pentobarbital also eliminate the spontaneous depolarizations but do not greatly effect the membrane potential.

The above results indicate that rhythmicity is inherent in the musculature of S. mansoni and the nerve net of the animal has only a modulatory influence on contractions. In addition, movement of ions across the tegumental surface may play a role in electromechanical coupling. (This work was supported by grants from the Edna McConnell Clark Foundation, World Health Organization, and the National Institutes of Health.)

591 POSITION LEARNING IN BEHAVIORALLY APPROPRIATE SITUATIONS. R. Forman* and G. Hoyle, Dept. Biol., Univ. of Oregon, Eugene, OR 97403 USA. Supported by NSF Grant BNS-75-00463; PHS 5-T-32 GM07257.

We have developed a flexible paradigm which enables ability to learn to control an environmental variable to be tested in a wide range of situations. In some invertebrates the tests can be carried out whilst recording intracellularly from identified neurons. The intact or headless animal is secured to permit movement of only a single leg segment whose position is monitored. Upper and lower limits are set electronically to define a "window" of any angle and position within the movement range. The environmental variable is directly linked to the window limits. In one mode the animal must learn to hold the segment out of a broad window that includes its initial position; in another it must be moved into a narrow window away from its initial position and held there. We linked the former to temperature by placing a locust in a cool room and coupling the window to applied heat (starts at arrow in A). The animal tolerates a close heat lamp until its temperature dangerously exceeds the preferred range. It then makes many extension/flexion movements, locates the extension edge of the window, and learns to maintain a position at its edge, crossing the threshold at intervals to turn the heat lamp on and off to maintain a preferred temperature (36-38° C). The new position was retained for up to a few hours after the heat lamp was turned off. When linked to a device that brought food to the mouth, regular feeding was learned, the segment being brought to the edge of the window and very slowly through it (B). Feeding time increased to 2-7 min at an average interval of 45 min. All movements were voluntary. The insect is able to learn both a passive element (position) and dynamic ones (movement away from a position and back; also dwell time) in either extension or flexion ranges.



592 TEMPERATURE EFFECTS ON THE ELECTRORETINOGRAPHIC CIRCADIAN RHYTHM IN CRAYFISH. Beatriz Fuentes Pardo and Javier Ramos C.* Depto. de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, México 20, D.F. MEXICO

The effects of temperature in the circadian rhythm of response to light in crayfish Procambarus bouvieri have been examined. In order to obtain information about the role of this factor on the clock mechanism underlaying the circadian electroretinographic rhythm, we have obtained long-term records of the ERG in intact crayfish in conditions of controlled temperature and complete darkness except for the test light pulses. It was observed that the τ period of the free running oscillations tends to be almost invariable under different constant temperatures. A phase-response curve for two-minute pulses of stimulation, has been, however derived from experiments in which individual crayfish were treated with both, increments and decrements in temperature. The phase advance or delay was related to the point in the oscillation's cycle exposed to the temperature signal.

These results suggest that the circadian electroretinographic rhythm must be depending on thermolabile oscillators responsible for the described effects of temperature.

593 AXONAL TRANSPORT OF FOREIGN TRANSMITTERS IN AN IDENTIFIED NEURON OF *APLYSIA*. Daniel J. Goldberg and James H. Schwartz. Depts. Pharm. and Physiol., Div. Neurobiol. & Behav., Columbia Coll. Phys. & Surg., New York, N.Y. 10032.

One of the central tenets of neurobiology is that each neuron releases the same transmitter at all of its terminals. There are several steps at which this selectivity could be determined: uptake of transmitter or its precursor across the plasma membrane, synthesis of transmitter, and packaging of transmitter into appropriate organelles with transport of these organelles to the terminals. Using intracellular microinjection, we have been able to circumvent the first two processes and study packaging in the living cell.

The giant cerebral neuron (GCN) in the central nervous system of the sea hare, *Aplysia californica*, has been shown to use serotonin as its transmitter. Thus, GCN synthesizes serotonin from tryptophan, contains serotonin in high concentrations, and releases serotonin from its terminals. In addition, the application of serotonin mimics the effects of GCN stimulation on follower cells and muscle. Previous work in our laboratory has shown that when ^{3}H-serotonin is pressure-injected into the cell body of GCN it is packaged in characteristic storage vesicles and transported along the axons by fast transport. We have now found that other neurotransmitters, when injected into GCN's cell body, also undergo fast axonal transport. ^{3}H-Dopamine and ^{3}H-histamine both are transported in amounts and at velocities similar to ^{3}H-serotonin. With both substances, the radioactivity appearing in the axons has been analyzed and shown to be predominantly in the form of the injected transmitter. ^{3}H-D, L-octopamine is transported when injected in small amounts, but there is virtually no transport when larger amounts are injected. When unlabeled D, L-octopamine is injected together with ^{3}H-serotonin, the subsequent transport of the latter is partially depressed. ^{3}H-choline, when injected into GCN, is converted into ^{3}H-phosphorylcholine and ^{3}H-betaine; these substances move into the axons in substantial amounts but with the kinetics of diffusion. Finally, previous studies have shown that the precursor of serotonin, 5-hydroxytryptophan, and the metabolite of serotonin, tentatively identified as a glucuronide conjugate, are not transported. These compounds,like the choline metabolites, have no net charge.

We believe that the foreign transmitters are transported by virtue of their ability to be packaged by the serotonergic storage vesicle. We would suggest that a molecule must have a net positive charge to be packaged. D-octopamine, which does not occur naturally, may inhibit packaging when present at sufficiently high concentrations.

594 EVIDENCE FOR NEUROHORMONAL ACTIVATION OF GALACTOGEN SYNTHESIS IN THE ALBUMEN GLAND OF THE SNAIL, HELIX POMATIA. Esther M. Goudsmit, Dept. Biol., Oakland Univ., Rochester, Michigan 48063.

An investigation is under way to test the hypothesis that the reproductive cycle of pulmonate gastropods is regulated by neurohormones. A bioassay has been developed that utilizes organ culture to detect a precise metabolic response of a target organ, the albumen gland of the snail, H. pomatia, to the action of putative regulatory molecules. The albumen (egg white) gland synthesizes galactogen, a galactose homopolymer, in preparation for summer egg laying. Galactogen is the sole carbohydrate component of the albumen gland's secretory product, perivitelline fluid, which envelops each freshly laid egg. In autumn, the gland instead accumulates glycogen as a food reserve for winter hibernation. Thus a clear-cut biochemical manifestation of the seasonal cycles of reproduction and hibernation occurs. Albumen gland explants are easily maintained in culture and experimental activation of galactogen synthesis within the quiescent gland of a hibernating Helix provides a bioassay for regulatory molecules that turn on an essential element of the reproductive cycle.

Previous experiments indicated that albumen gland explants incubated in the presence of a Helix brain (nine circumpharyngeal ganglia) show a 2- to 10-fold increase in the specific activity of galactogen-^{14}C when compared with duplicate explants from the same gland maintained in the absence of brain (Goudsmit, J. Exp. Zool. 191:193, 1975). The brain-specific substance is released, during high-$[K^+]$ depolarization, by a $[Ca^{++}]$-dependent mechanism (Goudsmit, Brain Research, in press). Present results show that the brain-specific substance retains activity after exposure to 100°C for six minutes, but not after treatment with protease.

Galactogen-^{14}C synthesis can be stimulated 2- to 4-fold by incubation of albumen gland explants with 10^{-3} M concentrations of either the dibutyryl or 8-bromo analogue of 3'5' cyclic adenosine monophosphate (cAMP). Theophylline (10^{-3} M) enhances activation. Concentrations of cAMP below 10^{-5} M have no stimulatory effect. The amount of cAMP in a single Helix brain is only 20 picomoles (Levitan and Treistman, J. Neurobiol., 8:265, 1977), therefore it is unlikely that the brain-specific substance assayed in organ culture is cAMP itself.

Evidence thus far indicates that the galactogen-stimulating substance possesses properties characteristic of known peptide neurohormones.

Supported by NIH grant GM-23240.

595 HIGHER-ORDER CONTROL OF THE FEEDING MOTOR PROGRAM OF THE SNAIL HELISOMA TRIVOLVIS. Bonnie Granzow and Stanley B. Kater. Dept. Zool., University of Iowa, Iowa City, IA 52242.

Our previous work demonstrated that a bilaterally symmetrical pair of neurons in the cerebral ganglia have an excitatory influence over the feeding motor program of the buccal ganglia in Helisoma. This excitatory influence is manifested as an increase in the rate of alternating bursts in protractor and retractor motoneurons. There is a direct relationship between the frequency of action potentials experimentally evoked in an individual cerebral cell and the rate of the buccal motoneuron bursting (Granzow and Kater, Neuroscience 2:1049-1063, 1978).

Now we have found that these effects of cerebral cell activity can be mimicked by bath application of 5-hydroxytryptamine (the putative transmitter of these cells). We have obtained a dose-response curve which indicates that there is a direct relationship between the concentration of 5-hydroxytryptamine in the bath and the rate of bursting in buccal ganglia motoneurons.

In addition, we have found indications that increasing the frequency of firing in an individual cerebral cell produces qualitative changes in the composition of the motor output of the buccal ganglia; activity appears to be initiated in motoneurons which are quiescent at lower levels of cerebral cell firing. In particular, at least some protractor motoneurons remain quiescent at lower levels of cerebral cell activity.

Buccal neuron #4 which innervates the ipsilateral salivary gland fires non-rhythmically at low frequencies of cerebral cell firing and rhythmically (in phase with retractor motoneuron bursts) at higher levels of cerebral cell firing. Bath application of 5-hydroxytryptamine also changes cell #4's activity from non-rhythmical to rhythmical.

596 ALTERATIONS IN THE NERVOUS SYSTEM OF DROSOPHILA MELANOGASTER IN MUTANTS OF ACETYLCHOLINESTERASE AND CHOLINE ACETYLTRANSFERASE. Ralph J. Greenspan, James A. Finn, Jr.* and Jeffrey C. Hall*. Dept. of Biology, Brandeis Univ., Waltham, MA 02154.

Genetic variants deficient in the enzymes acetylcholinesterase (AChE) and choline acetyltransferase (CAT) have been obtained in Drosophila melanogaster and employed to study nervous system function and development in the absence of breakdown or synthesis of acetylcholine, a putative central nervous system transmitter. AChE-null mutations (of the Ace locus found by Hall and Kankel, Genetics 83, 517, 1976) and CAT-null mutations (defining the Cat locus) are lethal when homozygous, killing the animals late in embryogenesis but permitting apparently normal formation of the nervous system at a gross level. There is no detectable AChE histochemical activity in Ace$^-$ embryos and no detectable CAT activity by a radiometric assay in Cat$^-$ embryos.

Conditional mutants at the Ace and Cat loci have also been isolated. Conditional Ace$^-$ mutant adults paralyze at high temperatures tolerated by wild-type flies, and the mutation also has a temperature-sensitive lethal period during embryogenesis. Cat$^-$ temperature-sensitive mutations are lethal during embryonic, larval and adult stages, and CAT enzyme activity in these mutants is temperature-sensitive in an in vitro assay.

Analysis of the Ace$^-$ mutations for long term effects on localized parts of the nervous system has been carried out using genetic mosaics in which expression of the mutation is permitted in only a portion of the animal. These mosaics show histochemically null patches for AChE in limited portions of the cortex and neuropile of the brain and thoracic ganglion. Null patches have anatomical defects associated with them that appear to be condensations of the neuropile. In a dorsal brain mosaic,half of which is mutant, the null tissue has a degenerate appearance. Preliminary histological results of silver-stained mosaic brains support the observation of neuropile condensation. Some Ace$^-$ brain mosaics exhibit deficits in visually driven behavior, and an absence of the "off" transient of the electroretinogram correlates with mutant tissue in the first order optic lobe, the lamina.

AChE-null tissue is permissable in all cortical regions of the nervous system. These mutant patches are not usually very extensive, and bilateral mutant tissue for symmetrical structures is extremely rare and restricted mainly to the optic lobes. Null patches in the neuropile are less obtainable and these are completely restricted to the dorsal brain unilaterally, and the optic lobes. These results suggest there is no single focus for Ace$^-$ lethality and that the presence of the wild-type enzyme unilaterally is sufficient to compensate for the mutant tissue in most parts of the nervous system.

597 LASER PHOTOSTIMULATION OF INDIVIDUAL NEURONS IN THE CENTRAL NERVOUS SYSTEM OF THE LEECH AND BARNACLE. A. Grinvald* (SPON: C.R. MICHAEL) Dept. of Physiol., Yale Univ. Sch. Med., New Haven, CT. 06510.

A search for unidentified neuron(s) may become exceedingly difficult even in a simple nervous system, if many pairs of cells have to be impaled, or if the neurons are small. Such a search might be easier if it was possible to stimulate neurons with focal laser light; thus scanning of the laser microbeam over the ganglia may be used to find the neurons which are presynaptic to a given monitored neuron(s).

My preliminary experiments are an attempt to develop a general method, for non-pigmented neurons, by improving the original experiments of Arvanitaki and Chalazonitis (1953). They were carried out on the segmental ganglia of the leech, Macrobdella decora and the supraesophagel ganglia of the barnacle, Balanus nubilus. A neuron was impaled with microelectrode to monitor its electrical activity. The ganglion was then perfused with normal Ringer containing 10-100 µg/ml of a photoreactive compound (Indolenine tiobarbituric acid derivatives). The perfusion was changed back to a normal Ringer after ∿20 minutes. A 2 mW He-Ne laser was used to form a spot of light having a radius of only 1 µ which was positioned on the impaled neuron with the aid of 40X/.7NA Zeiss water-immersion objective using a vertical top illumination. A light pulse (.1-5 sec) then triggered fast photochemical reactions leading to a depolarization and subsequent firing. Reversible subthreshold depolarization was observed for P, T and N cells in the leech, when shorter light pulses were used.

The following results were obtained: (a) The low intensity of laser light has no effect on the electrical activity of the ganglia prior to the incubation with the photoreactive compound. (b) Staining of the neurons did not significantly alter the resting potential, the action potential size or shape, and I did not observe a modification of the synaptic inputs onto neurons. (c) In all cases it was possible to depolarize the selected neurons. (d) The effect of light is localized to the illuminated area. It seems that the in-plane resolution for stimulation is 4-6µ, and the depth of field is 5-10µ. Thus small neurons probably can be selectively stimulated. (e) With appropriate conditions photostimulation is usually "reversible" in that repetitive firing stopped after 1-150 seconds depending on the cell type, and recovery of the resting potential was observed. It is not yet clear if genuine membrane recovery occurs or ionic pumps are responsible for the repolarization. Improved performance may be achieved by a correct combination of beam intensity, pulse duration, and type and concentration of the photoreactive compound. Supported by a fellowship from Muscular Dystrophy Association.

598 POWER SPECTRA OF VOLTAGE NOISE IN STATOCYST HAIR CELLS. Y. Grossman* and Daniel L. Alkon*. (SPON: E.F. MacNichol). Sec. on Neural Systems, Lab. of Bio., NINCDS, NIH, MBL, Woods Hole, MA. 02543.

Power spectra (PS) and autocorrelation functions of voltage noise were obtained from hair cells in the statocyst of the nudibranch mollusc Hermissenda crassicornis under different conditions. Membrane potentials were recorded on FM Tape (DC-1000Hz) and digtized data were analyzed (0.1-100 Hz) off line on a Fourier Analyzer. Forty percent of the cells (N=20) from normal statocysts had a maximum peak of the PS at frequencies between 6-8Hz. Frequency of hair movement was previously shown to be about 7Hz for hairs experiencing the weight of the statoconia. The cells from statocysts containing only one statoconium (N=5) had a maximum peak at frequencies between 4.5-5Hz. For this type of cell frequency of hair movement was previously shown to be about 5Hz. For cells located in front of the centrifugal force vector rotatory stimulation shifted the noise PS to higher frequencies, increased noise variance and decreased the relaxation time constant (τ_r). The opposite effect occurred for cells located behind the force vector. Treatments such as hypertonicity (1600-2600 mOsmole), cooling (19 → 9°C), low extracellular Na^+, and perfusion with chloral hydrate (3-12 mg/ml), slowed or eliminated hair movement (Grossman et al., 1977). These treatments greatly reduced the noise variance, shifted the PS to the lower frequencies and increased the τ_r (Fig. 1). Current clamp control experiments indicated that these results cannot be accounted for by changes of noise filtering properties of the hair cell membrane during these treatments. These findings suggest that modulation of the voltage noise due to hair cell membrane conductance is caused by the movement of the hairs against the statoconia.

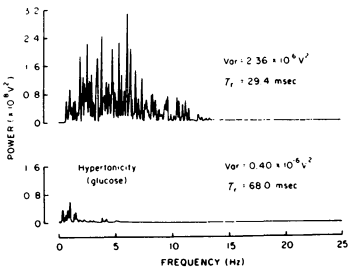


Fig. 1. Effect of hypertonicity on the power spectrum (PS) of a hair cell's voltage noise. Upper record shows PS before and lower record after treatment. Noise variance (var) was reduced, relaxation time constant (τ_r) was increased and the PS shifted to lowest frequencies.

599 PURIFICATION OF A NEUROACTIVE PEPTIDE FROM MOLLUSCAN BRAIN BY AFFINITY CHROMATOGRAPHY ON NEUROPHYSIN-SEPHAROSE. Anthony J. Harmar* and Irwin B. Levitan (SPON: B. Gähwiler). Friedrich Miescher-Institut, P.O.Box 273, CH-4002 Basel, Switzerland.

The mammalian posterior pituitary hormones oxytocin and vasopressin are members of a family of structurally related peptides, all of which possess a chain of 9 amino acid residues with a disulfide bridge linking cysteinyl residues at positions 1 and 6. Such hormones are present in the pituitary of all the (over 40) vertebrate species so far examined, but are commonly believed to be absent in invertebrates. However, this assumption may be incorrect, since:

1). Vasopressin and related peptides initiate or potentiate the "bursting" pattern of electrical activity in certain molluscan neurons.

2). Extracts of molluscan ganglia contain a protease-sensitive factor which has vasopressin-like effects on molluscan neurons.

We have purified a vasopressin-like factor from Helix brain by affinity chromatography on bovine neurophysin linked to Sepharose. The factor binds to neurophysin-Sepharose under conditions optimal for vasopressin binding (0.1 M ammonium acetate, pH 5.7), and like vasopressin may be eluted with 0.1 M formic acid. The purified material has vasopressin-like effects on the electrical activity of Helix cell F-1.

Labelled material, extracted from Helix ganglia following incubation with tritiated amino acids or with 35S-cysteine, can be eluted from neurophysin-Sepharose by 1 mM vasopressin, has a molecular weight of approximately 1000 daltons, and appears to be essentially homogeneous by 2-dimensional chromatography/electrophoresis. The radioactive peptide is distinguishable from all the known neurohypophyseal peptides by electrophoresis at pH 6.5. Physiological and structural studies on the labelled and unlabelled peptides are in progress.

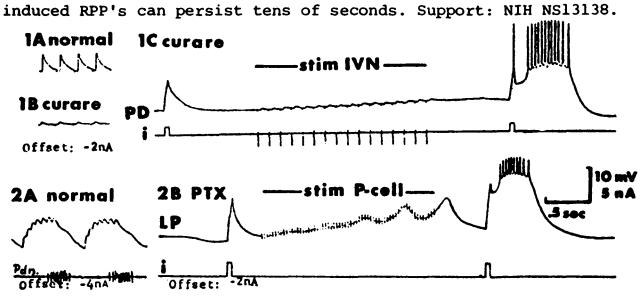
600 INDUCTION OF REGENERATIVE PROPERTIES IN NEURONS OF THE LOBSTER STOMATOGASTRIC GANGLION BY IDENTIFIED NEURAL INPUTS. D.K. Hartline and D.F. Russell. Biology Dept., B-022, UCSD, La Jolla, Ca 92093.

Several inputs induce a "regenerative plateau-potential property" (RPP) in specific neurons: (1) "ivn through-fibers" (IVN) in PD; (2) "hepatopancreas-duct nerve" units in PD and PE; (3) "P" units in LP and maybe other pyloric-follower [these also act as gastric rhythm command fibers]; (4) other "slow" inputs, producing no obvious PSP's, induce RPP's in pyloric-follower cells.

A single-fiber input can both evoke typical EPSP's and induce RPP's in a given postsynaptic cell; the EPSP may be blocked pharmacologically while the induction remains. In Fig. 1A, IVN stimulation evokes EPSP's in a PD; the PSP's are largely blocked by 5 x 10^{-4}M curare (Fig. 1B) yet IVN stimulation still strongly induces (enhances) an RPP in the PD (Fig. 1C).

RPP-inducing inputs may be organized into circuit loops, firing in coordination with a motor rhythm: In Fig. 2A P-cell inputs fire in bursts coordinated with the pyloric rhythm, causing EPSP trains in the LP. P-cell stimulation induces an RPP in LP even with the EPSP largely blocked by 5x10^{-6}M picrotoxin (Fig. 2B). P-cells may thus reinforce the pyloric rhythm by phasic induction, as well as by trains of EPSP's. In the gastric system, brief firing of Interneuron 1 (I1) appears to release a burst of firing in commissural ganglion neurons which then induces a brief RPP in the CP. This "central reflex" may help I1 "drive" CP bursts during the gastric rhythm, reinforcing the weak "direct" excitation of CP by I1.

Since regenerative properties can be induced by neural inputs, we speculate that changes in other cellular properties may also be neurally induced, e.g. (1) sensitivity to neurotransmitter; (2) voltage-dependent conductances (producing e.g. burst termination or phase-shifts in rhythmic patterns); (3) fast action potentials (e.g. in a "non-spiking" neuron to gate its output along an axon). Neural induction may contribute to theories of memory: e.g. induced RPP's can persist tens of seconds. Support: NIH NS13138.



601 SINGLE IDENTIFIED NEURONS PRODUCE PRESYNAPTIC FACILITATION OF THE GILL-WITHDRAWAL REFLEX BY INCREASING CA^{++} CONDUCTANCE IN SENSORY NEURONS OF APLYSIA. R. Hawkins, M. Klein,* and E.R. Kandel. Div. Neurobiol. & Behav., Depts. Physiol. & Psychiat., P&S, Columbia Univ., New York, N.Y. 10032.

Sensitization of the gill-withdrawal reflex in Aplysia is due to presynaptic facilitation at the excitatory synapses made by identified sensory cells on the gill motor cells. This facilitation can be produced in the isolated abdominal ganglion by stimulation of the connectives from the head ganglia. Klein and Kandel (1978) have found that connective stimulation produces 1) a small, long-lasting depolarization in sensory cells, and 2) an increase in the duration of the action potential in sensory cells in the presence of tetraethylammonium (TEA). This spike broadening has been shown to represent an increased Ca^{++} influx which leads to greater transmitter release from the sensory cells.

Hawkins et al. (1976, 1977) have previously identified two neurons in the abdominal ganglion (L28 and L29) which produce facilitation of the EPSPs at the sensory-motor synapses. We have now found that there are at least four other neurons which have similar inputs and are weakly electrically coupled to each other and to L29. We therefore propose to call the five L29-like cells L29A to L29E. Stimulation of a single L29 cell produces broadening of sensory neuron spikes in the presence of TEA, as well as a long-lasting depolarization in sensory cells. All L29 cells which have produced facilitation have also produced broadening of the sensory neuron spike, whereas other cells have not. As few as eight spikes in an L29 cell can cause a 100% increase in the duration of the action potential in a sensory cell. The spike broadening in sensory cells persists for several minutes, as does the facilitation produced by L29. Stimulation of one L29 cell can produce broadening in many (perhaps all) of the sensory cells, and two different L29 cells can produce broadening in the same sensory cell.

Thus, single facilitating neurons exert relatively widespread modulatory effects on the population of sensory neurons. Intracellular injection of electron dense marker into a facilitating neuron now allows a morphological analysis of how these widespread modulating effects are produced.

602 A NEW THORACIC LIMB INNERVATION PATTERN OF A DECAPOD CRUSTACEAN. Russell H. Hill* and Fred Lang. Boston Univ. Marine Pgm., Marine Biol. Lab., Woods Hole, Mass. 02543.

Experiments designed to study inhibition in the closer muscle of the claws and walking legs of Homarus americanus revealed that the inhibitory innervation differs from that previously described for the superfamily Nephropsidea. Earlier studies have suggested that the stretcher and closer muscles share a common inhibitory axon while the bender, extensor, and accessory flexor share another common inhibitor. The present evidence indicates that the stretcher and opener each receive a specific inhibitor while the closer muscle shares a common inhibitor with the bender and extensor. These conclusions rest on the following observations: 1) closer muscle tension from the slow closer excitor (SCE) was inhibited only when hyperpolarizing IPSP's were present in the closer, bender, and extensor muscles. 2) Inhibition of SCE tension was never observed with stretcher or opener IPSP's. 3) Each of three spikes recorded from the closer nerve could be identified as a response of the fast, slow, or inhibitory axon. Tension produced by SCE was inhibited only when the inhibitor spike was present. The appearance and disappearance of the inhibitor spike was always correlated with the presence or absence of IPSP's in the closer, bender, and extensor muscle fibers. 4) When the closer nerve was stimulated in the mid-propodite region, antidromically-evoked IPSP's were elicited in the bender and extensor. 5) Focal stimulation of nerve branches on the bender muscle resulted in inhibition of closer tension. 6) Stretcher and opener IPSP's were independent of each other and of those in the closer, bender, and extensor. 7) No IPSP's or EPSP's were seen in any of the antagonistic muscles concomitant with the IPSP's of the closer, bender, and extensor.

The hyperpolarizing IPSP's were found only in specific areas of each of the three muscles sharing a common inhibitor. Stimulation of the inhibitor had no apparent effect on tension or EPSP's produced by the fast closer excitor. In Homarus americanus a basis may exist for support of the much earlier proposal that antagonistic muscles are inhibited during contraction of agonists.

Supported by grants from NSF and NIH.

603 IONTOPHORETIC MAPPING OF ACETYLCHOLINE RECEPTORS ON THE SOMA, AXON AND IN TWO DISTINCT NEUROPILES OF A GIANT MOLLUSCAN NEURON. Philip. E. Hockberger and Michael B. Merickel, Neural and Behavioral Biology Program and Dept. of Physiol. and Biophysics, Univ. of Illinois, Urbana, IL 61801.

It is a well-established phenomenon that extra-synaptic receptors are found on the somata of molluscan neurons. There is, however, surprisingly little information on the distribution and properties of receptors on the axon, the region where the vast majority of synapses are known to occur. Specifically, we wished to determine whether receptors are localized to discrete regions, or whether they are more or less randomly distributed throughout the membrane.

We selected the gastroesophageal neuron (G-cell) in Anisodoris nobilis due to its accessible structure and because there is abundant information on its physiological and biophysical properties. The soma and axon are both large (approx. 350 um and 50 um, respectively), and consequently can be impaled with microelectrodes for intracellular recording and dye injection. In addition, the G-cell has two anatomically and physiologically distinct neuropiles (Gorman and Mirolli, J. Exp.Biol.53: 727-736, 1970) which can be readily investigated. The ability to directly visualize the G-cell axon and its neuropilar regions allows precise placement of the iontophoretic electrode.

Various technical and statistical considerations were incorporated into the analysis in order to normalize the ACh responses recorded distally from the iontophoretic locus. The results demonstrate that ACh receptors are localized within each neuropile but not on the axon adjoining them. Responses from the soma proper were less predictable, even though the axon hillock region was usually very responsive. All ACh responses were depolarizing and blocked by d-tubocurare (10^{-4}M), suggesting that the receptors are of the same type.

The demonstration that ACh receptors are localized to the neuropilar and somatic regions is very interesting, but not surprising, Most previous studies on molluscan neuron receptors have been based on the soma response characteristics only. Our results suggest that the lack of a somatic response to a putative neurotransmitter does not eliminate the transmitter from consideration. Additionally, it appears that molluscan receptors may not be as non-localized as previously throught. (Supported by RIAS grant #NSF SER 76-18255).

604 AXONAL ORGANIZATION OF THE DORSAL MESOTHORACIC NERVE INNERVATING THE DORSAL LONGITUDINAL FLIGHT MUSCLE OF DROSOPHILA MELANOGASTER Kazuo Ikeda and Takashi Tsuruhara*. City of Hope Natl. Medical Center, Duarte, CA 91010.

The arrangement of motor axons controlling the longitudinal flight muscle (DLM) was studied with light microscopic (K.I.) and electronmicroscopic (T.T.) methods. The six bilateral pairs of DLM fibers are referred to as 45-a through f from dorsal to ventral (Miller, 1950) and correspond to DLM 6 through DLM 1 in our previous report. These DLM fibers are innervated by five bilateral pairs of motor axons. Four of the five axons separately innervate muscle fibers f through c. The 5th axon innervates b and a commonly. The nerve containing these axons first bifurcates into medial and lateral branches at the ventrolateral surface of f so that the DLMs are innervated both on the medial and lateral sides. For a short distance proximal to the bifurcation, as well as subsequent ones, the axons were observed to lie in one plane parallel to the muscle surface. The medial branch made three major branches, i.e., anterior, central, and posterior. The anterior and posterior branches contained two axons innervating f and e. The central branch contained three other axons and further bifurcated, making anterior dorsal and posterior dorsal branches. The anterior dorsal branch contained two axons innervating the anterior portions of d and c. The posterior dorsal branch contained three axons, two innervating the posterior portions of d and c separately and one innervating b and a. The branches containing two axons (anterior and posterior for f and e, anterior dorsal for d and c) ran parallel to those muscle fibers along the border between them, then made many fine branches innervating their respective muscle fibers. The lateral branch bifurcated and innervated in a similar manner. Thus, particular sets of motor axons (f-e, d-c) run in close association with each other for fairly long distances so that activity in one axon might have an effect on the other. It is interesting to note that the amount of association of these axons correlates very well with the degree of interaction which is observed in the phase relationships between sets of muscle fibers innervated by these axons (Harcombe & Wyman, 1977; Koenig & Ikeda, 1977).

Supported by USPHS NIH grant NS-07442 and H.D. Foundation grant.

605 NEURONAL CIRCADIAN RHYTHM IN APLYSIA: TEMPERATURE COMPENSATION OF THE PHASE SHIFTING INDUCED BY A PROTEIN SYNTHESIS INHIBITOR. Jon W. Jacklet. Dept. Biol., Univ. at Albany, N.Y., 12222.

Anisomycin, a potent inhibitor of protein synthesis in eucaryotes, causes phase dependent shifts in phase of the circadian rhythm in electrical activity recorded from the eye of Aplysia in vitro (Science 198, 69). Delays in phase up to 15 hours or advances up to 5 hours are obtained depending upon the phase of the rhythm at which the 6 hour pulses of inhibitor (10^{-6}M) are given. Repeating these experiments at 12°, 15° and 17°C showed the effect of the inhibitor at these temperatures is the same: identical phase response curves (PRC's) are obtained. Thus, the inhibitor effects are temperature compensated as is the period length of the circadian rhythm over this range of physiological temperatures for Aplysia. This result supports the hypothesis that protein synthesis at the eucaryotic ribosome is a fundamental part of the rhythm generating mechanism. Similar studies on the circadian rhythm of Acetabularia (P.N.A.S. 73, 3216) by Karakashian and Schweiger using cycloheximide had shown that 5°C changes in temperature (20-25°C) produced quite dissimilar PRC's. The differences in temperature dependence of the PRC's in Aplysia and Acetabularia may be related to the differences in mechanisms for the rhythms or perhaps to differences in the inhibitors used.

Pulses of anisomycin applied to the Aplysia eye rhythm at 17°C near the critical point (circadian time 1-4) for cross over from delays to advances in the PRC produced unusual post-pulse periodicities of the rhythm. Initial delays of 8-10 hours were produced by the pulses but the next period of the rhythm after the initial delay was 50-53 hours (approximately double the normal period) before returning again to the normal period of 26 hours. The clock apparently skipped a beat. This behavior suggests a temporary state of desynchronization similar to splits in this rhythm previously induced by temperature and light pulses.

606 ACETYLCHOLINE FUNCTION IN THE NEMATODE ASCARIS. Carl D. Johnson* and Ira S. Kass* (SPON: P. Claude). Dept. of Zoology, Univ. of Wisconsin, Madison, WI. 53706

The role of acetylcholine in the nematode Ascaris lumbricoides is being investigated by combined techniques of biochemistry and pharmacology. As previously described, (Neurosciences Abstract #555, 1977)among the motorneurons in the body of Ascaris, choline acetyltransferase (CAT) is concentrated only in excitatory cells. Inhibitory motorneurons do not contain CAT. This result is obtained by measuring CAT in strips of hypodermis containing isolated pieces of identified motorneurons. The analysis includes five of the seven classes of motorneurons (Stretton, et al, PNAS in press). It has been extended to include at least five examples of each of 34 identified motorneurons in the animal. Of these 34, twenty have CAT, fourteen do not. There is a complete congruence of excitatory function and elevated CAT level.

Experiments to examine the pharmacology of motorneuron synapses are currently being performed. Each cell is separately stimulated and the effect of drugs on the post-synaptic response determined. Tubocurare ($3x10^{-5}$M) has been shown to block the responses of excitatory motorneurons, consistent with these cells being cholinergic. Excitatory motorneurons in both the ventral and dorsal nerve cords also synapse onto the dendrites of inhibitory motorneurons with output in the opposite cord. In preliminary experiments, these synapses are also blocked by curare.

Throughout the body of Ascaris, low levels of CAT activity were observed in strips of hypodermis which did not contain any nerve. In the anterior part of the first segment and in the tip of the head, this activity increases dramatically and obscures the expected elevated levels of CAT in excitatory motorneurons. The anterior 1mm of the animal (weighing 1mg out of a total 30gm) has about 50-100X as much CAT as the ventral and dorsal nerve cords. This CAT activity appears to be extraneuronal and its function is unclear.

We are also examining the distribution of cholinesterase activity with emphasis on distinguishing between multiple molecular forms. At least four forms of cholinesterase, separable by zone velocity sedimentation in sucrose gradients have been observed. These forms have similiar sedimentation coefficients and kinetic properties to separable forms of cholinesterase previously isolated from the soil nematode Caenorhabditis elegans. The two larger forms are distributed along the animals' length whereas the smaller forms seem to be concentrated in the head.

(Supported by NSF Grant BNS 76-09641 and a Postdoctoral Fellowship from the Muscular Dystrophy Association.)

607 NEUROTRANSMITTER MODULATION AND cAMP CORRELATES OF AFTERDISCHARGE IN NEUROENDOCRINE BAG CELLS OF APLYSIA. L. K. Kaczmarek, K. Jennings* and F. Strumwasser. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The neuroendocrine bag cells in the abdominal ganglion of Aplysia generate a long-lasting synchronous afterdischarge upon brief electrical stimulation of a pleurovisceral connective. Following their afterdischarge these cells become refractory to further stimulation. We find that synchrony, afterdischarge, and prolonged refractoriness are properties that can be expressed in the isolated asomatic neurites of the bag cells. We have distinguished two apparently independent types of refractoriness. The first (type I) is seen as a failure of action potentials generated in the tips of bag cell neurites to invade cell somata (Dudek and Blankenship, J. Neurophysiol. 40: 1312, 1977). This form of refractoriness may be prematurely induced by increases in temperature or by partial substitution of propionate for extracellular chloride ions. The second form of refractoriness (type II) controls the duration of afterdischarge in the neurites such that stimuli after the first afterdischarge (mean duration 30.2 min) produce only very short afterdischarges or fail to elicit an afterdischarge. We have found that the durations of the first and subsequent afterdischarges in each experiment are linearly related to the mean firing rate within 1 min of the onset of each afterdischarge. Type II refractoriness is sensitive to serotonin, dopamine, and the methyl xanthine phosphodiesterase inhibitors. Extracellularly applied serotonin and certain of its analogues suppress an ongoing afterdischarge while dopamine and the phosphodiesterase inhibitors, when applied at the end of the first afterdischarge generate a subsequent afterdischarges of long duration without further electrical stimulation. Moreover the mean duration of the subsequent afterdischarges induced by theophylline were found to be 7.5 times longer than those which could be generated by electrical stimulation at this time. None of these compounds influenced the degree of type I refractoriness. We have shown that both serotonin and dopamine increase cAMP levels in the pleurovisceral connectives (Cedar and Schwartz, J. Gen. Physiol. 60: 570, 1972) and in the bag cell clusters and that the occurrence of an afterdischarge is also associated with a specific increase in total cAMP in the bag cell clusters. cAMP levels peak 2 min after the onset of afterdischarge and thereafter decline to control values. Our data suggests that serotonin and dopamine may control bag cell afterdischarge and that activation of adenylate cyclase is linked to bag cell activity.

608 INCREASE IN PRESYNAPTIC CALCIUM CURRENT ASSOCIATED WITH PRESYNAPTIC FACILITATION MEDIATING BEHAVIORAL SENSITIZATION OF THE GILL WITHDRAWAL REFLEX IN APLYSIA. M. Klein,* and E.R. Kandel. Div. Neurobiol. & Behav., Depts. of Physiol. & Psychiat., Columbia Univ., New York, N.Y. 10032

Behavioral sensitization is an enhancement in the response to a constant stimulus after the presentation of a different (sensitizing) stimulus. Short-term sensitization of the gill-withdrawal reflex in Aplysia is due to a prolonged increase--lasting up to an hour--in transmitter release from the presynaptic terminals of sensory neurons in the abdominal ganglion. The presynaptic facilitation can be simulated with extracellular application of serotonin (5-HT) or with intracellular injection of cyclic AMP (cAMP) suggesting that the facilitation may be mediated by a 5-HT-sensitive adenylate cyclase in the presynaptic membrane (Kandel et al., 1976, Cold Spring Harbor Symp., Quant. Biol. XL:465). In the present study, we examined the hypothesis that presynaptic facilitation results from a cAMP dependent increase in the Ca^{++} current triggered by the action potentials in the sensory neuron.

The action potentials of sensory neuron cell bodies were found to have a Ca^{++} current contribution which is unmasked and enhanced when the K^+ current which normally terminates the action potential is blocked with 0.1m tetraethylammonium (TEA). In TEA the sensory neuron action potential (normally 2-4 msec) shows a slowly repolarizing plateau of about 50 milliseconds duration. This plateau behaves like a Nernst Ca^{++} electrode and serves as a good assay for changes in Ca^{++} current. Electrical stimulation of the neural pathway from the head that mediates sensitization, application of 5-HT or phosphodiesterase inhibitors (isobutylmethylxanthine or Ro-20-1724), as well as intracellular injection of cAMP all increase the calcium phase of the TEA action potential. There is also a strong parallel, both before and after sensitizing stimulation, between the duration of the calcium plateau of the action potential in the cell body of the sensory neuron and the transmitter released by its terminals as measured by the amplitude of the monosynaptic EPSPs evoked in the motor neurons by intracellular stimulation of a single sensory neuron.

These results are consistent with the idea that presynaptic facilitation consists of a cAMP-mediated increase in a voltage sensitive calcium current in the presynaptic terminals of the sensory neurons. This synaptic facilitatory action is novel in that it sometimes produces no change in the resting postential, is of long duration, and exerts its influence on a voltage sensitive conductance triggered by the action potential, rather than on the non-voltage sensitive conductances typical of synaptic actions.

609 CHARACTERIZATION OF THE CONCURRENT INTERVAL CORRELATION BETWEEN FIBERS OF THE BILATERAL PAIR OF DORSAL LONGITUDINAL MUSCLES IN DROSOPHILA. J. H. Koenig* and Kazuo Ikeda. (SPON: J. Holden). City of Hope National Medical Center, Duarte, CA 91010.

Simultaneous intracellular recordings were made from the 12 fibers comprising the bilateral pair of dorsal longitudinal flight muscles in Drosophila. Various analyses of this firing pattern have suggested there are two major influences determining interval size: (1) a common driving input which causes a positive concurrent interval correlation among the fibers of both sides and (2) an input involved in spacing out the firing times of the ipsilateral fibers. This second input would tend to obscure the influence of the first; therefore, a concurrent interval correlation analysis was performed which takes into consideration the influence of this second input, thus allowing a clearer visualization of the characteristics of the first.

Such an analysis reveals an interesting concurrent interval relationship between fibers of both the ipsilateral and contralateral sides. For example, if it is observed that three successive intervals of one fiber exhibit a long-short-long sequence, then the majority of the other fibers will also exhibit a long-short-long sequence. Furthermore, if the duration of the intervals of all the fibers (of both sides) are plotted successively by order of occurrence without regard to which fiber the interval represents, then the long-short-long sequence is observed as a progression of gradually decreasing and then increasing interval sizes.

The observations demonstrate an amazingly high concurrent interval correlation between both ipsilateral and contralateral fibers. Furthermore, the nature of this correlation suggests there is a periodicity to the common input driving this system which has an approximately 1 to 1 relationship with the motoneurons, that is, one period of the common input per one motoneuron interval.

Supported by USPHS NIH grant NS-07442.

610 ENDOGENOUS DOPAMINE IN THE LOBSTER STOMATOGASTRIC GANGLION AND A SINGLE, IDENTIFIED NEURON OF THE COMMISSURAL GANGLION. P.D. Kushner and J.K. Ono*. Dept. of Bio., U.of O., Eugene, Or 97403 and Div of Neurosci., City of Hope Med.Centr., Duarte CA 91010.

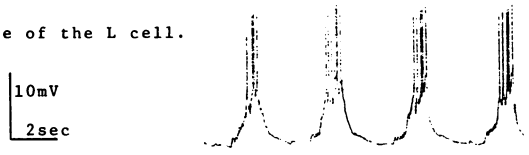
Catecholamines have been localized histochemically in particular nerves and ganglia of the stomatogastric system of spiny lobsters(Kushner and Maynard,'77). Dopamine synthesized from ^{3}H-tyrosine and ^{3}H-DOPA accumulates in those same structures (Barker, Kushner and Hooper,'78). Endogenous assays of dopamine were performed on nerves, ganglia and a single cell of the commissural ganglion according to the method of McCaman et al., '73.

Levels of dopamine found in the stomatogastric ganglion were 1.65 $\pm$ 0.89 pmole/gang. (n=8) and in the L cell 0.31 $\pm$ 0.17 pmole/cell (n=7). With a diameter of approx. 150 um the concentration of dopamine in this cell is estimated at 0.2 mM. Nerves, which in the histochemical assay had very low amounts of detectable catecholamines, gave low or no signal in this assay, although in the previous synthesis experiments there was significant accumulation of ^{3}H-dopamine. The commissural ganglion likewise gave no consistent signal but was found to possess a factor which inhibited the assay.

Significant values of endogenous dopamine in the stomatogastric ganglion confirm the dopaminergicity of the profuse neuropil histofluorescence. Endogenous dopamine values of the L cell of the commissural ganglion establish this cell as the first identified dopamine-containing neuron of arthropods.

The L cell has a constant position and axonal projection within the ganglion. From $CoCl_2$ backfills the cell does not have a process in the stomatogastric nerves (Kushner, submitted). Intracellular recordings have revealed the cell to fire tonically or to burst. Parameters involved in eliciting the tonic or bursting behavior are under investigation.

Burst mode of the L cell.



611 AXOTOMY OR PARTIAL AXOTOMY CAUSES A TRANSIENT INCREASE IN THE ELECTROGENICITY OF CRAYFISH NEURONS. John Y. Kuwada and Jeffrey J. Wine. Dept. of Psych., Stanford Univ., Stanford, CA. 94305.

Ionic channels are unevenly distributed in nerve cell membrane. It is not understood how regional variations in channels are produced and maintained, but under some circumstances the electrical properties of the membrane can be changed. For example, many invertebrate neurons have nonspiking somata and spiking axons. Pitman et al. (Science, 1972, 178, 507) recently demonstrated that nonspiking somata of cockroach motoneurons will support spikes following axotomy or implantation of colchicine; a finding that has been replicated in the locust (Goodman & Heitler, Soc. Neurosci. Abstr. 1977, #1357). We are assessing the effects of axotomy on the somata of neurons in the crayfish because: (a) trophic phenomena are sometimes unusual in this species, for example, distal portions of cut crayfish axons may live for many months and heal by fusion; and (b) in the experiments with insects entire limbs were removed or colchicine was applied to mixed nerves, so the possibility of changes caused by alterations in presynaptic elements could not be evaluated.

The first neuron chosen for study was the peripheral inhibitor to the fast flexor muscles of the crayfish abdomen. The inhibitor has many identified synaptic inputs, a known transmitter (GABA), a nonspiking soma, and an axon which runs in a pure motor root that contains only it and the axons of 6 to 10 fast flexor motoneurons. The main axon of the inhibitor or one of its axon branches can easily be cut through a small slit in the soft ventral cuticle of the abdomen; the axons of the other motoneurons are also cut, but no other neurons are injured. A homologous inhibitor on the other side of the ganglion serves as control.

In control neurons (either intact axon or acutely transected axon), a small (3 to 15 mV) passively conducted spike is recorded in the soma of the inhibitor following either orthodromic or antidromic stimulation. Two days after transection the membrane has changed so that overshooting impulses of up to 120 mV invade the soma. The soma membrane continues to support action potentials until about 2 weeks after axotomy, when spikes again fail to invade the soma. However, the amplitude of passively recorded soma spikes remains elevated for about 5 weeks, while the threshold for spike initiation to injected current is decreased for a similar period. The neuron's time constant increases to a peak about 3X normal and then declines with a time course roughly parallel to the changes in impulse amplitude. The same changes are produced when a single branch of the axon is cut. The implications of these results will be discussed.

Supported by NSF Grant BMS-75-17826, by an NSF Predoctoral Fellowship to J.Y.K., and a Sloan Foundation Fellowship to J.J.W.

612 NEUROTROPHIC INFLUENCES IN LOBSTER MUSCLE: CORRELATION BETWEEN OXIDATIVE CAPACITY AND PATTERN OF INNERVATION. Fred Lang, Mark M. Ogonowski*, W. J. Costello and B. Roehrig*. (SPON: J. Atema). Boston Univ. Marine Pgm., Marine Biol. Lab., Woods Hole, MA 02543

Muscle fibers from crustacean skeletal muscles have generally been characterized according to a dichotomy based on sarcomere length (SL). Fast fibers have short (2-4μm) SL while slow fibers have long (6-12μm) SL. However, we have observed that there is a striking heterogeneity in certain histochemical properties among fibers of a muscle which contains all long-sarcomere fibers. This variability is correlated with the pattern of innervation by the fast and slow motor axons.

The closer muscle of the crusher claw of the adult lobster (Homarus americanus) is composed entirely of long-sarcomere slow muscle fibers and is innervated by two motor axons, a fast and a slow. The majority of fibers (60%) are innervated by both axons while 15% are innervated solely by the slow axon and an equal amount solely by the fast axon. When conventional histochemical techniques are used to analyze the oxidative capacity of the fibers (as reflected by NADH diaphorase activity) there is a distinct staining pattern. Those fibers which receive both motor axons, or just the fast, have a low oxidative capacity while those receiving only the slow axon have a high oxidative capacity. When stained for myofibrillar ATPase activity, these tonic fibers all stain uniformly. Thus it appears that some properties of muscle fibers are correlated with the pattern of innervation by the motor axons while other properties are not correlated with this pattern.

During the early juvenile stages (2 weeks old) both claw closer muscles are virtually identical and each contains 30-40% fast muscle fibers and the remainder are slow (based on SL). The majority of fibers at this stage (> 90%) are innervated by both motor axons and these fibers have low oxidative capacity. Several molts later (4-8 weeks), the claws become asymmetric, with the fast fibers in one (the crusher) transforming into slow fibers. Whether the changes in oxidative capacity precede or follow the changes in innervation pattern is presently under investigation.

Supported by grants from NSF, Muscular Dystrophy Assoc. and by a NIH-NINCDS Research Career Development Aware (to F.L.).

613 CALCIUM AND ENDOCRINE REGULATION OF GENITAL NEURON ACTIVITY IN APLYSIA. F. J. Lebeda, J. T. Haskins and J. E. Blankenship. Marine Biomedical Institute and Dept. Physiol. & Biophysics, Univ. Tex. Med. Br., Galveston, TX 77550.

During late winter and spring months neurons in genital ganglia from A. californica were typically silent and exhibited accommodation to maintained intracellular depolarization in vitro (Lebeda and Blankenship, Soc. Neurosci. 3:183, 1977). However, during the summer and fall, a period of more frequent reproductive activity (Strumwasser et al., Comp. Biochem. Physiol. 29:197, 1969) some of the genital neurons from these animals were spontaneously active and lacked the accommodation response. In contrast, genital neurons from the more regularly egg laying species A. brasiliana did not usually show accommodation and were often observed to have repetitive activity. Thus, the possibility that a hormonal-induced transition occurs between these two electrical states was considered and served as the basis for the following experiments.

First, a reversible transition from the silent, accommodating state to the repetitively firing, non-accommodating state in some genital neurons was seen after bag cell excitation in vitro. Stimulation of these neurosecretory cells releases egg laying hormone, which is assumed to be a low molecular weight polypeptide (Arch, Am. Zool. 16:167, 1976). Second, a similar transition in some cells was observed during exposure to homogenates of the atrial gland (a portion of the large hermaphroditic duct caudal to the spermathecal duct). Homogenates of this tissue have been shown by Arch (J. Comp. Physiol. 1978, in press) to stimulate egg laying in vivo. Although the perfusates collected after bag cell discharge and the atrial gland homogenates were able to release eggs in isolated ovotestis preparations, the latter were much more effective. In a third series of experiments a reversible transition between electrical states was observed when isolated ganglia were bathed in zero-calcium artificial sea water containing 0.5 mM EGTA.

The hypotheses presently being considered are that one or more hormones may be: 1) responsible for species differences in the typically observed genital neuron activity, 2) associated with the experimentally observed transition in the electrical properties of the genital neurons and 3) involved with alterations in calcium binding and/or conductance.

Supported by NIH awards NS05831 (FJL), NS05830 (JTH), NS70613 (JEB), and NSF grant PCM76-18936.

614 BIOCHEMICAL IDENTIFICATION OF SEROTONIN IN VITAL-STAINED NEURONS FROM LEECH GANGLIA. Charles M. Lent, Kent T. Keyser* and Joyce Ono. SUNY Stony Brook 11794 and City of Hope, Duarte, Cal. 91010.

Seven widely-scattered neurons in leech segmental ganglia fluoresce yellow following Falck-Hillarp treatment. The fluorescence in the two colossal (60-80 μm) Retzius cells (RZ) has been shown to be produced by serotonin (5-HT). The remaining five -- an unpaired medial (M) and paired ventro-lateral (VL) and dorso-lateral (DL) -- cells are much smaller (10-15 μm). The presence of 5-HT within these small cells is inferred because until recently, they have been unidentifiable in living ganglia. Neutral Red dye selectively and vitally stains these monoamine cells rendering them identifiable for cellular and biochemical studies.

We measured 5-HT in neural tissues from Macrobdella decora by an enzymatic technique (Saavedra, et. al, J. Pharm. Exp. Therap. 186:508, 1973) which is sensitive to 50 femptomoles of serotonin (50×10^{-15}). The serotonin content of stained and unstained ganglia ranges from 9 to 10.5 picomoles, while that of stained and unstained Retzius cells is from 0.3 to 0.4 p moles. The 5-HT content of neither RZ nor ganglia are significantly affected by the staining procedure. We dissected 18 individual VL cells ($\bar{x}$ diameter, 10 μm) and detected 5-HT in 17 at 0.2 ± 0.2 p moles. Unstained control cells had no measureable serotonin. We calculate the minimum concentration of 5-HT in these small VL cells as 100 mM -- a value exceeding the aqueous solubility of serotonin.

We have also measured the emission curves of each of the identifiable MA cells in the ganglia by microspectrofluorometry after Falck-Hillarp procedures. The emission spectra of RZ, VL, DL and M cells have very similar curves with peaks at 515 to 525 nm. Further, the fluorescence of each of these cell classes fades during exposure to U.V. We suggest from these data that all the fluorescent neurons within leech segmental ganglia contain genuine serotonin. (USPHS fellowship NS05343, J.O; NIH, BMS 75-00464, C.M.L.)

615 RESPONSE PROPERTIES OF PARTIALLY DEAFFERENTED SENSORY INTERNEURONS IN THE CRICKET, ACHETA DOMESTICUS. R.B. Levine. Dept. Biol., SUNY, Albany, NY 12222.

Sensory interneurons in the cricket receive a highly specific set of synaptic inputs from cercal sensory receptors. Both the receptor types which will innervate a given interneuron and their relative strengths must be specified during development. Part of this specificity may depend upon the presence of a normal balance of synaptic inputs during development. In order to investigate this possibility, crickets were reared with only one intact cercus throughout post-embryonic development, thereby partially deafferenting the sensory interneurons. The characteristics of remaining, untreated synaptic inputs were compared to the characteristics of these pathways in animals reared with both cerci intact, by recording intracellularly from identified sensory interneurons. Examples of the types of results which were obtained are presented below.

The medial giant interneuron (MGI) normally receives weak excitatory synaptic inputs from receptors on the cercus contralateral to its axon. The strength of this contralateral excitatory pathway was significantly enhanced in animals reared with only one cercus. This result is in agreement with the similar observation of Palka and Edwards (1974), who used extracellular recording techniques.

Normally, no clear membrane potential changes are observed in the lateral giant interneuron (LGI) upon stimulation of the contralateral cercus alone. The existence of contralateral inhibitory inputs, however, was demonstrated by their ability to reduce the amplitude of antidromically activated spikes which passively invade the dendritic area of the interneuron. In addition, the size of an independently evoked e.p.s.p. was decreased by concurrent sound stimulation of the contralateral cercal receptors. In unilaterally reared specimens the ability of this contralateral input to reduce the size of the antidromic impulse was significantly enhanced. Coincident with this apparent conductance change was a prominent membrane hyperpolarization. Sound stimulation of other contralateral cercal receptors caused a long latency e.p.s.p. which was never observed in control animals.

In summary, the results suggest that the strengths of synaptic inputs to sensory interneurons are not rigidly controlled during development, but can be altered. The results are consistent with the hypothesis that a normal balance of synaptic inputs is essential for the proper development of synaptic specificity.

Supported by N.S.F. research grant No. BNS-7523454 to R.K. Murphey.

616 SEROTONIN RECEPTORS IN HELIX AND APLYSIA: ANALYSIS BY ADENYLATE CYCLASE STIMULATION AND 3H-LSD BINDING. Irwin B. Levitan and Alan H. Drummond*. Friedrich Miescher-Institut, P.O.Box 273, CH-4002 Basel, Switzerland.

Serotonin stimulates adenylate cyclase activity in crude membranes prepared from Helix and Aplysia ganglia. Half-maximal stimulation is obtained with approximately 2 μM serotonin. The stimulation ranges from 20% to 50%, and is usually observed only in the presence of 0.1 mM GTP. A similar response can be obtained using membranes prepared from isolated Helix and Aplysia neuronal cell bodies. The extent of the stimulation by serotonin does not differ significantly among different identified Aplysia neurons. d-LSD, an antagonist which blocks electrical responses to serotonin in molluscan neurons, also blocks serotonin stimulation of adenylate cyclase in membranes from whole ganglia and from isolated cell bodies (Ki = 2-5 nM). In contrast, the pharmacologically inactive stereoisomer l-LSD is without effect on the cyclase stimulation.

3H-LSD binds stereospecifically to dopamine and serotonin receptors in Helix and Aplysia nervous system. Under appropriate conditions each receptor type may be studied individually, using this single radioactive ligand (Drummond, Bucher and Levitan, NATURE 272: 368-370, 1978). A detailed pharmacological analysis is currently under way to determine the relationship between the serotonin receptors labelled by 3H-LSD, those responsible for serotonin-stimulation of adenylate cyclase, and those mediating electrical responses to serotonin in molluscan neurons.

617 INDUCED L9 ACTIVITY PREVENTS HABITUATION OF THE GILL WITHDRAWAL REFLEX EVOKED BY SIPHON STIMULATION IN APLYSIA. Ken Lukowiak* (SPON: J.F. MacDonald). Dept. Physiol., McGill Univ., Montreal, Quebec, Canada, H3G 1Y6.

The effect of induced activity in central gill motor neurons L_7, LDG_1, and L9 on the gill withdrawal reflex and its subsequent habituation evoked by tactile stimulation of the siphon was studied in 20 preparations. Each preparation was tested for habituation at least 2 times: 1 series with L9 depolarized to produce 1-5 AP's/sec and one series with L9 free running. In most preparations L_7 or LDG_1 was also recorded from and depolarized on a 3rd series. Each habituation series was followed by a rest period of at least 3 hrs. The intensity of the tactile stimulus was 1 gm. The following results were obtained with L_7 and LDG_1 depolarization: 1) induced low level activity (1-3 AP's/sec, which does not elicit an observable gill withdrawal movement) resulted in an increased gill reflex amplitude but had little, if any, effect on the rate or degree of gill reflex habituation; 2) the synaptic input (as evidenced by the number of AP's evoked per trial) continued to decrement as in the control series. Thus, induced activity in L_7 or LDG_1 does not affect gill reflex habituation or the synaptic decrement associated with it. The following results were obtained with L9 depolarization: 1) induced activity in L9 also affected gill reflex amplitude, though not to the same extent as did L_7 or LDG_1; 2) induced activity prevented the occurrence of habituation of the reflex and in some cases resulted in reflex potentiation over the series; 3) in preparations already habituated, induced L9 activity brought about its reversal; 4) the synaptic input to L9 as well as to L_7 or LDG_1 continued to decrement even though the reflex no longer habituated. L9 activity can thus prevent habituation of the gill withdrawal reflex or bring about its reversal without affecting synaptic decrement. This property appears to be peculiar to L9 and not of central motor neurons in general. L9's affect probably occurs in the periphery and not in the PVG. L9 may be part of the CNS control system which exerts both facilitatory and suppressive control over the PNS and gill reflex behaviours.

Supported by the Medical Research Council of Canada.

618 PRESSURE INDUCED CONVULSIVE SEIZURES IN SQUID. Diana W. Mann. Institute of Marine Biomedical Research, University of North Carolina at Wilmington, 7205 Wrightsville Avenue, Wilmington, North Carolina 28403.

The effects of pressure on the nervous systems of terrestial vertebrates have been extensively studies. The complex of symptoms (culminating in convulsive seizures) seen under high pressure has been called the High-Pressure Nervous Syndrome (HPNS).

Lologuncula Brevis, a local species of squid, were compressed in natural seawater, hydraulically, in the high pressure aquarium system. These invertebrates exhibit a clear and reproducible pattern of HPNS symptoms: Pre-seizure abnormal activity includes rapid chromatophore flashing, waxing and waning of very fast fin movements, often with fibrillation, uncoordinated swimming, tentacle tetany episodes and "sinking spells". Most of these symptoms are also seen in very stressed animals at 1 atm. The seizure onset is sudden and consists of a sequential pattern of most of the following symptoms: fast jet swimming, loss of body position in space, tentacle tetany, inking and mantle contracture. The seizure may be followed immediately by death, or recovery of activity after a quiet post-ictal phase and/or additional seizures. Rapid decompression led to successful recovery in a few individuals.

Convulsion threshold pressures (P_c) ranged from 30 to 130 atms, depending on temperature, compression rate, and individual variability. Temperature was controlled to the selected value ± 0.5° C throughout each experiment and each animal was run within 1-2° of the ambient seawater temperature at the time of capture. Oxygen concentrations were essentially saturated.

At 18° C there was a significant compression rate effect. With fast compressions of 10 atm/min, P_c averaged 97 atm (N=8) in strong contrast to P_c=54 atm (N=8) at a rate of 0.5 atm/min. Preliminary data suggests that temperature alters the compression rate dependence of P_c. The positive slope of the P_c/logP relationship is opposite to that seen in vertebrates (see Brauer et al, J. Appl. Physiol. 38:220-227, 1975). The apparent compression rate effect is due to some interaction between time at pressure and the pressure magnitude as shown by fast compression and hold experiments.

619 *EFFECTS OF VARIOUS POLLUTANTS ON THE RESTING MEMBRANE POTENTIALS OF BLUE CRAB MUSCLE FIBERS.* *Luis A. Marco and Wallace E. Jenkins* Dept. of Psychiatry, MUSC, and VA Hospital, Charleston, SC 29403.*

As part of a larger study of heavy metal, herbicide, and insecticide pollutants, the effects of various concentrations of mercuric chloride ($HgCl_2$), Diquat, and Mirex on the resting membrane potentials (RMP) of adult blue crab (Callinectes sapidus) muscle fibers were investigated. Glass micropipettes filled with saturated solutions of KCl and conventional electronic equipment were used. Small bundles of 50-100 fibers from various walking leg muscles were laid on the recording chamber and stretched to approximately rest length prior to impalement. RMP in artificial normal sea water averaged -78 mV±4mV. $HgCl_2$ at concentrations of 50, 25, 15, and 5 ppm gradually lowered RMP to final values of (-) 13, 24, 48 and 62 mV respectively. $HgCl_2$ at 2.5 ppm did not alter RMP during 60 min. periods of exposure. The largest drops in RMP were reached after 30-35 min. of exposure to the poison. No further deterioration was observed beyond 35 min. This effect was irreversible despite several washes with normal sea water. The herbicide Diquat (ethylene-dipyridylium dibromide) did not alter the RMP at concentrations of 1 ppm, or 1, 3, and 44 ppt. The insecticide Mirex ($C_{10}Cl_{12}$) was also ineffective in lowering RMP at the concentrations so far investigated (200 ppb, 1 and 20 ppm). The normal RMP obtained are equivalent to those published by Hayes et al. (Comp. Biochem. Physiol. 26:761, 1968), the only ones available for comparison. There are no published findings of the effects of these agents on muscle fibers of this crustacean. Shealy and Sandifer (Mar. Biol. 33:7, 1975) found concentrations of 0.0005-0.05 ppm of Hg to be toxic to larval grass shrimp. 50% mortality of adult brine shrimp is caused by $HgCl_2$ concentrations ranging from 0.1 to 10 ppm depending on exposure time (Brown and Ahsanullah, Mar. Pollut. Bull. 2:182, 1971). Doyle et al. (Environ. Contamin. & Toxicol. 16:422, 1976) reported 8 to 0% survival chances of adult crayfish after 96 h. exposure to 2.5 and 5 ppm of $HgCl_2$, respectively. Ambient concentrations of mercury are usually lower than those tested here but they increase sharply around industrial pockets, bottom and sewer waters. Although no symptoms of poisoning were observed in juvenile blue crabs during a 96 h. exposure to 0.1 ppm of technical Mirex in flowing sea water, a delayed effect (irritability, paralysis, death) occurred within 18 days after being placed in Mirex-free water (Lowe et al., U.S. Dept. Inter. Circular 335, 1970). A similar delay effect 17 h. after exposure to Mirex 1 ppm for 1 h. or following 19 h. exposure to Diquat 3 and 4 ppt could not be demonstrated in our fiber preparations. (Supported by South Carolina Sea Grant R/PS-1 and Slocum-Lunz Foundation.)

620 INHIBITORY RESPONSES EVOKED BY CHOLINERGIC AGONISTS IN CRUSTACEAN STOMATOGASTRIC GANGLION NEURONS. Eve Marder and Danièle Paupardin-Tritsch*. Laboratoire de Neurobiologie, Ecole Normale Supérieure, Paris 75005 France.

In order to use pharmacological agents as tools in transmitter identification in the stomatogastric ganglion and at other Arthropod central synaptic connections, we have undertaken to characterize the pharmacological properties of the responses to ACh and other cholinergic agonists of the cells of the stomatogastric ganglion of the crab, Cancer pagurus.

In addition to the previously described depolarizing and excitatory effects most often found with iontophoretic applications of ACh, or other nicotinic and muscarinic agonists(Marder & Paupardin-Tritsch, J Physiol, 1978, in press), we now show that the muscarinic agonist acetyl-β-methyl-choline(MeCh) can elicit inhibitory responses which are due to an increase in K^+-conductance.

Cells of the stomatogastric ganglion were penetrated with double barrel microelectrodes, and agonists were applied iontophoretically from single or double barrel electrodes placed in the neuropile region of the ganglion. Responses were studied under either voltage clamp or current clamp conditions. MeCh applications at some neuropile sites produced biphasic responses at resting potential(-60mV), with an early depolarizing phase(inward current) followed by a slow hyperpolarization(outward current). The reversal potential of the inhibitory phase was -80mV, and raising the external K^+-concentration two-fold shifted the reversal potential of the inhibitory potential 17mV in the depolarizing direction. The inhibitory responses were not blocked in 20 mM $CoCl_2$ or 0 Ca^{++}, high Mg^{++}, thus showing that these responses were not synaptically mediated. Preliminary pharmacological studies have shown that high concentrations of both nicotinic and muscarinic agonists were relatively ineffective in blocking these responses, possibly because of inaccessibility, or because the receptors are insensitive to those agents thus far tried.

On occasion MeCh biphasic responses were found at neuropile sites at which ACh applications elicited purely depolarizing responses. Our current hypothesis is that ACh applications activate a ubiquitous extrajunctional, nicotinic depolarizing receptor, and thus routinely mask cholinergic inhibitory potentials. E.M. is a postdoctoral fellow of the Helen Hay Whitney Foundation.

621 SENSORY DEPRIVATION IN THE CRICKET CENTRAL NERVOUS SYSTEM: EVIDENCE FOR A CRITICAL PERIOD. S.G. Matsumoto and R.K. Murphey. Dept. of Neurobiology, Harvard Medical School, Boston, MA. 02115 and Dept. Biol. Sci., S.U.N.Y. Albany, Albany, N.Y. 12222.

The cercal sensory system of the cricket, Acheta domesticus develops extensively during the organism's postembryonic period of maturation. The majority of the adult complement of cercal-sensory neurons differentiates during this time. Sensory input is important for the development of the normal physiological characteristics of some of the neurons in this system. Chronic sensory deprivation leads to a decrease in the responsiveness of an identified interneuron, the medial giant interneuron (MGI), to acoustic stimulation (Matsumoto and Murphey, 1977, J. Physiol. 268: 533-548.).

The severity of the loss in sensitivity of the MGI depends upon the developmental stage at which deprivation is initiated and its duration. Treatment regimens of constant duration (instars) have progressively weaker effects the later they are begun. Deprivation for five instars beginning in the first, second or third instar alters the response properties of the MGI while later treatments have no effect. A recovery process is not a factor since the period of normal activity following the last deprivation treatment is longest in those cases where the MGI shows the greatest modification. No sensory neuron degeneration was detected in the earliest instars after the application of the deprivation procedure.

Treatments initiated at hatching (i.e. first instar) required a minimum of four instars to produce an effect when the animals were tested as adults. Recordings from immature specimens indicate that a partial recovery from the effects of early deprivation can occur with long post-treatment periods. Specimens deprived for the first four instars exhibit a greater degree of depression when examined in the sixth instar than in the adult (tenth instar).

We conclude that there is a well defined period during the early postembryonic development of the cercal sensory system in which it is sensitive to a reduction in neuronal activity. Manipulations of the activity levels after this period have no effect.

This work was supported by an NSF grant #BNS 752345A01 awarded to R.K.M.

622 NEURONS OF THE CEREBRAL GANGLION OF APLYSIA WITH EXTENSIVE MONOSYNAPTIC EXCITATORY CONNECTIONS PROJECTING TO NEURONS IN OTHER GANGLIA. Richard E. McCaman and David G. McKenna*. Div. Neurosci., City of Hope Nat'l. Med. Ctr., Duarte, CA 91010.

Two bilaterally symmetrical clusters of neurons in the cerebral ganglion of Aplysia californica have been shown to project to follower cells in the abdominal, pedal, pleural and cerebral ganglia. The follower cell response is, in every instance, a monophasic EPSP which voltage clamp experiments demonstrate has an underlying increased conductance mainly to Na^+. The monosynaptic nature of these connections has been assessed by the persistence of the EPSPs in elevated Ca^{++}, graded reduction in elevated Mg^{++} and augmentation after intracellular injection of TEA into the presynaptic neurons. A portion of this highly divergent network has been previously described by Fredman and Jahan-Parwar, (Br. Res. 100, 209, 1975) who described connections between the neurons of the A cell clusters (presynaptic) and the B cell clusters (follower cells) within the cerebral ganglion. There are approximately 18-20 A cells in each bilaterally symmetrical cluster and a single A cell has been found to have as many as 30-50 follower cells in the various ganglia.

We have attempted to characterize the transmitter mediating these connections by combined chemical and pharmacological experimentation. Microchemical assays for various putative transmitters (5HT, ACh, DA, histamine, GABA and Oct) indicate that these substances are not present in the A neurons. The A-cell content of various amino acids (glu, asp, taur, gly) are in no way different from levels found in other pigmented Aplysia neurons.

Approximately 40 different compounds (including various biogenic amines and amino acids) were applied from microelectrodes by pressure ejection or iontophoresis to various follower cells. Only two compounds, 5HT and glu, were observed to produce a depolarization involving an increased conductance mainly to Na^+. 5HT does not appear to be the transmitter since a) bath perfusion of bufotenin (10^{-4}M) completely blocks the 5HT evoked response but has no effect on the EPSP recorded from the follower cells and b) as mentioned previously the A cells (presynaptic) do not contain measurable 5HT. Glutamate does not appear to be the transmitter since a) bath perfusion of glutamate (10^{-5}M) desensitizes and completely blocks the glutamate-evoked response while even at 10^{-3}M it has no effect on the EPSP; and b) bath perfusion of quisqualic acid (10^{-4}M) completely blocks the glutamate-evoked response but has no effect on the EPSP recorded from the follower cells. Thus, the transmitter utilized to mediate this extensive array of connections is unknown but there remains the possibility that it may be structurally related to glutamate. (Supported by N.S.F., BNS 76-06053 and U.S.P.H.S., NS 09339).

623 FEEDING AND REJECTION IN PLEUROBRANCHAEA: COMPARISON OF TWO BEHAVIORS USING SOME OF THE SAME MUSCULATURE. Andrew D. McClellan*. (SPON: R. Plonsey). Dept. Biomed. Eng. and Dept. Anat., Case Western Reserve University, Cleveland, Ohio 44106.

The organization in the nervous system of behaviors which use some of the same muscular and neural elements has been a long standing problem in neurobiology. Some muscles of the buccal mass of Pleurobranchaea are used in two similar behaviors, feeding and rejection (regurgitation). A typical feeding response involves proboscis extension and is accompanied by rhythmic jaw opening and radula movement, which brings food into the buccal cavity. A stereotypic rejection sequence, which may last 20-60 minutes, is comprised of periods of active rejection during which material is expelled from the buccal cavity. Rejection also involves proboscis extension as well as rhythmic jaw opening and radula movement; however, the net result is obviously different from feeding. The range of frequencies of radula movement during feeding overlaps with the range seen during rejection. During the rejection sequence and for several hours afterwards the feeding response is suppressed; this may represent modification of motivational state.

Appropriate muscles and nerves were recorded from during feeding and rejection to compare the motor programs. A substantial part of the motor programs for feeding and rejection are similar, as might be predicted from the resemblance of the two behaviors. The relationship between the phase of a major radula protractor muscle and cycle period (measured from a retractor muscle) is similar during feeding and rejection, suggesting that a common pattern generator is operating during both behaviors. Sharing of part of the motor program may be a mechanism for conservation of neural and muscular elements. Several of the components present during the feeding motor program are either altered or absent during rejection. The functional differences between two similar behaviors may represent an early step in the evolutionary divergence of behaviors in the nervous system. The two behaviors are presently being compared neurophysiologically to determine their organization in the central nervous system.

(Supported by NIH (PHS) traning grant GM-01090 to author, and NSF grant BNS 76-81233 to G. Mpitsos, Dept. Anat.)

624 METABOLISM OF TYRAMINE IN THE CENTRAL NERVOUS SYSTEM OF THE MOTH *MANDUCA SEXTA*. Marcia M. Moore*, Gerald D. Maxwell, and John G. Hildebrand. Dept. Neurobiol., Harvard Med. Sch., Boston, MA 02115

Tyramine, a prominent candidate for a role as a neurotransmitter or neuromodulator, is synthesized from tyrosine in the CNS of the moth *Manduca sexta* (Maxwell and Tait, Soc. Neurosci. Abstr. 3:409, 1977). We have now studied the metabolism of [^{3}H]tyramine in *Manduca* central nervous tissue *in vitro*. Structures were removed from pharate adult *Manduca* and incubated for 6 hr in 50 µl of modified Grace's insect tissue culture medium containing 94 µM [^{3}H]tyramine. After incubation metabolites were extracted from the tissues and separated by high-voltage electrophoresis at pH 1.9.

Three major metabolites of [^{3}H]tyramine were detected. One has been identified as N-acetyltyramine on the basis of the following evidence: (1) the compound co-migrates with authentic N-acetyltyramine when chromatographed on paper in 4 different solvent systems and when electrophoresed at pH 1.9, 6.4, and 9.3; (2) the compound is hydrolyzed to tyramine under the conditions of pH and temperature that convert standard N-acetyltyramine to tyramine; and (3) both the compound and standard N-acetyltyramine are converted to tyramine by an N-acyl amino acid hydrolase.

The second metabolite has several chemical properties compatible with its identification as tyramine-O-sulfate. Incubation of tissue with both [^{3}H]tyramine and $^{35}SO_4$ resulted in incorporation of tyramine and SO_4 into the metabolite in nearly equimolar amounts. This metabolite has no net charge at pH 1.9. When exposed to 1 N HCl at 100° for 1 hr, it was hydrolyzed to tyramine. This behavior is consistent with that reported for sulfated metabolites of monoamines from the lobster (Kennedy, J. Neurochem., 30:315, 1978).

The third metabolite is positively charged at pH 1.9 and exhibits electrophoretic and hydrolytic behavior consistent with the hypothesis that it is an N-aminoacyl derivative of tyramine, reminiscent of the β-alanyloctopamine found in lobsters (Kennedy, Soc. Neurosci. Abstr. 3:252, 1977).

N-acetyltyramine is produced in substantial amounts by nervous tissue, tracheae, and connective tissue, but not by muscle. Production of the other two metabolites is greater in nervous tissue than in tracheae, connective tissue, or muscle.

These findings support the view that acetylation, sulfation, and other modifications possibly including N-aminoacylation may be important processes of monoamine metabolism in Arthropods.

This research was supported by a Radcliffe DuPont Fellowship to MMM, an NIH Postdoctoral Fellowship to GDM, and NSF Grant BNS 77-13281 to JGH.

625 POSTEMBRYONIC DEVELOPMENT OF AN IDENTIFIED INTERNEURON IN THE CRICKET ACHETA DOMESTICUS. R.K. Murphey. Dept. Biol., SUNY, Albany, NY 12222.

A number of recent experiments focus attention on the postembryonic development of the medial giant interneuron (MGI) of the cricket. Two types of experiment suggest that normal synaptic inputs to the MGI are susceptible to modification during development. In one experiment removal of some inputs to the MGI resulted in enhancement of the remaining inputs. In a second experiment activity in the afferents which excite the MGI was lowered (sensory deprivation) during development, and this resulted in a decrease in the sensitivity of the MGI to standard stimuli. Both experiments suggest questions which can be answered only by recording from the MGI in very young specimens.

The techniques previously reported (e.g. Murphey 73) for recording from adults have been adapted, virtually unchanged, to immature specimens. Recordings from the MGI have been obtained in specimens as young as the fourth (of 10 instars). The MGI was injected with cobalt to confirm its identity. Morphologically the neuron is very similar to the adult MGI. As in the adult, the MGI was directionally sensitive, its response increased monotonically with increasing stimulus intensities, and it received its primary excitatory input from the contralateral cercus.

The recordings were unusual in one respect, all electrical activity appeared to be electrotonically closer to the somatic recording site. Passively invading action potentials had faster rise times in the immature specimen than in the adult. Synaptic potentials also exhibited faster rise times. In some preparations, the unitary potentials giving rise to the compound postsynaptic potential (psp) which occurs upon acoustic stimulation could be distinguished. Finally, unlike adults, hyperpolarization greatly augmented the psp produced by standard stimuli and blocked action potentials in MGI, while depolarization reduced the amplitude of the psp and increased the number of action potentials in response to a standard stimulus. These results suggest that the soma recording site is a better "window" into neuron function in the immature specimen than it is in the adult.

These results provide the techniques and the baseline data upon which studies of the effects of sensory deprivation on the developing MGI will be based.

Supported by NSF research grant BNS-7523454.

626 NEURAL REGENERATION IN THE SNAIL, HELISOMA TRIVOLVIS. A. Don Murphy and S. B. Kater. Dept. of Zool., University of Iowa, Iowa City, Iowa 52242.

The advantageous characteristics afforded by gastropod molluscan nervous systems for neurophysiological studies are well documented. However, only recently have such molluscan preparations begun to be exploited for neurodevelopmental studies. The snail, Helisoma trivolvis, offers the opportunity to study neural regeneration of identified molluscan neurons with defined synaptic connections and behavioral roles. We have examined neural regeneration of the paired salivary effector neurons (4R and 4L) which are located in the buccal ganglia. These neurons send axons out the esophageal nerve trunks and innervate the salivary glands. The use of the salivary neuroeffector complex combined with the development of an in vivo organ culture technique has facilitated these studies. The salivary glands of Helisoma display excitatory post-synaptic potentials or action potentials in response to action potentials produced in neuron 4 (J. Exp. Biol. 72, 77-90 and 91-106, 1978). Thus they provide a readily available electrophysiological assay for peripheral neural regeneration and functional reinnervation of target cells. The culture technique involves explanting the paired buccal ganglia, the muscular buccal mass, and the salivary glands of one snail and implanting this neuroeffector complex in the heamocoel of a host snail. The esophageal nerve trunks containing the axons of a pair of identified salivary effector neurons located in the buccal ganglia were crushed, thus severing connections between the ganglia and the salivary glands. Within as little as five days salivary neuroeffector neurons functionally reinnervated the glands such that action potentials in neuron 4 again evoke excitatory postsynaptic potentials usually suprathreshold for action potentials in salivary gland cells. The morphology of regenerate neurons was visualized following intracellular injection of the intensely flourescent stain Lucifer Yellow CH. This neural regeneration was manifested by the growth of extensive sprouts from the proximal axon stump to the salivary glands.

627 PARALLEL PROCESSING IN THE CRAYFISH OCCULOMOTOR SYSTEM: DATA AND A HYPOTHESIS. H.B. Nudelman and R.M. Glantz. Univ. of Tex. at San Antonio and Rice Univ., Biol. Dept.,Houston,Texas 77001.

The representation of information in nervous systems by parallel ensembles of neurons may be broken into two classes; one in which the input neurons receive the same information (e.g., spindle afferents from one muscle) and one in which the input neurons receive different information (e.g., compound eye). Here we are interested in the second class. The study of this problem requires that we must simultaneously record from many neurons at once since there may be information useful to the animal, in the timing between action potentials on different channels. This information cannot be obtained from sequential single unit recordings. Furthermore, it would be useful if we had identified units to work with so that hypothesis could be made and tested. The crayfish occulomotor system's response to light position satisfies both of these criteria.

Previous studies by the authors (Fed.Proc:Vol. 36,7-1977 and J. Neurophysiol. Vol. 39,6,1976) indicate that under high physiologic intensity broad field illumination pairs of sustaining units (SU) could be made to fire in near synchrony,i.e., their cross correlations showed peaks around zero. This cross correlation decreased as the intensity was decreased and disappeared at low intensity.

In order to establish the physiological significance of these cross correlations between SU's, behavioral measurements were made on crayfish under the lighting conditions that were known to produce them. As had been observed in other crustaceans, the crayfish oriented their eyestalks with respect to the position of the light source. This response was to the position of the light and not tracking of the light source, since the light was presented at random positions and moved in the dark between these positions. The eyestalk position response lost its statistical significance at reduced light intensity levels were the SU's lost their cross correlation.

While recording from a pair of SU's from an eye fixed to the carapace it was found that the cross correlation between the two units varied as a function of the position of the light source.

It is hypothesized that for different light positions different groups of SU's will show significant cross correlation around zero and it is in this fashion that the light position is represented by the ensemble of SU's. This scheme of encoding positions may be simply decoded. If the sustaining units project to the appropriate motor neurons decoding will take place by spatial summation of the convergent input.

Data to support this hypothesis will be presented.

628 SPECTRAL SENSITIVITY OF OPTOKINETIC TRACKING IN THE CRAYFISH, PROCAMBARUS. Richard F. Olivo and William M. Petkun*. Dept. Biol. Sci., Smith College, Northampton, MA 01063.

The function of the blue-sensitive receptors in the crayfish eye remains elusive; so does the answer to the question of whether crustacean motion-detection systems are color blind, as insect systems are. We partially answer the first question by showing here that the blue receptors contribute to the optokinetic tracking system.

Restrained crayfish (Procambarus clarkii) were suspended at the center of a sinusoidally-oscillating (9.9 cyc/min, 41° excursion) drum consisting of alternating opaque and translucent (20°) stripes. We previously showed that the amplitude of sinusoidal tracking depends on the luminance of the stripes (Neurosci. Abstr. 3:581, 1977). In the present experiments, monochromatic light at intervals from 420 nm to 680 nm illuminated the drum evenly from outside; at each wavelength, neutral density filters were placed in the light path to vary the stripe radiance (range: 2 - 100 nW/cm^2), and the corresponding amplitude of optokinetic tracking was measured. The illuminating light was monitored continuously with a calibrated radiometer.

For each experiment, the stripe radiance required to elicit a criterion response was determined for each wavelength, and a log relative quantum sensitivity spectrum was calculated. Spectra from 14 experiments on 6 animals were averaged to obtain the mean spectral sensitivity; the average spectrum matches published spectra of the dark-adapted ERG.

Since the blue receptors' contribution to the ERG spectrum is not detectable without selective adaptation to red light, we repeated our experiments with blue or red adapting lights flooding the inside of the drum. The normalized blue-adapted spectrum is the same as the non-adapted spectrum; both represent essentially the response of the yellow receptors alone. The red-adapted spectrum has a second peak in the blue, and thus shows that the blue receptors do contribute to the optokinetic system.

629 CRAYFISH GIANT FIBERS ARE COMMAND AND DECISION NEURONS. Gene C. Olson* and Franklin B. Krasne. Dept. of Psychology, UCLA, Los Angeles, California 90024

Recently, it has been argued that in order for a neuron to be considered a command neuron, its firing should be both necessary and sufficient for producing behavior. It has also been pointed out that a neuron that is considered a command neuron by virtue of these criteria could be either the initial issuer of the order (i.e., a decision neuron) or merely the conveyer of an order issued elsewhere. (See Kupfermann, I. and Weiss, K.R., The Command Neuron Concept, The Behav. and Br. Sci., in press 1978.)

The crayfish lateral giant fibers (LGs) are often considered to be among the best established examples of both command and decision neurons. LG firing is perfectly correlated with the occurrence of short latency tail-flip escapes to mechanical stimulation of the abdomen, and directly applied shocks which fire the LGs produce tail-flips. It is widely believed that the LGs are necessary for this tail-flip behavior, but the available evidence does not exclude the possibility that LG firing merely serves to increase the rate of intersegmental propagation of excitation to motor neurons and that should the LG firing be prevented, tail-flips, though imperfect, would still occur, -- i.e., the LGs might not be necessary. Nor does available evidence exclude the possibility that, though the LGs receive numerous excitatory synapses directly from mechanoreceptors and first-order sensory interneurons, a substantial additional input from a specific pre-LG decision neuron is necessary to carry the membrane potential over firing threshold -- i.e., the LGs might not be decision neurons.

Therefore: (1) We have studied the effects on tail-flip behavior of hyperpolarizing both LGs so that they cannot fire. We find that a shock to primary afferents that causes fast flexor contractions when the LGs are permitted to fire does not do so when hyperpolarization prevents LG firing. (2) We have examined afferent fiber shock-evoked EPSPs in the hyperpolarized LGs just above and below the stimulus level that causes firing of the unpolarized LG. This should uncover possible decision neuron inputs whose rising portions merge smoothly with rising spikes. We find no sign of any special boost of excitation to the LGs (from a possible decision neuron) as the stimulus strength crosses threshold. We conclude that the LGs do appear to be both command and decision neurons. (Supported by USPHS Grant NS08108.)

630 A COMPARATIVE STUDY ON THE INFLUX OF RADIOACTIVE TRYPTAMINE AND 5-HYDROXYTRYPTAMINE BY SNAIL (HELIX POMATIA) NERVOUS TISSUE. Neville N. Osborne* and Volker Neuhoff* (SPON: L.T. Graham) Forschungsstelle Neurochemie, Max-Planck-Institut für experimentelle Medizin, 3400 Göttingen, Germany.

Isolated snail ganglia possess the ability to concentrate tryptamine from an external medium by a process which is saturable at very high concentrations of amine in the medium and temperature sensitive, thus able to produce a net uptake of tryptamine. Kinetic analysis of the influx of tryptamine shows that the amine is taken up by a single transport system with K_m value of 1.4×10^{-4}M. The influx of tryptamine into snail ganglia is thus fundamentally different from that of its hydroxylated analogue 5-HT (5-hydroxytryptamine), where the accumulation shows rapid saturation kinetics, is sodium dependent, slightly inhibited by ouabain and also temperature sensitive. Kinetics of 5-HT uptake show the influx to be divided into a high affinity mechanism with a K_{mH} value of 8.5×10^{-8}M (sodium sensitive component) and a low affinity mechanism with a K_{mL} value of 1.8×10^{-4}M (sodium insensitive component). The influx of tryptamine is thus, in principle, similar in character to the low affinity uptake mechanism for 5-HT, i.e. sodium insensitive with relatively high K_m value. Further evidence that the tryptamine influx mechanism is similar in character to a low affinity uptake system for 5-HT is shown by the fact that the tryptamine influx and low affinity 5-HT uptake are not influenced by a number of pharmacological agents, analogues of tryptamine and ATP. The high affinity uptake mechanism for 5-HT in contrast is very sensitive to pharmacological agents (e.g.: chlorimipramine, imipramine), analogues (e.g.: tryptamine) and a variety of other substances.

631 OCTOPAMINE MODULATES SENSITIVITY OF IDENTIFIED INSECT INTERNEURONS. Michael O'Shea* and R. K. Murphey (SPON: B. C. Abbott) Dept. Biol. Sci. USC, Los Angeles, CA 90007 & SUNY, Albany, N.Y. 12222.

The terminal ganglion of the cricket, Acheta domesticus, contains the somata and dendrites of giant auditory interneurons with axons ascending in the ventral nerve cord. These neurons receive excitatory input monosynaptically from sensory neurons which innervate sound sensitive hairs on the antennae-like anal cerci. Electronmicrographs of one of the giant interneurons, the Medial Giant Interneuron (MGI), reveal a close association between the MGI dendrite and another neuron containing large neurosecretory-type vesicles. These vesicles are similar to those of dorsal aminergic neurons in the locust, a related insect.

Neutral Red dye, which stains monoamine containing neurons, revealed about 40 large neuronal cell bodies on the dorsal midline of the terminal ganglion. These putative aminergic neurons are similar to dorsal neurons in the locust, some of which are known to be octopaminergic. For example, they have overshooting soma action potentials and project axons bilaterally to peripheral nerves. In the locust, one of these efferent aminergic neurons functions peripherally as a modulator of neuromuscular transmission; central nervous functions have not yet been attributed to them. Some of the dorsal neurons in the cricket do not project peripherally but ramify extensively in the neuropile of the terminal ganglion. Central functions of the dorsal neurons in relation to the MGI are now being sought.

We have not yet found a dorsal neuron which affects the sensitivity of the MGI but to date less than 10% of the neurons have been tested. Octopamine, however, has a specific sensitizing effect. Local perfusion of octopamine (10^{-5}—10^{-4}M) produces an increase of 10 to 20% in the response of the MGI to sound. The following amines are without effect at 10^{-4}M; tyramine, noradrenaline, dopamine and 5HT. Only synephrine is an effective agonist of octopamine. The sensitizing effect is blocked by the α-adrenergic blocking agent, phentolamine. These results are consistent with the pharmacology of the peripheral effects mediated by an octopaminergic neuron in the locust and suggests that an octopaminergic neuron, possibly among the dorsal group, causes sensitization of the cricket MGI in the terminal ganglion.

Supported by: Cellular Biology Section, University of Southern California (M.O.) and NSF Grant #BNS 7523454A02 (R.K.M.).

632 IS ADAPTATION DEPENDENT ON Ca^{++}? L. Donald Partridge. Dept. of Physiology, Univ. of New Mexico, Albuquerque, N.M., 87131.

Spike frequency adaptation is a process basic to the encoding mechanisms of neurons. In Archidoris neurons, adaptation has been shown to result from the gradual increase of a slowly activating potassium conductance during a train of spikes (Partridge & Stevens, J. Physiol. 256:315). Similar slow potassium currents have been found to be activated by Ca^{++} ions in numerous other molluscan neurons (Meech & Standen, J. Physiol. 249:211). The potassium conductance responsible for adaptation in Helix pomatia has been indirectly attributed to such a Ca^{++} activating mechanism (Colding-Jorgensen, Acta. Physiol. Scan. 101:382). The experiments reported here were designed to test for such a role of Ca^{++}.

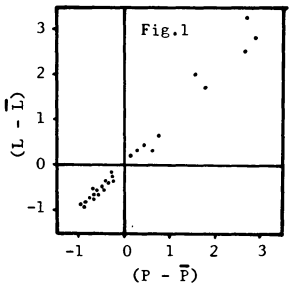
Ganglion cells from Helix aspersa were studied. In an attempt to block inward Ca^{++} currents, experiments were done using the following bathing solutions: 7mM Co^{++} and 0 Ca^{++}; 2 mM Cd^{++} and 7 mM Ca^{++}; 1mM EGTA limiting free Ca^{++} to 10^{-9} M. In each case either the progressive diminution of spike amplitude and increase in spike width or the decrease in amplitude of peak inward current was used as a test for effective blockage of inward Ca^{++} current. Although there was considerable variability amongst cells, spike frequency adaptation was generally found to persist in the absence of inward Ca^{++} currents.

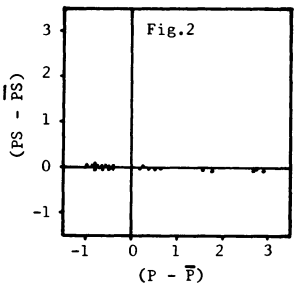
It is concluded that there exists a Ca^{++} independent process that is capable of producing spike frequency adaptation in these neurons. This is consistent with the original model proposed by Partridge & Stevens of a voltage dependent activation producing a gradual summing potassium conductance during a train of spikes.

633 A CENTRAL MOTOR PROGRAM FOR UROPOD BEATING IN THE ANOMURAN CRAB, EMERITA ANALOGA. Dorothy H. Paul. Dept. of Zoology, Oregon State University, Corvallis, OR 97331.

Two rhythmic motor patterns in the same motoneurons underly beating of the pair of swimming appendages (uropods) in the sand crab, Emerita analoga. In motor pattern no.2, associated with treading water, generation of bursts in the power-stroke motoneuron (PS) is dependent on proprioceptive feedback from return strokes (1). The previous attribution of motor pattern no.1, associated with swimming, to a central pattern generator is now supported by the recording of rhythmic activity in the appropriate motor nerves (central motor program) in excised portions of the ventral nerve cord including from 4 to 7 of the most posterior ganglia. The temporal organization of this central program shares 2 salient features with both the behavior (2) and motor pattern no.1 (1;2): the positive correlations of PS latency and period of return-stroke motoneuron bursts (RS) have similar slopes (Fig.1; 1;2) and PS durations are nearly invariant (Fig.2;2). The central motor program appears to be organized around a PS burst generator, since PS bursting may occur without RS bursts. Two differences of the central program compared to the behavior and motor pattern no.1, the brief and labile RS bursts and the longer periods, suggest an important role of sensory afferents and/or anterior neural centers in prolonging RS bursts and in maintaining normal frequencies of uropod beating.

(1) D. H. Paul. J. exp. Biol., 65, 243-258, 1976.
(2) D. H. Paul. Z. vergl. Physiol., 75, 233-258, 1971.

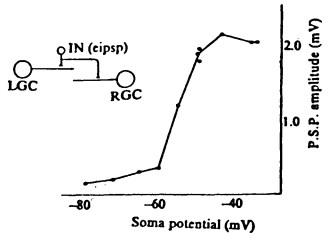




Variation in PS latency (L-$\bar{L}$), Fig.1, and variation in PS duration (PS-$\bar{PS}$), Fig.2, correlated with variation in period (P-$\bar{P}$). $\bar{L}$ = 4.17+1.04 s.; $\bar{PS}$ = 0.41+0.04 s.; $\bar{P}$ = 4.87+1.03 s.; N = 28.

634 CONTROL OF TRANSMITTER RELEASE BY SOMA POTENTIALS IN THE APLYSIA CNS. B. Peretz° and T. Shimahara*. Labo. N. B. C. of CNRS 91190 Gif s/ Yvette, France. °Dept. of Physiology, U. of Ky., Lexington, Kentucky 40506.

In an Aplysia monosynaptic pathway, imposed hyperpolarization of the pre-cell soma decreased the size of the PSP amplitude and depolarization increased it (Shimahara & Peretz, 1978). In the isolated pleural ganglion, the post-cell, left giant cell (LGC) and the interneuron (IN), which evoked EIPSPs in LGC, were each impaled with two microelectrodes. When the IN soma potential was displaced from -30mv to -110mv, and the LGC soma at its resting level of -55mv, the relationship between IN soma potential and PSP amplitude was described by an S-shaped curve (see fig.). In the linear region of the curve, -45 to -60mv, the "control constant" is 0.2mv (PSP)/mv (soma potential). At IN of -45mv (resting), PSP amplitude was close to maximum. Polarization for less than 50ms, sufficient time to alter IN spikes, had no effect on PSP size; synaptic delay of 7 ms was unchanged with imposed soma polarization; these results exclude the possibility that altered spikes are sufficient to explain the effect. With spike blockade by 94μM of TTX in the bath, soma depolarization at about +5mv initiated release and was sustained for at least 2 sec; electrotonic coupling between IN and LGC was excluded with TTX. We conclude that soma polarization controls release at the terminals. Control probably is by electrotonic spread to the terminals and involves Ca: Increased Ca++, 33mM, partially protected release from hyperpolarization and enhanced the effect of depolarization; decreased Ca++, 3.3mM, had the reverse effect. Post-tetanic potentiation, characteristic of this synapse, was enhanced by soma depolarization and suppressed by hyperpolarization. One electrical function then of large molluskan somata, those electrically close to their terminals, is to modulate transmitter release by changes in membrane potential even though a spike successfully initiates the release. (Macy Found., INSERM, NIMH).



635 EFFERENT INFLUENCE OF THE CEREBRAL GANGLION ON PHASE SETTING OF THE CIRCADIAN OSCILLATOR IN THE APLYSIA EYE. Robert G. Prichard* & Marvin E. Lickey. Dept. of Psy., U of OR, Eugene, OR 97403.

The eye of Aplysia is a self contained circadian oscillator. Circadian rhythms change their phase in response to light pulses applied at different phases of their oscillation (PRC). Most circadian rhythms are also reset by an LL→DD transition produced by transfering the organism from continuous light (LL) to continuous darkness (DD). An LL→DD transition acts as a "dusk signal" and sets the phase of most oscillators to subjective dusk (ct. 12) if at least 12 h of LL preceed the transition. Aplysia eyes were exposed to an LL→DD transition after increasing durations of LL (500 lux) to determine 1) if they too could be set to ct. 12 by a transition and 2) if the other oscillators and photoreceptors Aplysia are known to possess contribute to the resetting of the ocular oscillator. Animals were exposed to at least 5 cycles of LD 12:12 before receiving from 1 to 69 h of continuous light. Just before the end of LL the eyes and the cerebral ganglion were dissected and placed in vitro for recording. One eye was isolated by cutting the optic nerve; the other was left attached to the cerebral ganglion. The preparation was then placed in DD and the phase of both eye rhythms determined. For durations between 1 and 12 h, the transition had little effect on either isolated or attached eyes. For durations between 24 and 69 h, the transitions set both isolated and attached eyes to ct. 12 ± 3h; the phase to which the eyes were set oscillated ± 3 around ct. 12 once for each additional 21 h of LL. This shows that continuous light did not stop the oscillator. For durations between 12 and 24 h, attached and isolated eyes responded differently to the LL→DD transition. This caused a phase difference to develop between the attached and detached eyes. For example, following 21 h of LL the phase difference was around 12 h. This demonstrates an important contribution by the cerebral ganglion to the phase setting of the eye. The time course of the contribution has been examined following 21 h of LL by cutting the attached eye's optic nerve at hourly intervals from 1 to 10 h after the transition. Initial results indicate that the first 5h of neural communication produces only a minor phase difference between the attached and isolated eyes. Ten hours of communication produces the full 12 h phase difference. Therefore the major influence of the cerebral ganglion on the eye occurs between the fifth and the tenth hour after the LL→DD transition. Since the first 5 h of communication has little effect, cerebral photoreceptors are unlikely to be the direct source of the efferent influence. Experiments are being performed to determine if a cerebral oscillator may be involved. Supported by NS 12374

636 GRADED SYNAPTIC TRANSMISSION BETWEEN SPIKING NEURONS DURING THE GENERATION OF A CENTRAL MOTOR PATTERN. Jonathan A. Raper* (SPON: J. Lamborghini). Dept. of Neuroscience. UCSD, La Jolla, CA 92093.

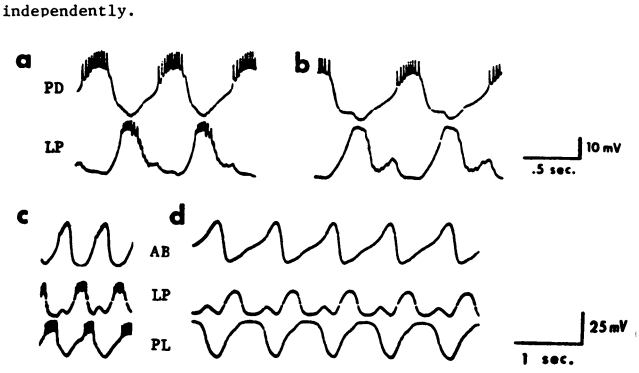
Graded synaptic transmission between spiking neurons can be shown to exist during spontaneous cyclic activity in the pyloric subsystem of the spiny lobster stomatogastric ganglion. Localized TTX perfusion across the posterior margin of the ganglion suppresses pyloric spiking but preserves slow wave activity and central activating connections. Graded inhibitory synaptic actions can be observed from neurons which normally fire action potentials (control fig. 1a, localized toxin blocks LP in 1b).

Bath application of TTX sufficient to block spikes also halts spontaneous cycling. Bath applied TTX plus 1 mM Dopamine induces patterned cyclic activity without action potentials, but in other respects similar to normal behavior (control fig. 1c, DA + TTX 1d).† Induction involves the activation of endogenous bursting properties in the AB, and possibly plateau potentials in the LP. Graded transmission is responsible for the proper phasing of units. Its presence can be inferred by the reversal of postsynaptic waveforms with injected current and postsynaptic disinhibition with presynaptic hyperpolarization.

These results suggest that graded synaptic transmission could play an important role in the physiological functioning of a central pattern generator composed of spiking neurons.

(Support: PH 55-732-607--7153 and NIH NS13138 to D. Hartline.)

†W. Anderson and D. Barker have made similar observations independently.



637 SENSORY MODULATION OF RHYTHMIC FEEDING IN LIMAX MAXIMUS. Stephen C. Reingold & Alan Gelperin. Department of Biology, Princeton University, Princeton, NJ 08540.

We are using feeding behavior of the terrestrial mollusk Limax maximus to study modulation of a rhythmic behavior by sensory input.

Feeding is largely accomplished by rhythmic protraction and retraction of a rasping radula and supportive odontophore, each cycle of which results in ingestion of small amounts of food. The central neurons responsible for generation of the feeding motor program incorporate a pattern generator within the buccal and cerebral ganglia.

In intact, behaving animals, radula-odontophore movements during feeding on an artificial diet (carrot, $CaCO_3$, vitamins in an agar matrix) can be recorded with a movement transducer. A feeding bout from a 2-weeks starved animal consists of as many as 1000 bites over 20 minutes, with a characteristic warm-up period (5-30 cycles) during which instantaneous bite frequency increases slightly. A plateau phase of near constant bite frequency follows. Termination of feeding can be abrupt or can show a slight decrease in bite frequency.

Parameters of feeding behavior such as meal duration, amount of food eaten, and bite frequency can be measured as a function of characteristics of the food (type and concentration of food substance; hardness of agar matrix). When hard foods (15% agar) and soft foods (3% agar) were presented alternately to animals, bite frequencies were consistently lower for hard foods than for soft foods. Changes in bite frequency as a function of food hardness imply afferent influences on the output frequency of the CNS pattern generator for feeding.

In highly dissected preparations consisting of lips, brain, and buccal complex (musculature, medial tooth, radula and odontophore) feeding motor program and appropriate buccal complex movements can be triggered by food extracts delivered to the lips. Extracellular electrodes record sensory input from the buccal complex and motor output underlying feeding movements. Artificial loads added to buccal complex structures in such preparations mimic the effects of food hardness in intact animals. In many cases, changes in load on the medial tooth and on the radula result in changes in frequency of feeding motor output which are similar to changes seen in intact animals presented with foods of different hardness.

Supported by NIH grant 5F32NS95188 and NSF grant BNS 76-18792

638 SCORPION LEG MOTOR SYSTEM: MICROSCOPIC ANATOMY AND ELECTROPHYSIOLOGY. Thomas M. Root and Robert F. Bowerman. Dept. of Zoology, Univ. Wyoming, Laramie, WY 82071.

Seven different muscles in the walking leg of the scorpion (Paruroctonus mesaensis) were studied with light microscopy, electron microscopy and intracellular recording. This represents the broadest such study of an arachnid neuromuscular system to date. These muscles control the three most active leg joints (T-F,F-P & P-T) during walking (Root & Bowerman 1978, Bowerman & Root 1978). Compared to crustacean and insect muscles, scorpion leg muscle fibers are strikingly uniform in sarcomere length both within and between muscles, and are relatively short (2-4 microns). The fibers are small (15-30 microns diameter) and are closely packed within the muscle. The fibers consist of a central region of nuclear material and mitochondria surrounded by a radially arranged pattern of contractile elements. The radiating myofibrils bifurcate near the fiber surface and may constitute up to 80% of the fiber cross-sectional area. The sarcoplasmic reticulum forms alternating dyads with the T tubules as has been previously described for Limulus, spider walking leg muscles, and the scorpion claw closer muscle. Data were collected from each of the seven muscles on fiber numbers, fiber cross-sectional area, thin-thick filament ratios, banding patterns and synaptic ultrastructure. Intracellular recording together with tension measurements have elucidated neuromuscular characteristics for each muscle, and innervation patterns for the entire leg. Values were obtained for resting potential, input resistance, number of motor axons per muscle fiber and per muscle. Features such as facilitation and fatigue are also described.

639 MOLT-RELATED "NEUROSECRETORY" CELLS IN THE ABDOMINAL NERVE CORD OF THE CRAYFISH, PROCAMBARUS CLARKII. Richard L. Roth* (SPON: Jeffrey J. Wine). Dept. Biol. Sci., Stanford Univ., Stanford, CA 94305.

Near the rostral margin of each thoracic and abdominal ganglion of the crayfish, Procambarus clarkii, there are two pairs (one on either side of the midline) of apparent neurosecretory cells. In the 6th abdominal ganglion there are an additional 16 such cells along the caudal margin. As a class, these cells are unique among neuronal somata of the ventral nerve cord in that their cytoplasm contains two systematically and inversely fluctuating populations of granules. The granules of one of these populations are about 0.5um in diameter, are fuchsinophilic by Gomori's aldehyde fuchsin method, and those in each cell are distributed into about 80 compact clusters of several hundred granules. The granules of the other population are about 0.25um in diameter, are intensely argyrophilic by the Nauta method and are uniformly dispersed except where they are displaced by clusters of fuchsinophilic granules.

The fuchsinophilic granules are discharged (apparently directly from the cell body rather than via an axonal channel to a neurohemal organ) near the time of ecdysis. There is some diminution in granular content in the day or two preceding the molt, and depletion is nearly total by 12 hours after ecdysis. Reaccumulation of fuchsinophilic granules is apparent by the 3rd post-molt day and appears to progress at a uniform rate for 4-6 weeks.

The argyrophilic granules are maximally demonstrable 1 day after ecdysis and appear to decline in number as fuchsinophilic granules accumulate. Whether the argyrophilic granules represent a separate secretory product or a precursor or part of the synthetic machinery involved in the formation of the fuchsinophilic granules is not yet known.

There is indirect evidence from coablt backfills that these apparent neurosecretory cells may also serve as ascending interneurons. Efforts to confirm this suggestion by intracellular recording with marker-filled microelectrodes are now in progress.

It is also of some interest that a brief transition from aggressive to escape behavior occurs in conjunction with the discharge of the fuchsinophilic product. Whether the behavioral shift is causally related to the neurosecretory activity is under investigation.

Supported by NIH Grant NS 02944 to D. Kennedy.

640 FIRING PATTERNS OF APLYSIA WHITE CELLS: EFFECTS OF TEMPERATURE AND MENTHOL. Ronald L. Seaman, Dept. of Physiology, Univ. of Texas Health Science Center, Dallas, Texas 75235.

Intracellular recording was used to monitor the firing rates of the neurosecretory white cells (R3-R14) in the abdominal ganglion of Aplysia californica. The rate of spontaneous firing was measured for constant temperatures between 0 and 28 °C. After a temperature change, the firing rate was allowed to stabilize before a measurement was made. Some firing patterns did not fully equilibrate after 20 minutes at a constant temperature, especially at lower temperatures. Such long equilibration periods may account for some of the differences in firing rates reported for the white cells. The response of most white cells (67%) contained a maximum rate of 0.024 to 0.263 per second between 15 and 21 °C. The others (33%) responded with faster firing rates as temperature was increased, reaching a rate of 0.6 to 0.8 per second at 22 °C. Menthol (0.1 and 1 mM) in the bathing medium affected the peaked response by increasing the magnitude of the peak, by shifting the peak to a higher temperature, or both. The peak was increased by as much as 2.5 times. Menthol (0.1 mM) did not significantly change the second type of response.

A regular rate was the most commonly observed firing pattern; however, in some cells there were instances of grouped activity resembling that exhibited by the ganglion's bursting pacemakers. The white-cell bursting patterns were much slower. They consisted of groups, or bursts, with 2 to 100 action potentials and of duration as long as 250 seconds. The interburst intervals ranged from 30 to 450 seconds. Bursting patterns were seen in the 18 to 27 °C range for both types of temperature responses. The bursting was distinct from firing patterns which developed as the result of temperatures above 30 °C. These abnormal patterns were repetitive but the firing was irregular and often contained attenuated action potentials. Bursting was seen in the presence of menthol (0.1 and 1.0 mM) in the same temperature range in the cells bursting without menthol.

In addition to bursting activity, the menthol-induced changes in the peaked responses strengthen the case for those white cells being models for "cold" thermoreceptors. The white cells showing the menthol-insensitive monotonic response may be models for "warm" thermoreceptors. The similarity between the firing patterns of white cells and bursting pacemakers seems to indicate that there is little difference in electrical properties between them other than the dynamics of slow potentials. The firing patterns of both groups of neurons depend on temperature, suggesting temperature-sensitive rate processes. The term "beating pacemaker" may be appropriate for the white cells only under certain conditions.

(Supported by a Biomed. Res. Sup. Gr. administered by UTHSCD)

641 INKING IN APLYSIA: CENTRAL LOCUS OF SELECTIVE SENSITIVITY TO LONG DURATION STIMULI. E. Shapiro,* J. Koester, and J. Byrne, Dept. Physiol., Columbia Univ., New York, N.Y., and Dept. Physiol. Univ. of Pittsburgh, Pa.

Aplysia californica release ink in response to strong noxious stimuli. Ink release has been shown to have a high threshold and a steep input-output relationship when measured as a function of stimulus amplitude (Carew and Kandel, 1977). We have found that ink release also exhibits a steep input-output relation when measured as a function of stimulus duration.

Three types of behavioral experiment demonstrated that ink release is selectively sensitive to long-lasting noxious stimuli (electrical shocks). In two experiments stimulus amplitude was kept constant and we measured either amount of ink released as a function of stimulus duration or percentage of animals that released ink at given stimulus durations. In the third experiment we determined a strength-duration curve for the threshold for a "full" ink response (50% of releasable ink). In all three experiments, which covered a stimulus duration range of up to five sec, a sharp, steplike increase in stimulus effectiveness occurred for stimuli greater than about two sec.

To determine the cellular mechanisms that underlie this low-pass property of inking behavior, we recorded from and voltage-clamped the ink motor neurons (L14 cells). These are three nearly identical abdominal ganglion cells previously described by Carew and Kandel (1977). The release of ink in response to a constant frequency spike train of up to five sec duration in cell L14A was found to be a linear function of train duration with a 0-intercept of the Y-axis. Thus peripheral facilitation cannot account for the behavioral results. A five sec electrical stimulus to the head or to the connectives that carry sensory input to the ganglion produce a spike pattern in the L14 cells consisting of a brief 1-2 sec pause followed by an accelerating burst of spikes. The initial pause has previously been shown to be attributable to a rapidly activating outward current (Byrne, et al., 1976, Neurosci. Abstr.) We have now found that the late accleration in L14 spike activity in response to a five sec noxious stimulus is due to an increase in synpatic effectiveness: a slow, decreased-conductance EPSP that is recruited with some delay accounts for the late increase in firing rate. The presence of this EPSP was demonstrated by voltage-clamp examination of L14 synaptic currents. An increase in L14 input resistance and a positive shift in PSP reversal potential was correlated with L14 burst behavior.

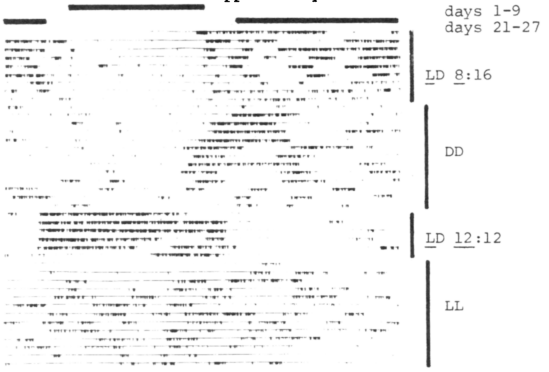
In the companion abstract Byrne describes in greater detail the ionic mechanisms responsible for the anti-accommodation firing pattern of the L14 cells.

642 PLANARIA CAN BE ENTRAINED TO LIGHT CYCLES AND EXPRESS A FREERUNING RHYTHM OF LOCOMOTOR ACTIVITY IN CONSTANT DARKNESS. John T.F. Smith, C.M. Loer*, and J.L. Larimer. Department of Zoology, University of Texas, Austin, TX 78712.

It is known that Planaria express daily cycles of feeding by making more cannibalistic attacks at night than in the day (Best, 1960). They have a daily cycle of sensitivity to electrical shock (Becker-Carus, 1970). Lunar cycles of orientation in magnetic fields have also been demonstrated (Brown, 1962). We found that Planaria entrain to light cycles and can express a freerunning circadian rhythm of locomotor behavior.

Dugesia were collected locally. Locomotor activity of single animals was monitored in small rectangular glass chambers (55mm x 15mm x 5mm) fabricated from micro slides. Activity was detected as the animal moved through an infrared beam and recorded on an event recorder. The detector could be useful for other small animals.

Shown here is the 41 day actograph of a single Planarian given different light regimen. The first 9 days were light cycles of 6 hrs light (1 lx), indicated by the bar above the record, and 18 hrs of darkness, LD 8:16. Activity was limited to the dark period. The next 12 days constant darkness, DD, prevailed and the animal appeared by inspection to freerun with a period of 23.6 hrs. The following 6 days were LD 12:12. The animal was again active during the dark. When placed in constant light, LL, activity occured in bursts of about 1 hr seperated by 2 hr "rest" periods. This behavior persisted throughout the day with no circadian component. No tidal or lunar rhythms in the behavior of Planaria were evident. Supported by NIH NS 05423.



643 SECRETORY SOURCES OF EGG-LAYING INDUCTION ALSO INFLUENCE THE ISOLATED APLYSIA HEART. T. Smock‡ S. Arch‡ and P. Lloyd* (SPON: D. Rhodes). Biological Laboratories, Reed College, Portland, OR 97202, and Zoology Department, University of Washington, Seattle, WA 98195.

Recently there has been interest in endogenous peptides that excite the heart of certain gastropods. Experiments by P. Lloyd (pers. comm.) on the Helix aspersa heart in vitro reveal that one of the peptides has a molecular weight of ca. 6000 and an alkaline isoelectric point. The biochemical similarity of the molecule to egg-laying hormone (ELH) of a related mollusc, Aplysia californica, suggested the hypothesis that the cardio-accelerating substance was a homologue of ELH. To evaluate this hypothesis, a preparation consisting only of the ventricle, auricle and cristae aorta was isolated from a freshly killed Aplysia and perfused with filtered sea water. The ventricle was cannulated through a slit approx. 5mm from the junction with the auricle. Cotton thread secured a small amount of heart muscle to the cannula. A tight ligature was necessary as the heart would not beat without positive internal fluid pressure. A hook through the cristae aorta was connected to a force transducer. Moderate tension between the hook and the cannula induced the heart to beat regularly after 1-2 hours of perfusion. As expected, 5-HT (10^{-9}M) and acetylcholine (10^{-8}M) had excitatory and inhibitory effects, respectively, when applied to the inside of the ventricle through the cannula. A survey of Aplysia tissue homogenates revealed two major loci of cardio-accelerating activity: the neuroendocrine bag cells of the parieto-visceral ganglion and the "atrial" gland of the large hermaphroditic duct. These two organs are also the sources of egg-laying factors in Aplysia. Gel filtration chromatography of extracts of each tissue revealed that the cardio-excitatory activity co-elutes with egg-laying activity at about 6000 daltons. In both cases the activity was sensitive to digestion with bacterial protease. Though the heart was much more sensitive than egg-laying as a bioassay, the relative amounts of cardio-accelerating activity in the two tissues paralleled the amounts of egg-laying activity as determined by serial dilution. Further, we found that electrical stimulation could increase release of the active substance from the atrial gland. Since the heart responds to samples that contain only 1/20 the material needed to induce egg-laying, it seems likely that it will prove to be a useful preparation for rapid and accurate bioassay. Since bag cell secretion also affects electrical activity of identified neurons that alter heart rate (Mayeri et al., J. Neurophysiol., in press) it seems that the neurodocrine effector may intervene at several levels of organization in the physiology of the animal.

644 SYNAPTIC PHYSIOLOGY AND PHARMACOLOGY OF A WHITE, PUTATIVE COMMAND INTERNEURON (C2) IN TRITONIA DIOMEDEA. Robert Snow. Dept. of Zoology, Univ. of Wash., Seattle, WA 98195.

A pair of visually identifiable neurons (C2) in the cerebral ganglia of Tritonia diomedea are thought to be command cells for escape swimming. Their activity is apparently required for the initiation of each swim cycle (Getting, J. Comp. Physiol. 121: 325, 1977: Taghert and Willows, J. Comp. Physiol. 123: 253, 1978). Antidromic stimulation of all of the brain roots suggests that C2 is an interneuron having no peripherally directed processes. C2 make contralateral synaptic connections with all cells sampled (N>60) in the pedal and pleural ganglia. Evidence that these connections are monosynaptic includes 1) they are not blocked by high Ca^{++} and Mg^{++} ASW, and 2) presynaptic polarization affects the efficacy of all psp componants (Shimahara and Tauc, J. Physiol. 247: 299, 1975).

Four different psps have been identified, and all four have been recorded together in some of the post synaptic neurons. These psps are 1) a fast (40 msec to peak), Cl^- dependent ipsp that is blocked by 10^{-4} M curare (dTC) and reverses at -56 mV, 2) a slow (100 msec to peak), rapidly facilitating, Na^+ dependent epsp that is not blocked by dTC, 3) a slow (2.5 sec to peak), K^+ dependent ipsp that is blocked by intracellular tetraethylammonium (TEA) ions and by cooling to 1° C. This psp is the most common potential seen, and 4) a very slow (15 sec to peak) epsp that is reduced as the post synaptic neuron is hyperpolarized, disappearing at -80mV. This psp is also blocked by cooling to 1° C. The first three of these potentials are due to conductance increases since they are accompanied by a marked drop in the input impedence as measured in the soma. No change in the input impedence is detectable in the soma during the fourth psp.

Positive evidence for the transmitter of C2 is still lacking. However, bath application of up to 10^{-3} M putative transmitter candidates to desensitize the receptors suggests that the transmitter is not likely to be any of the compounds for which receptors have been identified in gastropods (Carpenter et.al., J. Neurobiol. 8: 119, 1977). The transmitter candidates tested were 5-hydroxytryptamine, acetylcholine, dopamine, γ-aminobutyric acid, phenylethanolamine, octopamine, histamine, aspartic acid and glutamic acid. Also, except for the ionophore blockers dTC and TEA, no pharmacological agents have been effective on any of these psps. Pharmacological agents tested were hexamethonium, methysergide, bicuculline, phentolamine, cimetidine and fluphenazine. This evidence suggests a novel transmitter or pharmacology. (Supported by NSF grant BNS75 13597 A02.)

645 THE PEPTIDERGIC BAG CELL NEURONS OF APLYSIA: MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL STUDIES OF DISSOCIATED CELLS IN TISSUE CULTURE. F. Strumwasser, L. K. Kaczmarek and D. Viele*. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The bag cell (BC) neurons exhibit interesting discharge properties in the intact BC system such as prolonged afterdischarge and postactivity refractoriness. In order to further dissect these interesting electrophysiological properties we have explored the possibility of producing primary cell cultures of BC neurons. The BC neurons also synthesize a polypeptide, egg-laying hormone, in the intact cluster. Cell cultures have the potential for eventually producing a cell line of invertebrate peptidergic neurons since Coggeshall (J. Neurophysiol. 30:1263, 1967) presented evidence that their numbers approximately triple between juvenile (5 g, body weight) and adults (200 g).

We have used a variety of individual enzymes and combinations for dissociation of cells (collagenase, elastase, neutral protease, pronase, trypsin). Our best results were obtained incubating abdominal ganglia in neutral protease (1.25% w/v solution in filtered seawater [FSW]) for 6 hours at 22°C. After removal of the BC clusters from the abdominal ganglion, the connective tissue capsule is removed and the bag cells are disaggregated using a Siliclad-coated pasteur pipet. Typical yields, with neutral protease, of single BCs (from 2 BC clusters) vary between 250 and 500 cells. We estimate about a 30-50% recovery during dissociation of the cluster. We also obtain glial and other unidentified cells.

Many of the BCs and other cells attach to the plastic surface of the dish (Falcon, 35 mm) and develop neurites, within 24 hr, in Eagle's minimum essential medium made up in FSW. This medium has been shown to support normal resting, action and synaptic potentials in the intact abdominal ganglion (Strumwasser and Bahr, Fed. Proc. 25: 512, 1966). It is interesting that some BCs exhibit as many as 6 processes emanating from their cell body indicating that a heteropolar neuronal condition can be expressed, at least in tissue culture, and is not unique to vertebrate neurons.

Intracellular recordings from BC neurons without neurites in primary culture have allowed us to conclude that the soma itself is capable of supporting a regenerative action potential (AP). The soma also supports repetitive firing, with applied transmembrane current. In our best experiments overshoot-to-undershoot values of APs are 80 mV and firing frequencies, to applied currents, are as high as 8 spikes/sec (14°C). The specific membrane resistance and specific capacitance of ∿60 µm diameter BCs (without neurites) are approximately 30,000 $\Omega.cm^2$ and 0.5 $\mu F/cm^2$. The surface receptors of BC neurons are being explored with iontophoretic applications of various transmitter candidates. We find that many BCs respond to serotonin (creatinine sulfate) with depolarization and in those BCs with APs serotonin may cause firing.

646 SITES OF ACTION OF THE POLYPEPTIDE EGG-LAYING HORMONE (ELH) IN THE HEAD GANGLIA OF APLYSIA CALIFORNICA. Duncan K. Stuart and Felix Strumwasser (SPON: C. A. G. Wiersma). Division of Biology, California Institute of Technology, Pasadena, CA 91125.

ELH is a polypeptide of about 6,000 m.w. synthesized in the bag cell neurons of the abdominal ganglion of Aplysia. When injected into an animal, ELH induces egg-laying along with characteristic behaviors, such as a nodding and weaving motion of the head as the eggs are attached to the substratum. It is not known whether these behaviors are mediated by a direct effect of ELH on the nervous system, by a secondary factor(s), and/or by a reflex response to the eggs' extrusion.

We have shown that a suppression of feeding occurs during egg-laying, as previously shown in Pleurobranchaea by Davis et al. At 20°C, starved Aplysia (n=7) injected with bag cell extract stopped eating a 1 gm piece of red algae at 17+4 min post-injection and their eggs first appeared at 29+4 min. Six of seven controls, injected with head ganglia extract, ate all their algae.

We have also studied the effect of ELH in vitro on the isolated buccal ganglia, which control the feeding movements, and on the combined head ganglia (buccal, cerebral, pleural, and pedal). ELH applied to the paired buccal ganglia activates a pair of neurons into a tonic pacing mode (∿1 spike/sec). These neurons each have an ipsilateral axon in buccal nerve 3. At 20°C, this effect is apparent between 5 and 10 min after addition of ELH to the chamber. By 15 to 25 min their rate of firing, as recorded from each buccal nerve 3, reaches a maximum. The correlation between this 15-25 min period and the time at which Aplysia, injected with ELH, stop feeding suggests that this pair of neurons may be exciting buccal muscles which retract the feeding apparatus or inhibiting others which protract it.

ELH also increases the rate of firing of a second pair of buccal neurons (∿10 spikes/min before to ∿30 spikes/min after), each with an ipsilateral axon in the cerebro-buccal connective.

When applied to the head ganglia, ELH causes large bursts of neuronal activity in the pedal ganglia nerves to the foot, and increased activity in the nerve to the penis. These pedal nerve effects may relate to changes in locomotion or the head weaving movements that occur during egg-laying.

These above neuronal effects were produced either by ELH released from activated bag cells in disconnected abdominal ganglia, then fractionated by gel filtration, or by ELH partially purified (in collaboration with Arlene Y. Chiu and Eri Heller) using ammonium sulfate precipitation, an anion exchange column, and gel filtration. The results suggest that at least some of the characteristic behavior during egg-laying is mediated by a direct, hormonal effect of ELH upon the nervous system.

647 PLASTICITY OF FEEDING IN THE OPISTHOBRANCH MOLLUSC NAVANAX INERMIS. A.J. Susswein* and M.V.L. Bennett (SPON: I. Kupfermann). Division of Cellular Neurobiology, Department of Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y.10461

Several forms of plasticity of feeding behavior were investigated in Navanax. Navanax is a carnivore which ingests prey with suction caused by rapid pharyngeal expansion. Swallowing movements then move prey into the esophagus. Feeding is little affected by handling or posture, and is more resistant to noxious stimuli than in some other gastropods. By contrast, ingestion is readily affected by recent feeding history: 1)Arousal. Feeding latency is decreased following exposure to food. 2)Habituation. Repeatedly eliciting feeding responses and then rapidly withdrawing the prey causes a gradual increase in response latency dependent upon the rate of prey presentation. The latency increase is not due to motor fatigue, as shown by dishabituation with an alternate prey species. 3)Satiation. Continued feeding causes increased latency and eventual cessation of feeding after a mean weight gain of 42.5% of initial animal weight (N= 12). Effects of satiation remain after the gut has emptied, suggesting that gut distension does not alone account for satiation.

When presented with food too large to swallow whole, Navanax either ceases to respond after a few presentations, or maintains suction on the partially swallowed prey for several days while digesting the more deeply engulfed portion. Switching between rejection and maintained suction is due to activation of mechanoreceptors in the posterior pharynx, as shown by similar switching produced with non-prey, neutral objects. This behavior persists in animals in which the buccal ganglia are surgically isolated from the rest of the C.N.S. Since the neurophysiology of the buccal ganglia has been extensively studied, the behavioral switching in the isolated buccal ganglia is an attractive preparation for neurophysiological study.

648 PRESYNAPTIC ACTION OF GLUTAMATE AT A CRAYFISH NEUROMUSCULAR JUNCTION. M. THIEFFRY* AND J. BRUNER* (SPON: H. KORN). Laboratoire de Neurobiologie cellulaire du C.N.R.S., 91190 - Gif-sYvette and Laboratoire de Neurobiologie, Université de Picardie, 80039 - Amiens, France.

It is largely admitted that glutamate depolarizes crustacean muscle fibers by acting on muscle membrane receptors. However, in recent work we could demonstrate that "acute denervation" induced by isotonic calcium[1] strongly depressed the glutamate response of fast abdominal flexors of crayfish Procambarus clarkii. This observation which is in favour of a presynaptic action of glutamate, previously suggested by other authors[2] was further supported by a direct demonstration of the sensitivity of terminals to glutamate.

To this end, small axonal branches were impaled close to the point where they penetrate between muscle fibers. Another intracellular electrode was inserted into a muscle fiber at about 500 µm from the site of intra-axonal recordings.

Bath application of glutamate (0.1mM) depolarized both the muscle fiber and the axon by about 20 mV. Glutamate ionophoretically applied on to the axon far enough from the muscle still induced the response in the axon thus implying the existence of glutamate receptors on the axonal membrane.

The presence of such receptors in the vicinity of nerve terminals was further demonstrated by ionophoretic injection of glutamate on the sensitive spots of the muscle membrane. In those conditions depolarizing responses were simultaneously recorded in the muscle fiber and in the axon. Both responses disappeared when the glutamate electrode was moved from the sensitive spot.

The above observations, together with the fact that other procedures can lead to a blockade of the glutamate response with unmodified sensitivity of the post-synaptic membrane to the natural transmitter, suggest that the glutamate response of the muscle fiber could be mediated partially, by a presynaptic mechanism (supported in part by an I.N.S.E.R.M. grant n° 387670).

1. Heuser, J., Katz, B. and Miledi, R. (1971) Proc. R. Soc. Lond. B., 178, 407.

2. Colton, C.K. and Freeman, A.R. (1975) Comp. Biochem. Physiol., 51C, 285.

649 THE NATURE OF MULTIPLE α-BUNGAROTOXIN BINDING COMPONENTS IN THE BRAIN OF LIMULUS POLYPHEMUS. W. Eric Thomas* and James G. Townsel (Sponsor: John S. Thomas) Departments of Biochemistry and Physiology, Meharry Medical College, Nashville, Tennessee 37208

The binding of ^{125}I-αbungarotoxin to membrane fragments prepared from Limulus brain tissue has been reported (Thomas, et al., 1978, Arch. Biochem. Biophys. 187:53). The kinetics of toxin binding indicate multiple binding sites. However, the nicotinic cholinergic ligands d-tubocurarine and nicotine greatly inhibited toxin binding at a concentration of 10^{-5}M. Sucrose gradient sedimentation of solubilized toxin binding activity revealed the presence of three toxin binding components. The presence of multiple toxin binding components raises doubts as to the specificity of α-bungarotoxin binding in this preparation. Therefore, the nature of the three toxin binding components from Limulus brain tissue has been investigated by sucrose gradient sedimentation.

The three toxin binding components have sedimentation coefficients of 9.0S, 15.4S and 17.4S. All three components were degraded by chymotrypsin. The total toxin binding activity of the solubilized extract was inhibited 72.1%, 46.8%, 8.5% and 0% by 10^{-5}M d-tubocurarine, nicotine, scopolamine and pilocarpine, respectively. Phenylmethylsulfonylfloride and ethylenediaminetetracetate diminish the amount of the 9.0S component with a corresponding increase in the 15.4S and 17.4S components. Isolation of the 9.0S component, followed by addition to brain extract, resulted in the formation of the 15.4S and 17.4S components. Addition of isolated fractions of either the 15.4S or 17.4S components to brain extract led to the production of the 9.0S component. These findings suggest that the 15.4S and 17.4S components are separate proteins and that the 9.0S component is a subunit common to both and is the site of α-bungarotoxin binding. Thus, the binding of α-bungarotoxin in Limulus appears to be specific.

(This study was supported by NIH Grant 1 R01 HL 17370 and Public Health Service Minority Biomedical Support Grant RR-08037.)

650 THE RESPONSES OF CERCAL RECEPTORS AND IDENTIFIED INTERNEURONS IN THE CRICKET (ACHETA DOMESTICUS) TO AIR STREAMS. Martha Tobias* (SPON: H.V.B. Hirsch) SUNY at Albany, Albany, N.Y. 12222

In this study, the cricket cercal to giant interneuron pathway in response to air stream stimulation was examined. The physiology of cercal displacement receptors (filiform hairs), as well as the directional sensitivity of the medial and lateral giant interneurons was characterized.

Intracellular recordings from filiform hair afferents revealed that the sensory neurons are excited when deflected in one direction, and inhibited when deflected in the opposite direction. Each filiform hair is associated with a single sensory neuron. There are two morphological classes of filiform hairs, with planes of movement oriented transversely (T-hairs) or longitudinally (L-hairs), with respect to the long axis of the cercus. I have divided each class of filiform hairs into two physiological types. T-hair afferents are composed of two populations, with preferred directions toward, or away from, the midline. Similarly, L-hair afferents are composed of two populations, with preferred directions toward, or away from, the body.

The directional sensitivity of the giant interneurons in response to air stream stimulation reflects differences in the strengths of excitation from the four receptor types described. Each member of the bilateral pair of giant interneurons elicits peak responses when air streams are directed ipsilateral to the giant interneuron's ascending axon. Furthermore, they are most responsive to ipsilateral T-hair sensory neurons excited by movement towards the midline, and ipsilateral L-hair sensory neurons excited by movement toward the body.

The directional responses of the giant interneurons to air stream stimuli were distinct from those previously obtained in response to acoustic stimuli. Directional responses of the giant interneurons to acoustic stimuli, resulted in a weak excitation when stimulating T-hairs. In contrast, the magnitude of response to air stream stimulation of either T-hairs or L-hairs is the same, suggesting an equal strength of excitation from both classes onto the giant interneurons.

The unidirectionality of the sensory neurons, as well as the circuitry from filiform receptors onto the giant interneurons, described in this study, are distinguishable only when a unidirectional stimulus, such as air streams, is employed. Supported by NSF research grant BNS 752345 A01 to R.K. Murphey.

651 PHARMACOLOGY OF PRESYNAPTIC FACILITATION OF THE GILL-WITHDRAWAL REFLEX IN APLYSIA. Thomas K. Tomosky-Sykes. Div. Neurobiol. & Behav., Dept. Physiology, P&S, Columbia Univ., New York, N.Y.10032

Various lines of evidence suggest that 5-HT may be the neurotransmitter mediating facilitation of the gill-withdrawal reflex in Aplysia. For example, of the three compounds (5-HT, octopamine, and dopamine - Cedar & Schwartz, J.Gen.Physiol. 60:570, 1972) known to increase ganglion levels of cAMP (which is also implicated in mediating facilitation) only 5-HT caused facilitation of the monosynaptic EPSP from gill and siphon sensory neurons to the motor neurons (Brunelli et al., Science, 194:1178, 1976). Both 5-HT and connective stimulation broaden the action potential in the sensory neuron by increasing Ca^{++} influx - a mechanism believed to underlie facilitation (Klein & Kandel, Abstract, Soc. for Neurosci., 1978).

I have tested the selectivity of the sensory neuron action potential broadening effect to determine other possible candidates for the facilitating neurotransmitter. Twenty-four putative transmitters have been examined, including the common monoamines, amino acids and derivatives, carbachol, adenosine, and ATP. The drugs were bath applied to a final concentration of 1 x 10^{-4}M in artificial sea water containing 10 mM tris buffer (pH 7.6) and 100 mM TEA (tetraethylammonium chloride). TEA was used to eliminate delayed rectification and make the effect of an increased Ca^{++} influx easy to measure.

The results indicate that only 5-HT and 6-HT cause significant increases in spike width. For example, 5-HT prolonged the sensory neuron action potential from a half-width of 15-30 msec to one of 125-300 msec. 6-HT was somewhat less effective, prolonging the action potential to 90-95 msec. In contrast, all other drugs produced either no increase or only inconsistent small increases (generally less than 50%) in spike width.

These data indicate that of the drugs examined, 5-HT is the most likely candidate for the facilitating transmitter. However, since more exotic amines or peptides were not examined, direct biochemical evidence is still needed to support this hypothesis.

An additional finding is that dopamine decreases the sensory neuron spike width. This suggests that this neurotransmitter may perhaps be involved in presynaptic inhibition at this synapse. Advokat et al. (Abstract, Soc. for Neurosci., 1976) have recently found that arousal by food produces attenuation of siphon withdrawal by an as yet unknown mechanism. It is attractive to think that this attenuation may be mediated by presynaptic inhibition of the sensory neurons, perhaps by dopamine.

This research was supported by NIH fellowship #NS05617.

652 SPONTANEOUSLY ACTIVE MOLLUSCAN NEURONS: SPIKE INITIATION ZONE LIES IN AXON. Steven N. Treistman. Department of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

Spontaneously active molluscan neurons have been thought to differ from other molluscan neurons, in that their pacemaker and spike initiation zones lie in the cell body membrane, rather than in an axonal site. The major lines of evidence for this conclusion were drawn from studies in which the cell bodies of spontaneously active cells were isolated by either ligation or enzymatic dissociation from their axons, and still exhibited patterned spike outputs.

By recording simultaneously from cell body and axon of intact pacemaker neurons R15, and left upper quadrant cells of Aplysia, I have found that the spike is initiated in the axon of these cells. This conclusion is based upon both the sequence and the rate of rise of the spikes in the two regions. Thus, in the intact cell, spike initiation occurs in the axon, and pacemaker neurons do not appear to differ from other cells in this respect. (This work was supported by NSF grant BMS 77-01548).

653 IDENTIFICATION OF MOTOR NEURONS MEDIATING OPALINE SECRETION IN APLYSIA CALIFORNICA. Susan Tritt* and John H. Byrne (SPON: G. Fromm) Dept. of Physiology, School of Medicine, University of Pittsburgh, Pittsburgh, Pa., 15261.

In response to noxious stimuli Aplysia californica characteristically releases two substances, ink and opaline, which are believed to act as defensive mechanisms against potential predators (Eales, 1921). Whereas there is now a fairly good understanding of the neural control of inking behavior (Carew and Kandel, 1977) little is known regarding the control of opaline secretion. Using a combined morphological and electrophysiological approach we have now identified cells which make a large contribution to the motor component of this behavior.

Electrical stimulation of tegumentary nerve 7 which innervates the region of the opaline gland (Eales, 1921) produced massive contractions of the opaline gland. Cobalt back-filling of the proximal end of this nerve resulted in the staining of a large number of cell bodies in the right pedal ganglion and three approximately 150 u diameter cell bodies in the right pleural ganglion. Intracellular recordings from the region of the stained cell bodies, revealed cells which could be antidromically activated by electrical stimulation of tegumentary nerve 7. Intracellular stimulation of the pedal ganglion cells primarily resulted in movements of the body wall near the gland, while stimulation of cells in the pleural ganglion produced marked contraction of the opaline gland itself. Like the motor neurons in the abdominal ganglion which mediate inking behavior, the pleural ganglion cells have relatively high resting potentials and are normally silent with occassional background subthreshold EPSPs. Another similarity between the ink motor neurons and those mediating opaline secretion is that the opaline motor neurons are electrically coupled with a coupling ratio of about 0.3. A train of electrical stimuli to the right pleural-abdominal connective produces large summating EPSPs in the opaline gland motor neurons which cause the cells to fire and lead to contraction of the opaline gland. Hyperpolarizing a single motor neuron can block spike activity produced by connective stimulation and reduce the gland contractions by up to 90%. Similarly firing individual neurons with intracellular current pulses to mimic firing rates produced by connective stimulation results in comparable glandular contractions, indicating that these identified neurons make a large contribution to the motor component of this behavior. The identification of cells mediating opaline secretion makes it possible to compare the neural circuits and biophysical properties of neurons mediating two similar behavioral responses to adversive stimuli. Supported by NIH grants NS13511 and NS00200.

654 SYNAPTIC INTERACTIONS BETWEEN MOTORNEURONS OF ASCARIS. J.P. Walrond*, J.E. Donmoyer*, P.A. Desnoyers*, and A.O.W. Stretton. Dept. of Zool., Univ. Wisconsin, Madison, WI 53706

Our investigation of the nervous system of the nematode Ascaris lumbricoides has employed a combination of anatomical and physiological techniques. The shapes of neurons which comprise the ventral and dorsal nerve cords have been completely reconstructed from serial 10µm sections in the light microscope. Criteria for identifying synapses in the light microscope have been developed and confirmed by examining selected sections containing putative synapses in the electron microscope. The neurons are divided into seven classes, based solely upon shape (Stretton et al., 1978 P.N.A.S.). Synapses between each of the 7 neuronal types and muscle have been identified. In the dorsal and ventral nerve cords, neurons communicate with one another through en passant synapses. In the dorsal cord, the excitatory motorneurons (DE1, DE2, and DE3) synapse directly onto the ventral inhibitory motorneurons (VI) while in the ventral cord the presumed ventral excitatory motorneurons (V-1 and V-2) synapse onto the dorsal inhibitors (DI). In the ventral nerve cord six neurons extend the entire length of the animal. They do not contact muscle, but synapse onto the excitatory motorneurons.

The synapses between the dorsal excitors and the ventral inhibitory dendrite have been investigated electrophysiologically. Activation of the dorsal excitor produces a depolarization in the dorsal musculature followed by a hyperpolarization in the ventral musculature. Stimulating various combinations of dorsal excitors and ventral inhibitors has produced a map of the synaptic interactions between dorsal excitors and ventral inhibitors.

By stimulating a single intact neuron and recording intracellularly from muscle cells along the length of the dorsal cord, the conduction velocities of each of the dorsal cord excitors has been found to be 25 cm/sec. The muscles send arms to the nerve cord where they have electrical synapses with one another in a region called a "syncytium". This arrangement permits the conduction of an action potential through the syncytium. Recordings taken posterior to neurons whose processes extend anteriorly in the dorsal cord, demonstrate a conduction velocity of 12 cm/sec in the muscles. Since a wave of propagation moves along the body of an intact animal at about 1 cm/sec, we can rule out the simple model in which the velocity of propagation of an action potential along either an excitatory motorneuron or the syncytium corresponds to the velocity of propagation of a body wave. (Supported by PHS Grant NS10509, NSF Grant BNS 76-09641, The Sloan Foundation, and The Research Fund of the Hraduate School, Madison)

655 CONFLICT AND RESPONSE SELECTION IN THE LOCOMOTOR SYSTEM OF APLYSIA. E.T. Walters,* T.J. Carew, and E.R. Kandel. Div. Neurobiol. & Behav., Depts. of Physiol. and Psychiat., P & S, Col. Univ., New York, N.Y. 10032

Locomotion in Aplysia californica is a complex centrally commanded motor sequence (Hening et al., 1977) which is modulated by a variety of internal and external stimuli (Kupfermann, 1968; Advokat et al., 1977; Weiss et al., 1977). We here explore the long-term consequences of noxious stimuli (which either facilitate or depress this motor program) on response selection in a subsequent "conflict" situation.

Aplysia respond to noxious stimulation with one of two defensive programs: a strong generalized withdrawal ("freezing"), or escape walking ("fleeing"). In order to obtain reliable stimulus control of walking we first examined the effect of the site of noxious stimulation on the immediate selection of a defensive program. In all experiments, walking was measured using a blind procedure. We found that animals receiving tail shock (N=16) rapidly began escape walking (median latency=21 sec), whereas animals receiving head shock (N=15) froze for several minutes, turned and only began walking after a significant delay (median latency=380 sec, $p < .001$). The head-group also walked more slowly than the tail-group ($p < .01$). Similar results have previously been described by Wachtel and Impelman (1973) who found that walking elicited by salt to a parapodium can be terminated by a noxious stimulus to the head region.

We next examined how previous experience with either head or tail shock affected the animal's long-term response selection in a "conflict" situation 24 hours later. The "conflict" stimulus was a compound stimulus consisting of a sequence of shocks applied alternately to the head and tail for 20 seconds. In response to the "conflict" stimulus control animals with no previous shock (N=16) began walking with a median latency of 140 sec. By contrast, animals which previously received tail shock walked significantly sooner (median latency=79 sec, $p < .05$) and those that received head shock walked significantly later (median latency=435 sec, $p < .002$) than controls. The groups also differed significantly in the amount of walking they exhibited ($p < .01$ and $p < .002$ respectively).

These results indicate that an identical "conflict" stimulus can produce opposite effects on escape walking depending upon the animal's past experience. Unlike sensitization of simple defensive responses such as gill and siphon withdrawal in Aplysia, which depend only upon the intensity and quality of the sensitizing stimulus, sensitization of escape walking, a more complex defensive behavior, can be specific to the history of previous stimulation to a particular site.

656 INTERNEURONS MEDIATING SPATIAL POSITION IN A COCKROACH. W. William Walthall and H. Bernard Hartman. Dept. of Biol. Sci., Texas Tech University, Lubbock, TX 79409.

The equilibrium receptor system for the cockroach Arenivaga consists of four interneurons, each excited by primary sensory neurons from one of four rows of tricholiths. Tricholiths are sensilla suspended from the ventrobasal region of the cerci, specifically adapted to signal information regarding the insect's spatial orientation.

Interneurons mediating spatial position are inactive when the insect is in the primary orientation. Displacements from that position evoke responses from one or two of the interneurons. These interneurons fire tonically to maintained displacements of the insect at other than primary orientation. In each instance, the frequency of firing and the rate of adaptation is proportional to the angular displacement. Physiologic analysis of their responses indicates that each interneuron independently responds to rotation in a different ca. 110° quadrant. Discharge from two interneurons occurs in regions where adjacent quadrants overlap, these being roll left and right, and pitch forward and backward. However, resolution of angular position to rotations within a quadrant is not possible due to the restricted area of overlap of the receptive fields, and the symmetry of the response patterns of each of the four interneurons.

Arenivaga is a burrowing insect unable to utilize orientation cues such as vision, differential limb loading and proprioception. The insect is nocturnal and "swims" through fine quasi-fluid soil which collapses about it and does not provide a firm foothold. The discrete equilibrium receptor system of the cerci is a particularly desirable adaptation. The employment of giant fibers to mediate this modality suggests that this information is of considerable importance in modulating locomotory behavior. Our physiologic results add support to the suggestion by Fraser (Nature 268: 523, 1977) that the cerci of cockroaches are equilibrium in function.

Supported by National Science Foundation grant #BNS77-22283 and National Aeronautics and Space Administration grant #NSG-7435.

657 A HISTAMINERGIC SYNAPTIC POTENTIAL PRODUCED BY A VOLTAGE-DEPENDENT APPARENT DECREASE OF CONDUCTANCE IN THE METACEREBRAL CELL OF APLYSIA. K.R. Weiss, E. Shapiro*, J. Koester and I. Kupfermann. Div. Neurobiol. & Behav., Coll. P & S, Columbia Univ., N.Y., N.Y. 10032

The output of the metacerebral cell(MCC) to buccal muscle and central neurons has been extensively studied. We now report that the MCC receives an excitatory synaptic input from the multiaction, histaminergic neuron C2 (Weinreich et al., 1975; Weinreich, 1977; McCaman and McKenna, 1978). This input has a number of unusual characteristics. A brief train of spikes in C2 produced a smooth excitatory synaptic potential that could persist for up to 60 sec. On occasion, the EPSP was preceded by a brief hyperpolarization, but the present analysis is exclusively on the later EPSP. Our data suggest that the EPSP is chemically mediated and is monosynaptic. It was enhanced by solutions with high Ca++ and was depressed by high Mg++. It persisted in a solution of high divalent cations (Mg++ and Ca++) which raises the threshold of possible interneurons between C2 and the MCC. It could be enhanced by injection of TEA into C2 and by depolarization of C2. Finally, even in high Mg++, histamine produced a slow EPSP in the MCC. Hyperpolarization of the MCC decreased the size of the EPSP, and voltage clamp studies of the EPSP elicited by C2 and by bath application of histamine revealed that the EPSP is associated with an apparent decrease of membrane conductance. Furthermore, unlike conventional synaptic potentials, the conductance change is highly voltage dependent. At approximately -90 mV, the synaptic current and apparent conductance decrease is no longer present. Further hyperpolarization, however, does not invert the current. Steady-state I-V plots under voltage clamp conditions suggested that the MCC normally possesses a region of negative-slope conductance that is mediated by calcium, as evidenced by the fact that it is blocked by cobalt or calcium-free solution. The negative-slope is enhanced in the presence of histamine. We are currently studying the ionic basis of the synaptic current.

Our evidence indicates a number of similarities between the synaptic potential comprising an input to the MCC and the synaptic potentials resulting from the activity of the MCC itself. In both cases a biogenic amine is involved and the action is of a modulatory type involving long duration of action and voltage-sensitive ionic conductances. Previous data suggested that the modulatory synaptic actions of the MCC are specifically related to its role in modulation of biting responses during food arousal. The present evidence suggests that the synaptic actions of neuron C2 are appropriate for it to be part of the neural circuit that modulates feeding.

Supported by grants NS 12492, 5 PO1 GM 23540 Scope D and 1 RO1-NS 14385.

658 GIANT FIBER ASSOCIATED WITH MUSHROOM-BODY NEUROPILES IN AN INSECT BRAIN: A POSSIBLE ONE-CELL FEEDBACK LOOP. Mitchell J. Weiss. Department of Biology, Livingston College, Rutgers University, New Brunswick, NJ 08903.

The corpora pedunculata or "mushroom bodies" of insects are paired brain centers of elaborate structure, whose primary role may be the higher-level processing of complex antennal sensory information (J. Morph. 142:21, 1974). In the "short-horned" or acridid grasshoppers (order Orthoptera), these centers are well developed and include the usual major neuropilar subdivisions: a calyx region mounted upon a descending, stalk-like pedunculus, which bifurcates at its lower end into a medial beta lobe and a reascending alpha lobe. In a light microscopic study of reduced silver preparations from the acridid grasshopper Melanoplus femurrubrum, I have discovered a single giant neuron on each side of the brain which interconnects different subdivisions of the ipsilateral mushroom body.

The unipolar soma of each giant neuron is located laterally in the protocerebrum, near the base of the optic lobe. After traversing the cortical layer of neuronal somata, the neurite enters the protocerebral neuropile and extends to a point close to the lateral surface of the ipsilateral pedunculus. Here the fiber expands in a T-shaped fashion into a giant process, which extends both anteriorly and posteriorly from this point. The posterior extension ascends the surface of the pedunculus, meanwhile emitting branches into it. At the top of the pedunculus the fiber ramifies widely into the "primary calyx" of the calyx region. The anterior extension of the giant process passes anteriorly and begins to bifurcate repeatedly in the general protocerebral neuropile. These branches enter the alpha lobe, in which they ramify. Adjacent to the pedunculus the giant process is flattened, with a typical width of 20-32u; the thickness varies in approximately inverse proportion from ca. 4.4u to as much as 10-15u. Although at least one minor branch of the neuron does not enter the mushroom body, the giant process connecting calyx (and pedunculus) with the alpha lobe clearly seems to represent the main conduction pathway within the neuronal tree. Similar giant neurons have been found in Schistocerca and other genera and apparently typify the entire family.

Conduction within a mushroom body is believed to extend from the calyx region (which receives the inputs) down the pedunculus and along the alpha and beta lobes (whose connections are believed to be efferent). The giant neuron may bring information from the alpha lobe back into the calyx region and thus form a striking one-cell feedback loop for the mushroom body.

Supported by NSF Grant BNS 76-09645 and the Busch Fund of Rutgers University.

659 EXCITATION OF CYCLICALLY PATTERNED FEEDING MOTOR OUTPUT BY CONSTANT CURRENT INTRACELLULAR STIMULATION. A. O. D. Willows. Friday Harbor Laboratories, Univ. of Wash., Friday Harbor, WA 98250.

The roles of various re-identifiable neurons in the brain and buccal ganglia of the mollusk Tritonia diomedea in the control of feeding behavior were determined using intact or isolated buccal mass, buccal ganglia and brain preparations. In addition to motor neurons driving various aspects of movements of the jaws, radula and odontophore, a group of interneurons were found which exert powerful, direct control over the co-ordinated output of the entire system. Brief (less than 2 s.) stimulation of particular individuals within this group of interneurons released single, co-ordinated cycles of impulse bursts in motor neurons lasting 10-20 s. These single cycles ressembled closely the cycles of impulse bursts seen in these same neurons during feeding movements. Similarly brief stimulation of other neurons in this group using differing current strengths elicited shorter or longer series of feeding motor output cycles depending upon current strength. A third class of these interneurons acted to drive feeding motor output so long as the constant current stimulus was maintained. In this last mentioned case, the duration of the cyclic output was strictly dependent upon the duration of the interneuronal stimulation whilst the frequency of the bursts in the motor output was nearly independent of the stimulus strength over a wide range. During spontaneously occurring feeding movements, the cyclic motor output (and the behavior) could be blocked, or reset by the application of hyperpolarizing currents to these interneurons.

It was found that these interneurons were electrically coupled to certain of the motor neurons, and in a few cases, connected by chemically mediated synaptic contacts as well.

It was observed also that sinusoidally varying subthreshold depolarizing currents in certain of the interneurons were capable of eliciting spikes in other neurons of the motor system. Thus spikes in the interneurons may not be essential to driving the motor output. The implication is that these interneurons are somehow closely associated with the motor pattern generator for feeding. They do not however exert exclusive control over that generator since other experiments indicate that feeding motor output can be elicited via routes independent of these interneurons.

660 EFFERENT INHIBITION OF THE LOCUST MOVEMENT DETECTOR SYSTEM DURING OPTOMOTOR NYSTAGMUS. Malcolm D. Zaretsky* (SPON: C.H.Fraser Rowell). Dept. Zoology, Univ. of California, Berkeley, CA 94720.

In the movement detection system of the locust, the lobula giant movement detector neuron (LGMD) and its post-synaptic cell, the descending contralateral movement detector neuron (DCMD), respond best to stimuli of small area in their 180 degree visual field. Rapid turning of the head with respect to the thorax by the locust is invariably associated with strong inhibition of the locust movement detector system. Chronic simultaneous single unit recordings from the DCMD and dorsal neck muscles of a tethered but otherwise freely moving locust demonstrate that this inhibition is of efferent origin. Inhibition occurs regardless of whether head movements are of optomotor or spontaneous origin.

Proprioceptive information is not required for inhibition of the DCMD response during head movement. Head turning movements are invariably preceded and accompanied by a burst of spikes from muscle 51 on the side ipsilateral to the direction of head turning. Inhibition of the DCMD persists during each burst of muscle spikes and 200-300 msec beyond regardless of whether the head is allowed to move or is prevented from moving by fixing it to the thorax. Head movements do not occur when the muscle firing rate is low, and the response of the DCMD is not inhibited when the muscle fires at a low rate, suggesting that a command for head movement is responsible for efferent inhibition of the DCMD.

The efferent inhibition of the movement detector system is distinct from two inhibitory mechanisms of visual origin which allow for discrimination of small field stimuli: (1) Feed-forward inhibition to the LGMD posterior to the site of convergence of the afferent pathway, produced by large field stimuli and (2) lateral inhibition presynaptic to the dendritic arborization of the LGMD which suppresses large field stimuli and reduces habituation at the excitatory afferent-LGMD synapses (O'Shea and Rowell, NATURE 254:53, 1975; Rowell, O'Shea and Williams, J. EXP. BIOL. 68:157, 1977).

It is biologically necessary for an animal to avoid confusion between changes in visual contrast generated by its own eye movements and those generated by moving objects. These results suggest that efferent inhibition serves this function in the locust movement detector system.

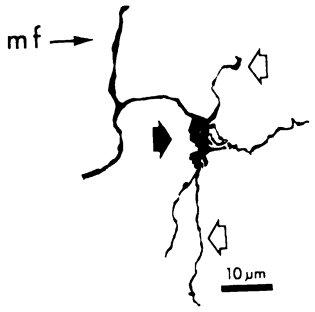
661 CALCIUM SENSITIVITY OF THE ULTRA-SLOW POTASSIUM CURRENT UNDERLYING ADAPTATION IN APLYSIA NEURONS. K. L. Zbicz* and W. A. Wilson. Dept. Pharmacol., Duke Univ. Med. Ctr., Durham, N. C. 27710 and Epilepsy Ctr., V. A. Hospital, Durham, N. C. 27705.

Certain neurons of Aplysia californica, notably R2 of the abdominal ganglion and the giant cell of the left pleural ganglion, adapt to prolonged electrical stimulation. The decrease in firing rate with time is mediated by a slowly developing potassium current which is activated when the cells are depolarized beyond approximately -50 mV. The rate of development of this current and rate of adaptation are enhanced by low concentrations of barbiturate (Cote, Zbicz, and Wilson, Nature, in press). We now report that this potassium current is calcium dependent. Exposure of these cells to low calcium or calcium free solutions reduces or abolishes the slow potassium current. In calcium free solutions, the normal tendency of the cells to adapt to electrical stimulation is lost and the cells respond to stimulation with rapid rates of firing which are occasionally interrupted by periods of prolonged depolarization. Elevation of extracellular calcium enhances the development of the slow potassium current and decreases the excitability of the cells. The metabolic inhibitor 2,4-dinitrophenol enhances the development of the slow potassium current and the tendency of the cells to adapt in a manner similar to that seen with barbiturate.

LIMBIC SYSTEM

662 SYNAPTIC EXTENSIONS FROM THE MOSSY FIBERS IN THE HILUS OF THE FASCIA DENTATA. D.G. Amaral and M.A. Connelly. Dept. Anat. and Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

In an earlier Golgi study which dealt primarily with the cell types in the hilar region of the rat hippocampus, occasional filamentous extensions were observed extending from the *en passant* swellings on the axons of the dentate granule cells, usually referred to as mossy fibers. These extensions, ranged in length from about 1 µm to 30 µm, were often branched, and appeared to contact the processes of various cell types in the hilar region. As shown in the drawing below, mossy fibers (mf) give rise to *en passant* swellings (filled arrow), sometimes by way of a short collateral branch. These swellings, in turn, give rise to several filamentous or clavate extensions (open arrows). In the 28-day-old rat, there are between 4 and 9 such extensions from most mossy fiber swellings, and the total length of the extensions from any one swelling is of the order of 75 µm. Serial electron micrographs through both normal and Golgi-impregnated mossy fibers has confirmed that these extensions are, indeed, presynaptic processes; each contains one or more vesicle-rich foci along its length and associated with these are asymmetric membrane specializations where the extensions are in synaptic contact with dendrites and dendritic spines of as yet unknown origin. A quantitative analysis of these extensions in Golgi material from rats of different ages indicates that their length is greatest in young animals and declines through the first post-natal month. For example, although there is no difference in the mean *number* of extensions in 14- and 28-day-old animals, the combined length of the extensions per mossy fiber swelling is some three times greater in the 14-day-old brains.



663 CONVERGENCE OF INPUTS FROM LIMBIC AND MIDBRAIN AREAS UPON HYPOTHALAMIC NEURONS. S. Anschel, M. Alexander and A. A. Perachio Yerkes Reg. Primate Res. Ctr., Emory Univ., Atlanta, GA 30322

There is substantial neuroanatomical evidence for overlapping projections from the preoptic-anterior hypothalamic area (POA-AHA), lateral septal area (LS), amygdala, and midbrain to the mediobasal hypothalamus (MBH). Electrophysiological studies of MBH and preoptic neurons have demonstrated both converging afferent and diverging efferent connections with extra-hypothalamic areas (Renaud, L.P., J. Physiol. 264(1977), 541-564; Dreifuss, J.J. and Murphy, J.T., Brain Res. 8(1968), 167-176). The purpose of the present study was to determine the type and origin of converging projections that produce short latency, excitatory responses in single neurons in preoptic area, MBH, dorsal and posterior hypothalamus, and to identify antidromically the projection path of their axons.

A transpharyngeal approach to the basal hypothalamus was used in urethane-anesthetized male rats. Bipolar stimulating electrodes were placed on the surface of the median eminence and in limbic and midbrain sites. Approximately 700 hypothalamic neurons were tested. Of 380 cells which were responsive to single pulse (1 Hz) electrical stimulation, 99 were demonstrated to have multiple inputs. The projections of 20 of these neurons were determined by antidromic activation. Histological localization has been completed for 83 of these multiple-input neurons.

POA-AHA and cortical amygdala (ACO) convergence was observed primarily in MBH (n = 21). Of these neurons, three were antidromically identified to project to the midbrain. Converging inputs from LS and amygdala were detected in 26 preoptic and hypothalamic neurons. Eighty-one percent of these neurons were found in the MBH or preoptic area; five were antidromically activated by stimulation of POA (n = 2), midbrain (n = 2) and median eminence. In addition, two neurons, one each in the posterior hypothalamus and premammillary region, were orthodromically activated by stimulation of LS and ACO and antidromically driven by stimulation of POA. Half of the neurons that responded to stimulation of afferents from the midbrain (n = 20) converged with inputs from the amygdala or POA.

These findings demonstrate a major concurrence of afferents to the MBH from POA, LS, amygdala and midbrain. Furthermore, a number of neurons receiving these diverse inputs were found to project to extra-hypothalamic sites. These types of neurons might subserve integrative roles related to the multiple physiological and behavioral functions that have been attributed to the MBH.

(Supported by NIH Grants #NS 09688 and #RR 00165)

664 HETEROSYNAPTIC MODIFICATION OF NEURONAL TRANSMISSION IN THE DENTATE GYRUS OF THE RAT. S.Y. Assaf*, and J.J. Miller, Dept. Physiology, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5

Paired-pulse stimulation of the perforant path (PP) results in a short term potentiation of the extracellularly recorded population EPSP and population spike in the dentate gyrus. However, relatively little is known concerning the effects which extrinsic afferents to the dentate exert on these PP evoked population responses. The present investigation examined the excitability changes in these responses following conditioning pulses applied to the commissural-dentate projection. Laminar profiles of the characteristic field potentials and unit activity elicited by single pulse stimulation (1-7v) of the PP and commissural pathway (contralateral CA_3) were recorded from the dentate in urethane anaesthetized rats. The population spike evoked by the PP test stimulus was markedly enhanced (150-500%) when preceded by a conditioning stimulus to the commissural input, however the amplitude of the population EPSP was not significantly altered. The time course of the potentiation (20-300 msec) coincided with the period of inhibition of spontaneously discharging granule cells elicited by commissural stimulation. These effects were abolished by acute and chronic transection of the contralateral commissural pathway. On the basis of these data it may be suggested that potentiation of the population spike by a heterosynaptic input occurs in the absence of changes in synaptic current and that inhibitory mechanisms may underly this potentiation.

This work was supported by the Medical Research Council.

665 NEUROGENESIS AND MORPHOGENESIS IN THE RAT SEPTAL REGION. Shirley A. Bayer. Dept. Biol. Sci., Purdue Univ., W. Lafayette, IN 47907.

Neurogenesis in the rat septal region was examined autoradiographically on postnatal day 60 after exposure to 3H-thymidine on two consecutive days during both the embryonic (E13+14, E14+15,.. E21+22) and neonatal (The day of birth and postnatal day 1, P2+3, P3+4) periods. The percentage of labelled cells and the proportion of cells added during each day of formation were determined at several anatomical levels within each septal region nucleus. There were significantly different waves of neurogenesis within and between nuclei. The neurons of the midline nuclear group (diagonal band, medial, and triangular septal nuclei) formed between E13-17, the lateral septal nucleus between E15-19, the bed nuclei of the stria terminalis and anterior commissure between E14-18, the nucleus accumbens between E17-P2. All nuclei and nuclear groups showed characteristic gradients of formation. The earliest forming neurons of both the midline nuclear group and the bed nucleus of the stria terminalis were in the vicinity of the decussation of the anterior commissure; younger neurons were located both rostrally and caudally. The lateral septal nucleus formed along a strong mediolateral gradient, and the nucleus accumbens formed along a ventrodorsal gradient.

Morphogenesis of the septal region was examined in normal rat embryos from E10-22. The septal region was postulated to form from two separate anlage located in the telencephalon by E13 and E14. The anterior ventromedial telencephalic wall presumably forms the midline nuclear group and the lateral septal nucleus. A posterior ventrolateral telencephalic ridge presumably forms the bed nuclei of the stria terminalis and anterior commissure. On E15, the early differentiating cells in these anlage fuse in the same region where the anterior commissure will ducussate on E17. Later, cells accumulate rostrally, caudally, and laterally to the early fused area. In the anterior portion of the developing midline nuclear group, a prominent subependymal zone appears on E16 and E17, presumably to form the nucleus accumbens. A quantitative analysis was made of the developing septal region using matched sections from both normal and X-irradiated brains surviving 6 h. after a single exposure to 200 R. The radiosensitivity of the neuroepithelium decreased significantly after E19; the subependymal zone was highly radiosensitive throughout; the differentiating cell zone was radioresistant throughout. The significance of the morphogenetic findings is discussed in the light of the autoradiographic results. (Supported by NSF Grant #BNS 77-12622)

666 EFFECTS OF SEPTAL LESIONS ON THE RENAL SODIUM GRADIENT. Wail A. Bengelloun, Khadija Baddouri[x], and Mohamed El Hilali[x]. Dept. de Biol., Fac. des Sciences, Univ. Mohammed V, Rabat, Morocco.

Hyperdipsia subsequent to septal lesions was first described by Harvey and Hunt (1) in 1965. Several reports had suggested that serum sodium (Na) concentrations remained unaltered following septal lesions in rats (2,3). In the present study we attempted to establish whether this was also the case for renal and urinary Na concentrations. In particular, we hoped to determine the effects of septal lesions on the normal Na concentration gradient in the kidney. Our interest was justified by the reports that septal stimulation increases ADH levels in the blood (4), whereas ADH replacement eliminates septal lesion-induced polydipsia and polyuria (5). This latter finding suggests a lesion-induced decrease in ADH secretion (see also 6). Such an ADH deficit would be expected to diminish the renal cortico-medullary Na concentration gradient (7).

Water intake of septal and sham-operated rats was monitored starting at 1 wk prior to surgery and continued for 2 wks post-surgery. At 1 wk post-surgery rats were placed in metabolism cages to facilitate urine output measurement and collection. 1 wk later, rats were sacrificed by cervical fracture and kidneys removed for sectioning (7) into cortex, external medulla, and combined internal medulla and papilla. Na concentrations in urine and in renal samples were analyzed by flame photometry.

In accordance with previous studies, septal lesions resulted in pronounced hyperdipsia and polyuria. Urinary Na concentrations were however unaffected by septal lesions, irrespective of whether the urine was collected in metabolism cages or directly taken from the bladder at sacrifice. Similarly, Na concentrations in the renal cortex and external medulla of septal rats did not differ from operated control levels. In the combined renal internal medulla and papilla sample, however, septal rats exhibited a significant decrease in Na concentration relative to controls.

While this relative flattening of the renal Na concentration gradient is concordant with the ADH hypothesis, our data does not preclude other factors (see 7) being responsible for the observed effects. Our results therefore strongly suggest that a more complete examination of renal function in the septal rat is imperative to understanding the role of the septum in water intake.

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667 RECIPROCAL ANATOMICAL CONNECTIONS BETWEEN HIPPOCAMPUS AND SUBICULUM IN THE RABBIT. Theodore W. Berger, Gerald W. Swanson*, Gary S. Lynch and Richard F. Thompson. Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717.

Horseradish peroxidase injections were made into the dorsal hippocampus of rabbit. Using the benzidine dihydrochloride method of development, orthograde and retrograde products were examined in the subicular cortex. A previously undescribed projection to hippocampus was seen to have its origin in dorsal prosubiculum and subiculum. Injections restricted to rostral levels of the dorsal hippocampus resulted in retrograde-filled cells first appearing in prosubiculum, several mm's posterior to the injection site. In more caudal sections, the retrograde field enlarges, extending medially and ventrally to include subiculum and limited areas of dorsal presubiculum. HRP-positive neurons were always seen contained within a well-circumscribed region of subicular cortex and were present following injections involving either hippocampal CA1 or CA3 cell fields.

Distribution of orthograde product first appeared at more caudal levels than retrograde-filled cells, and was regionally distinct from the retrograde field at rostral levels of the subicular complex. In anterior sections, labelled hippocampal terminals occupied the ventral-most part of the dorsal subiculum, near the ventricular surface. In more posterior regions, the density of orthograde product increased, as did the area over which terminals were distributed. Only in these caudal sections was considerable overlap seen between orthograde and retrograde fields, with labelled hippocampal terminals extending to subiculum and a significant portion of presubiculum. No orthograde product was ever seen in prosubicular or parasubicular areas. Finally, certain evidence indicated that cytoarchitectonic differences may exist with regard to hippocampal-subiculum projections. In total, the results strongly suggest a reciprocal, feedback anatomical relationship between hippocampus and subicular cortex.

668 EFFERENT CONNECTIONS OF THE POSTEROMEDIAL HYPOTHALAMIC NUCLEAR REGION OF THE PIGEON, COLUMBA livia. Mitchell L. Berk* (SPON: R. Adair). Dept. of Anatomy, The George Washington Univ., Washington, D.C. 20037.

The efferent projections of the rostral part of the posteromedial hypothalamic nuclear (PMH) region were determined by use of the autoradiographic technique. Iontophoretic injections of tritiated leucine were placed in PMH and the adjacent hypothalamic area of seven pigeons. Many heavily labeled fibers from PMH passed rostrally in the anterior hypothalamic area and entered the lateral preoptic area. Both the paraventricular nucleus, which lies ventral to the anterior commissure, and the medial preoptic nucleus received a moderate density of label. A few fibers passed dorsally into the lateral septal area, while grains were not found over the medial septal region. The density of grains, which occurred in the periventricular and dorsal portions of nucleus dorsomedialis anterior thalami, progressively decreased in nucleus dorsomedialis posterior thalami. Very few grains were seen in the stria medullaris and the lateral habenular nucleus. Fibers from the region dorsolateral to nucleus periventricularis magnocellularis were seen streaming dorsolateral to the occipitomesencephalic tract and entering nucleus dorsolateralis anterior thalami, pars magnocellularis (DLAmc). A few fibers passed laterally on the dorsal border of the ventrolateral thalamic nucleus and reached DLAmc ventrally.

A prominently labeled pathway coursed dorsolaterally from PMH towards, but not entering into, nucleus ovoidalis. At levels posterior to nucleus ovoidalis, these heavily labeled fibers projected laterally and densely innervated the substantia grisea et fibrosa periventricularis and nucleus intercollicularis of the midbrain. Label was also found in the central gray of the midbrain, extending posteriorly to the level of the Edinger-Westphal nucleus. Grains, concentrated medial to the lateral mammillary nucleus, continued posteriorly into the ventral tegmental area, lateral to the oculomotor nerve. Significantly, no grains appeared in the median eminence or in nucleus taeniae--archistriatum posterior and mediale, which are considered comparable to the mammalian amygdala, based on their projections to the medial hypothalamus (Zeier and Karten, 1971).

669 EFFECTS OF ELECTRICAL STIMULATION OF THE AMYGDALA UPON THE EXTENT OF THE TRIGEMINAL SENSORY FIELD PRESENT DURING ATTACK BEHAVIOR IN THE CAT. C.H. Block*, A. Siegel, and H. Edinger. Departments of Neurosciences and Physiology, College of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, New Jersey 07103.

It has been clearly demonstrated that trigeminal somatosensory input from the region of the lipline constitutes an important element for the occurence of the quiet biting attack response in the cat (MacDonnell and Flynn, 1966). It has also been shown that the amygdala exerts a powerful modulatory role upon this form of aggression (Egger and Flynn, 1963). The present study was undertaken to determine whether amygdaloid control of attack behavior is manifested through its regulation of the sensory fields supplied by the trigeminal nerve or upon motor components associated with this response.

Electrodes for stimulation and recording were implanted bilaterally into the amygdala and hypothalamus under aseptic conditions in cats. Postoperatively, stage I of the experimental procedure consisted of identifying sites in the amygdala which significantly modulated ($p<0.05$) hypothalamically-elicited quiet biting attack. In stage II of the experiment, the lateral extent of the lipline that, when probed, could elicit the jaw opening component of the attack response was determined for a given intensity of hypothalamic stimulation. Subsequently, the effect of dual stimulation of the amygdala and hypothalamus upon the "effective" extent of the lipline was determined.

The results indicate that sites in the amygdlala which significantly inhibited quiet attack also significantly reduced ($p<0.01$) the lateral extent of the lipline from which jaw opening could be generated. Other observations suggest that the amygdala does not significantly modulate motor components of the attack response. For example, latencies to initial movement during the attack sequence following either single stimulation of the hypothalamus or dual stimulation of the amygdala and hypothalamus were not significantly different ($p>0.1$). Further, latencies to jaw opening following midline probing of the lip were not significantly different when compared under conditions of single and dual stimulation ($p>0.1$).

It thus appears that amygdaloid modulation of the attack response is achieved, in part at least, through its effects upon the sensory component of this behavior.

{Supported by NIH Grant NS 07941-09}

670 HIPPOCAMPAL INFLUENCE ON AFFECTIVE COMPONENTS OF FEMININE SEXUAL AND AGGRESSIVE BEHAVIOR IN THE RAT: DORSAL-VENTPAL DISTINCTIONS. William R. Cameron, Fred H. Gage, III, Cheryl L. Boedeker* and John C. Hitt. Chemistry of Behavior Program, Dept. of Psychology, Texas Christian University, Fort Worth, Texas 76129.

Recent research has revealed that limbic structures (i.e., septal area & hippocampus) exert modulatory influence on feminine sexual behavior. In addition neuroanatomical, neurochemical, electrophysiological, and behavioral studies all indicate that the hippocampus may be both, functionally and structurally organized along a dorsal-ventral axis.

On the basis of this information, we assessed the influence of the hippocampus on feminine sexual and aggressive behaviors focusing on dorsal-ventral distinctions in mediation of these behaviors. Bilateral radio-frequency lesions were made in either the anterodorsal (DHL) or posteroventral (VHL) aspects of the hippocampal formation of ovariectomized rats. Sham-lesioned and unoperated rats served as controls. In the first phase of the study, subjects were tested with varying dosages of estrogen alone and then retested with a combination of estrogen and progesterone 6 hrs. later. The second phase consisted of testing the subjects under varying dosages of progesterone with estrogen held constant. Measures of lordosis, rejection, soliciting and aggression were taken.

Lesions of the anterodorsal and posteroventral hippocampus had differential effects on feminine sexual behavior. DHL animals displayed a lower probability and intensity of lordotic behavior than controls on both pre-and post-progesterone tests. Soliciting was also decreased in post-progesterone tests. The DHL animals also displayed little active rejection of the male and little fighting behavior. Conversely, the VHL animals demonstrated heightened probability and intensity of lordosis in both pre-and post-progesterone tests, although the most dramatic effects were in the post-progesterone tests. Soliciting was also somewhat decreased. Additionally, the VHL animals showed increases in active rejection of the male and increases in fighting behavior. Moreover, the VHL animals were reactive to touch by both the male rats and the experimenter.

The results suggest that the dorsal and ventral hippocampus have differential input into the diencephalic structures mediating feminine sexual and aggressive behavior. This input may best be characterized as mediating an affective component of female sexual and aggressive behavior.

671 OESTROGENIC INFLUENCES ON THE ELECTRICAL ACTIVITY OF THE OLFACTORY PATHWAY. L. Cartas-Heredia*, R. Guevara-Aguilar and H.U. Aguilar-Baturoni. Departamento de Fisiologia, Facultad de Medicina, U.N.A.M., Mexico.

The influence of the oestrogenic hormones over the spontaneous and induced activity of the olfactory pathway was studied in normal female cats. Electrodes were placed chronically or acutely in the olfactory bulb (OB), olfactory tubercle (OT) and in the prepyriform cortex(PPC). Oestrogenic hormones were applied locally in the posterior hypothalamic region. Recordings were made during the two different phases of the oestral cycle. In addition, another group of castrated animals was studied. The oestral phase was induced in these cats by the subcutaneous administration of 17-ß-oestradiol. Results indicate that the pattern of the electroencephalographic spontaneous activity as well as the response induced by hypothalamic stimulation changed. The number of the "bursts" for each 10 sec of trace, was higher in oestrus than in anoestrus for all the structures studied. The duration of each burst also changed, being shorter in oestrus than in anoestrus cats. The threshold for significant bursting in the olfactory structures following hypothalamic stimulation was lower in oestrus than in anoestrus. The evoked potentials recorded in the same 3 olfactory structures by hypothalamic stimulation exhibited changes in correlation with the hormonal administration. In all the structures studied the amplitude of the different components of the evoked potentials increased immediately after the hormones were administered. However the most dramatic increase was observed in the olfactory tubercle. In order to further investigate these changes in acute preparations, a study evaluating the excitability changes was conducted. Applied pulse pairs, with different interpulse intervals between 200 to 1000 m sec were delivered in the hypothalamus before and after 200 γ of the hormone were administered into the posterior hypothalamus. Results illustrated that a significant increase in the amplitude of evoked potentials occurred following the smaller interpulse intervals in the OB. In the OT, the administration of the hormone resulted in a decrease in the amplitude of the potentials for all interpulse intervals studied. A relatively smaller decrease in evoked potential amplitude was observed for longer interpulse intervals in the PPC.

672 NON-PYRAMIDAL LAYER PROJECTION NEURONS OF HIPPOCAMPUS. Robert B. Chronister. Dept. Anat. Sch. Med., Univ. S. Ala., Mobile, Ala. 36688

With the advent of the axoplasmic transport methods, the understanding of hippocampal hodology has changed considerably. Emphasis has shifted from the CA fields to subiculum. Throughout this research appears to be the assumption that only cells within the stratum pyramidale project out of hippocampus. This view has been buttressed by the recent observations (Ribak et al, 1978) that GAD positive neurons exist in stratum oriens and radiatum of hippocampus. No comment has been made concerning the total distribution and number of these GAD positive neurons.

In order to ascertain the distribution of projection neurons in hippocampus, discrete injections (0.02-0.05µℓ) of horseradish peroxidase were made in the various regions of the septal complex in adult Sprague-Dawley derived rats. After a 36 hour survival time, the animals were prefused with dilute Karnovsky's fixative in phosphate buffer (pH 7.4). They were sectioned at 80µ and reacted with DAB. Examination of these sections revealed numerous HRP positive neurons in stratum oriens and the alveus. An occasional positive neuron was also found in stratum radiatum. Characteristically, the highest density of positive neurons were found in the regions of stratum oriens and the alveus of the fundus of the hippocampal fissure.

Examination of standard Nissl and Heidenhain sections of adult rat revealed that there are more neurons in stratum oriens and the alveus of CA2-CA3 than in the adjacent CA1 (especially CA1c). This distribution was even more pronounced in stratum radiatum. The neurons also characteristically were found in small cluster or islands of cells. The results of these studies indicate that neurons other than those in stratum pyramidale project outside the hippocampus. The HRP technique does not allow statements to be made of the the terminations of these efferents but the involved neurons clearly cannot be considered interneurons or short-axoned cells. Supported in part by an Intramural Research Grant from the College of Medicine.

673 THE ROLE OF ENTORHINAL CORTEX DURING CLASSICAL CONDITIONING: EVIDENCE FOR ENTORHINAL-DENTATE FACILITATION. Gregory A. Clark*, Theodore W. Berger, and Richard F. Thompson. Dept. Psychobio., UCI, Irvine, CA 92717.

Previous investigations[1,2] have demonstrated an early, rapid and substantial increase in hippocampal neuronal activity induced by a behavioral learning paradigm. While certain anatomical and electrophysiological evidence suggested the entorhinal-dentate synapse as a potential site of facilitation, the possibility remained that such increases in hippocampal neural activation merely reflected corresponding changes in preceding structures. In the present study, electrophysiological analysis of entorhinal cortex was coupled with simultaneous recordings from hippocampus during learning to discriminate between these two hypotheses.

Microelectrodes were implanted in the CA1 or dentate granule cell layers of the hippocampus and in layers II or III of entorhinal cortex of New Zealand white rabbits. Entorhinal and hippocampal activity were recorded subsequently during a classical conditioning paradigm in which a tone CS was associated with a corneal air puff UCS to evoke nictitating membrane extension.Unit activity and behavior were analyzed for 250 msec prior to CS onset (Pre-CS background period), 250 msec between CS and UCS onset (CS period), and for 250 msec following UCS onset (UCS period).

Results showed that, while entorhinal and hippocampal activity were similar within trial periods, recordings from hippocampus and entorhinal cortex proved markedly different across trials (that is, over the course of learning). Within trials, neuronal records from both structures exhibited a heightened neural discharge (compared to spontaneous rates) during the UCS period early in training--prior to behavioral conditioning. Unit discharges in both areas shifted into the CS period as behavioral conditioning developed. Across trials, however, entorhinal discharges remained constant or increased slightly, yet rapid and substantial increases occured in the magnitude of the hippocampal discharge. Such hippocampal neuronal increases cannot be accounted for solely on the basis of activity either in entorhinal cortex or, as previously reported[3], in medial septum (the other major afferent to the hippocampus). Thus, these findings suggest facilitation across the entorhinal-dentate synapse and indicate the initial locus of these learning dependent neural changes to be the hippocampus per se.

1. Berger, T.W., Alger, B.E., and Thompson, R.F. Science, 1976, 192, 483-385.
2. Berger, T.W., and Thompson, R.F. Brain Res., 1978, 195, 323-346.
3. Berger, T.W., and Thompson, R.F. Science, 1977, 197, 587-589.

674 FINK-HEIMER STAINING FOLLOWING KAINIC ACID LESIONS: A NEW MODIFICATION OF AN ESTABLISHED TRACING METHOD. Timothy J. Collier,* Russell E. Ruth and Aryeh Routtenberg (SPON: R. Santos-Anderson). Cresap Neuroscience Laboratory, Northwestern University, Evanston, ILL 60201.

In tracing fiber systems emanating from a given anatomical locus, traditional electrolytic and chemical lesion techniques coupled with the Fink-Heimer silver staining method possess the drawback of damaging fibers traversing the area of the lesions, thus producing a degeneration pattern which combines fibers of passage and the degenerating system of interest. This problem can be circumvented through the use of autoradiographic tracing, using labelled amino acids and providing information about terminal fields and cells of origin. These methods do not, however, allow detailed tracing of the trajectory of fiber systems, nor do they provide data on the caliber and detailed pattern of terminal innervation of the fibers comprising the system of interest.

We propose that the use of kainic acid lesions in combination with Fink-Heimer silver staining of degenerating axons and terminals provides a method which is potentially as selective as autoradiographic tracing and horseradish peroxidase techniques, while, in addition, allowing detailed study of the course of the degenerating fiber system. Since local injections of kainic acid destroy nerve cell bodies but leave axons of passage intact, the Fink-Heimer method can be used to reveal unambiguously the course, trajectory, caliber and terminal field of the fiber system under consideration.

We have previously described the distribution of islands of dopamine fibers and terminals in the ventral lateral entorhinal cortex, and the apparent association of this dopamine innervation with cell clusters found at anterior levels of this cortical area (Collier and Routtenberg, Brain Research, 128, 1977, 354-360). Kainic acid lesions of these cell clusters, followed by Fink-Heimer silver staining, provides a technique for revealing the trajectory of this pathway along its course through the hippocampal formation as well as the details of the system's terminal ramifications. In addition, Vibratome® sections, prepared for detection of catecholamines, enable evaluation of the extent to which presynaptic dopamine islands remain undamaged following cell destruction by kainate. (Supported by MH 25281 and NSF 19388 to A. R.)

675 AUTORADIOGRAPHIC INVESTIGATION OF THE CENTRAL PROJECTIONS OF THE OLFACTORY TRACTS IN MACROPODUS OPERCULARIS (L.) (OSTEICHTHYES: BELONTIDAE). Roger E. Davis, Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

The distribution of the lateral and medial olfactory tracts was investigated using radioautography. Twentysix adult male Macropodus were administered 0.2 to 0.5 μCi of L [^{3}H-2,3] proline on a dry 60 to 80 μm diameter bead of Dowex resin which was implanted unilaterally in the olfactory bulb. As a control for diffusion of labeled amino acid from the bulb to the telencephalon, which could result in direct uptake into telencephalic neurons, in 2 additional males the bead was implanted in the rostral pole of the area dorsalis telencephali, or pallium. Following a 5 hr or 10 day survival period, the male was sacrificed, and the brain was embedded in paraffin. Horizontal or transverse, sections 10 μm thick were mounted on glass slides, coated with NTB-2 emulsion and kept in the dark at 5°C for 8 or 21 days. The slides were developed, fixed, and the sections stained with cresyl violet acetate. The sections were examined with dark and bright field microscopy to locate labeled axons and terminals. Reduced silver grains were distributed ipsi- and contralaterally in the medial and ventrolateral subpallium and in nucleus taenia and area Dp in the basolateral and posterior pallium. Extensive portions of the dorsal, central and posterior pallium contained no label or only scattered label suggesting that these areas do not receive primary olfactory input. The median forebrain bundle from the posterior telencephalon to the posterior medial thalamus was lightly labeled. A large labeled tract was seen in the ventrolateral subpallium projecting extensively to the neuropil adjacent to the nucleus lateral tuberis, nucleus of the posterior recess, and ventromedial to the nucleus of the lateral recess. Implantation in the rostral-pallium resulted in intense labeling in the area of the bead, the medial and lateral subpallium, and the projections to the posterior thalamus and hypothalamus. However, nucleus taenia and area Dp were only slightly labeled.

676 CHOLINERGIC MECHANISMS AND POST-TETANIC POTENTIATION. J.F. DeFrance, J.C. Stanley, J.E. Marchand, P. Divakaran and Y. Clement-Cormier. Department of Neurobiology and Anatomy and the Department of Pharmacology, The University of Texas Medical School at Houston, P.O. Box 20708, Houston, Texas 77025.

Acutely prepared rabbits were used to study, electrophysiologically and biochemically, the contribution of cholinergic mechanisms to post-tetanic potentiation in hippocampal field CA1.

Rabbits were acutely prepared under urethane or urethane-chloralose anesthesia. The dorsal aspects of the hippocampal formation and septal region were exposed by removing the cortex and corpus callosum. This allowed for precise positioning of the stimulating and recording electrodes.

Monosynaptic responses were recorded in hippocampal field CA1 with microelectrodes following activation of: (1) the septal-hippocampal pathway which takes its origin primarily from the medial septal region (MSR) (i.e., the medial septal nucleus (MSN) and the nucleus of the diagonal band of Broca (nDBB)), and (2) from the contralateral hippocampal field CA3 (CCA3). Stimulation of MSR and CCA3 was done with microelectrodes (1-50 megohms).

To study the influence of various drugs on the normal and potentiated responses, multibarrel electrodes were used. This array included a recording barrel and a current-summing barrel in addition to the drug containing barrels. The compounds studied were: acetylcholine (ACh: 0.1-0.5M, pH 6.7), guanosine 3', 5' monophosphate (cGMP, 0.1-0.5M, pH 6.0), atropine sulfate (Atp 0.1M, pH 6.2), physostigmine (Phys: 0.5M, pH 6.5), isobutyl methyl xanthine (MIX: 0.1M, pH 6.5), K^+Cl^- (1.0M, pH 6.8). Theophylline was administered peripherally (2.0-6.0 mg/kg). ACh, physostigmine, and cGMP each had an excitatory effect upon pyramidal cell responses when applied in stratum radiatum. The time-course studies showed that these effects outlasted the duration of the injection current for many minutes.

Phosphodiesterase inhibitors (e.g., isobutyl methyl xanthine) prolonged the time course of recovery with test responses which were post-tetanically potentiated. K^+, on the other hand, selectively enhanced tetanic potentiation.

It was suggested, with respect to the potentiation phenomena, that K^+ acted primarily presynaptically to facilitate transmitter release, whereas cGMP acted primarily postsynaptically for the enhancement of pyramidal cell excitability.

The electrophysiology findings were supported by biochemical analysis for cyclic GMP and cyclic AMP levels. Hippocampal tissue showed over a twofold increase in cyclic GMP levels following trains of stimuli which maximally produce post-tetanic potentiation. Cyclic AMP levels were only slightly depressed. Supported by NSF #GB35532 and NIH #1F32 NS05874-01.

677 EFFECTS OF A PAINFUL FOOTSHOCK ON THE PSYCHOPHYSICS OF SELF-STIMULATION BEHAVIOR. Philippe De Witte* and Michel Meulders. Lab. Neurophysiol., Université de Louvain, UCL 5449, 54, av. Hippocrate, B-1200 Bruxelles, Belgium.

In a study on the psychophysics of self-stimulation a rewarding hypothalamic stimulation served as a conditioned stimulus (C.S.) in an avoidance paradigm in the rat. Generalization tests were conducted by modifying the electrical parameters of the C.S. forming a set of substitute stimuli (S.E.). Results show that a Stevensian power function relates the reinforcing sensation, measured by the generalization tests, to the intensity of the rewarding stumulation (calculated by the bar-pressing rate for all the S.S.).

The effects of a concomitant painful footshock upon the reinforcing sensation as estimated by the power function were then studied. Data show that the greater the intensity of the footshock, the greater the length of each self-stimulation volley behavior, showing that animals attempt to compensate aversive properties of the footshock by procuring more intracranial reward. Further studies showing that injection of naloxone (5×10^{-4} mg) increases significantly the time elapsed to self-stimulate, suggesting the release of an intracerebral morphine-like substance during compensatory behavior when concomitant painful footshock is added during self-stimulation behavior.

678 BEHAVIORAL EFFECTS OF SYSTEMIC L-DOPA AND DIRECT APPLICATION OF DOPAMINE TO MESOLIMBIC FOREBRAIN IN NONHUMAN PRIMATES. Russell E. Dill, Daniel L. Jones,* J. Christian Gillin, and Greer Murphy.* Dept. Anat., Baylor Coll. Dent., Dallas, Tx. 75246 and NIMH, Bethesda, Md. 20014.

Six adult rhesus and four adult squirrel monkeys were given L-DOPA, 100 and 50 mg/kg i.p. respectively in combination with 20 mg/kg carbidopa and 5 mg/kg ascorbic acid. All animals were then observed in their home cage or a plastic observation cage. Both primates showed an initial period of depressed activity having mean durations of 24 and 23 min followed by increased locomotion, hypervigilance and in the case of the squirrel monkeys, protracted rubbing of the face alternating with "searching" activity. Both species developed a compulsive gnawing syndrome. The duration of these activities was 4 to 5 hours.

The nucleus accumbens (NA) was cannulated bilaterally in the 4 squirrel monkeys and 1 rhesus monkey. All animals were pretreated 18 hr prior to intracranial (i.c.) injection with the monoamine oxidase inhibitor tranylcypromine 1 mg/kg i.p. The NA of squirrel monkeys was injected bilaterally with 100 µg dopamine (DA) and observed as before. These animals showed an initial period of depressed activity lasting 79 min followed by increased locomotor activity characterized by "searching" activity, grooming and rapid stereotyped submissive or juvenile posturing. No gnawing and less face rubbing was seen as compared with systemic L-DOPA. The i.c. injection of 300 µg DA in each NA of the rhesus monkey produced a depressed period followed by intense stereotyped pacing. No other unusual behavior was seen.

Haloperidol, 0.1 mg/kg i.p. markedly delayed the onset of locomotor stimulation and prevented the appearance of all stereotypic and gnawing activity induced by either systemic L-DOPA or i.c. DA.

The i.c. injection of 100 µg L-norepinephrine into each NA of the squirrel monkeys produced a period of depressed activity lasting about 2 hr followed by minimal locomotor activity consisting of retropulsion in a sitting position. All animals displayed a marked loss of extensor muscle strength and tone in the lower extremity. The latter effect lasted between 6 and 12 hr. The i.c. injection of saline in all animals produced no unusual behavior.

These results confirm the locomotor stimulating and stereotypy-inducing properties of systemic L-DOPA in primates and suggest that some of the activities could be related to increased dopaminergic activity in the mesolimbic nucleus accumbens.

679 ELECTROPHYSIOLOGICAL EVIDENCE FOR THE PERFORANT-CA1 PATHWAY IN THE HIPPOCAMPAL SLICE. Herbert J. Doller* and Forrest F. Weight (SPON: A. P. Oliver). Laboratory of Neuropharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

A hippocampal pathway originating in the entorhinal cortex, traversing as part of the perforant pathway and terminating on the apical dendrites of the CA1 pyramidal cells has been described anatomically (Acta Anat 35:202, 1958; Brain 88:963, 1965). However, electrophysiological identification remains inconclusive (Exp. Neurol. 35:541, 1972; The Hippocampus, Isaacson and Pribrum (eds.), p. 161, 1975). We investigated this perforant pathway using electrophysiological techniques in the hippocampal slice (Epilepsia 18:543, 1977). Stimulation of the perforant pathway resulted an evoked potential with a latency of approximately 10 msec in the CA1 cell layer. This response was inhibited by media containing 0.1 mM Ca and 5 mM Mg suggesting synaptic mediation. Two types of experiments suggest this CA1 response was not the result of activation via the mossy fiber and Schaeffer collateral pathways. First, the sum of the latencies of the perforant pathway to the granule cells, the mossy fiber pathway, and the Schaeffer collateral pathway was approximately twice the latency of the perforant stimulation directly to the CA1 cells. In a second series of experiments, the mossy fiber pathway was transected. Despite this, evoked potentials were recorded in CA1 following perforant stimulation. Another possible interpretation of the data is that Schaeffer collaterals are being antidromically stimulated. To test for this possibility, 1 mm sections were cut out of the CA1 region of the slice between the stimulating and recording electrodes. The section was from the alveus to, but not including, the perforant pathway. This includes the stratum lacunosum which contains Schaeffer collaterals. With this lesion, evoked responses were still recorded in CA1 with perforant stimulation. These results provide electrophysiological evidence for a synaptic input to CA1 cells from the perforant pathway.

680 GENETIC AND NEUROCHEMICAL ASPECTS OF THE ACQUISITION OF AVOIDANCE BEHAVIOR FOLLOWING SEPTAL LESIONS IN THE MOUSE. Richard J. Fanelli*, Peter J. Donovick and Richard G. Burright*. Dept. of Psychology, SUNY-Binghamton, Binghamton, NY 13901.

One of the well defined behavioral alterations that occurs in animals with septal lesions is enhanced acquisition of a two-way active avoidance task. Previous work from our laboratory showed that many of the effects of septal lesions can be altered by manipulations of environment, experience and genotype. Recently, we reported that enhanced acquisition of the two-way active avoidance task associated with septal lesions is dependent on the strain of mouse tested. The present investigation examined the levels of brain catecholamines and their relation to the genotype dependent behavior following septal damage in mice.

Mice of the C57BL/6J and RF/J strains were assigned to either receive control operations or bilateral electrolytic lesions of the septal area. Following a ten day recovery period, animals were tested on a two-way active avoidance task in standard shuttle boxes. After the behavioral testing, the mice were sacrificed. For most of the mice, brains were removed, weighed and frozen, and a fluorometric analysis of catecholamine levels was done later. Remaining mice were perfused and examined for the extent of the lesion.

The mice of the C57 strain, which normally perform poorly in the two-way active avoidance task, showed an enhanced acquisition following septal lesions. The RF mice, which normally acquire the task rapidly, did not show the reported septal enhancement. The forebrain, hindbrain and total brain weights of the two strains were very similar across surgical conditions, but brains of lesioned mice tended to be somewhat lighter then the controls of both strains. There was a slight decrease in norepinephrine levels in the forebrain of the lesioned animals of both strains, however, there was no major lesion effect upon catecholamine levels, nor a lesion-by-strain interaction. Neither norepinephrine nor dopamine transmitter levels predicted the behavior of these two strains of mice or the septal effects.

From this data, it seems that the strain differences in the behavioral response to septal damage can not be explained exclusively on the basis of differences in catecholamine levels. The fact that differences do exist may implicate differential fine effects of the lesion, and their interaction with existing genotypic and experiential factors.

681 EFFECTS OF STIMULATION OF SUBSTANTIA INNOMINATA AND OTHER CORTICAL AND SUBCORTICAL SITES ON THE ELECTROPHYSIOLOGY OF AMYGDALOID NEURONS. Philip A. Femano*, Henry M. Edinger, Allan Siegel, and Stanley Z. Kramer*. Departments of Physiology and Neurosciences, College of Medicine and Dentistry of New Jersey - New Jersey Medical School, Newark, NJ 07103, and Department of Biology, Seton Hall University, So. Orange, NJ 07079.

The effects of electrical stimulation of neocortical and subcortical structures on the electrophysiology of amygdaloid neurons were examined in the ketamine anesthetized cat. Glass microelectrodes (3M NaCl, 8-10 MΩ) were used to record extracellular unit responses within the amygdala. Square wave stimulus pulses (25-1000 µA) were delivered through stainless steel electrodes located in the lateral olfactory tract, sylvian cortex, substantia innominata, and ventromedial hypothalamus.

All sites produced both excitatory and inhibitory influences on amygdaloid neurons. Of the sites tested, the substantia innominata and sylvian cortex exhibited the most powerful excitatory inputs upon amygdaloid neurons. Onset latencies were in the range of 5-30ms. Stimulation of the lateral olfactory tract and ventromedial hypothalamus elicited both a short latency excitatory response of approximately 20-50ms, plus a second population of longer latency excitatory responses. Orthodromic activation of single units could occasionally be elicited by more than one stimulus site. The preliminary evidence also suggests the presence of more complex convergent interactions of cortical and subcortical afferents on amygdalar neurons.

(Supported by NIH Grant # NS 07941-09)

682 HIPPOCAMPAL FIELD POTENTIALS AND SINGLE UNIT ACTIVITY EVOKED BY STIMULATION OF MEDIAL SEPTAL NUCLEUS, ENTORHINAL CORTEX AND VENTRAL HIPPOCAMPAL COMMISSURE IN FREELY-MOVING RATS. S. E. Fox and J. B. Ranck, Jr., Dept. of Physiology, Downstate Medical Center, State University of New York, Brooklyn, NY 11203.

Using aseptic stereotaxic surgery, rats were prepared with three independently moveable microelectrodes for chronic recording in the hippocampal region, and three stimulating electrodes in medial septal nucleus (MSN), entorhinal cortex (EC) and ventral hippocampal commissure (VHC) as reported previously (Fox and Ranck, Neuroscience Abstracts 3: 198, 1977). Electrodes for recording neocortical EEG and neck-muscle EMG were also implanted to facilitate behavioral monitoring. Prior to surgery, rats were trained to walk continuously on a motor-driven treadmill. They were also taught to drink continuously, immediately on presentation of water, by allowing them access to the water spout only a few minutes at a time, several times a day. They were housed in the treadmill for several days, so that after recovery, recordings could by obtained during both slow wave and paradoxical sleep.

Several days after surgery, field potentials and unit activity evoked by stimulation of MSN, EC and VHC were recorded from the hippocampal region during walking, drinking, slow wave sleep and paradoxical sleep, taking care to keep behaviors constant during each bout. These behaviors represent two theta-mode behaviors and two non-theta behaviors, one each during sleep and wakefulness. Field potentials evoked at fixed time of day were stable over many days once electrodes were in place. Three categories of results were obtained. 1) Field potentials in response to MSN stimulation did not correspond to their description in the literature. Strength/duration curves indicated that the chronaxie of the elements stimulated in MSN to produce the field potential in hippocampus was about 1 msec. This suggests that unmyelinated axons, dendrites, somas or some combination were being stimulated. 2) Dual pulse facilitation was observed in the VHC to CA1 afferent system, and its characteristics were similar to the facilitation in the EC to fascia dentata system. 3) Behavioral dependence of amplitude of components of field potentials from EC stimulation, reported by Winson and Abzug (Science 196: 1223, 1977) was confirmed, and extended to include other afferent systems and unit activity.

(Supported in part by NS 12664, NS 14497 and BNS 77-09375 to J. B. Ranck, Jr. and NS 05773 to V. E. Amassian.)

683 THE INTERCALATED NUCLEI OF THE AMYGDALA: A GOLGI STUDY IN THE RAT. JoAnn E. Franck, Dept. Psych., Univ. Rochester, Rochester, NY 14627.

In Nissl stained material the intercalated masses (MI) are clusters of medium-sized (15-20μ) cells in the external capsule and longitudinal association bundle (LAB), fiber tracts surrounding the lateral and basolateral amygdala. One large island appearing beneath the anterior commissure's lateral wing, is displaced ventromedially by the basolateral nucleus. Caudally, it lies in the fibers of the stria terminalis. The strategic location of the MI within the major fiber bundles of the amygdala prompted this analysis.

The appearance of the MI in Nissl material belies their heterogeneity and the formidable extent of their processes. Intercalated cells within the external capsule and LAB have several sinuous primary and secondary dendrites oriented parallel to the entering and exiting axons in frontal sections. The cells of the MI in the stria terminalis are identical except the dendritic axis is perpendicular to the strial axons. The dendritic spines are often so dense they obscure the dendritic shaft. Individual spines are extremely ornate; as many as four separate peduncles and twigs arise from a single stalk. Somatic spines are also observed. The axons give off several collaterals which ramify near the parent cell. The main axon continues dorsally or ventrally in the passing fiber bundle. Along their course the collaterals have numerous varicosities and twigs suggesting presynaptic specializations. A second type of MI cell associated with passing fibers also is observed medial to the ventral tip of the basolateral nucleus. Two or three dendrites emerge from the soma and are oriented parallel to the LAB. The most striking feature is the length of the primarily unbranched dendrites; some are often over 600μ long. Thus, these cells, which are more than 1mm long from tip to tip, form a formidable dorsal-ventral band between the basolateral and lateral nuclei and more medial areas. The spines of these cells, while fewer in number, are ornate and unusually long (10μ).

Many cells in the single large MI are not in the way of passing axons and are stellate in appearance. Dendritic spines are numerous but much shorter and simpler. The axons of these cells also emit several local collaterals. A single branch goes dorsally towards either the central amygdala or LAB.

Nothing is known of the function of the MI, however, many are located so that fibers exiting and entering the amygdala pass through them. In turn, the MI cells give off numerous short axon collaterals which may modulate function in neighboring amygdaloid nuclei. In addition, it is possible that some of these cells may project to extra-amygdaloid structures via the LAB.

(Supported by NIMH grant R03 MH 28678.)

684 ATTENUATION OF SEPTAL HYPERREACTIVITY BY INTRACRANIAL INJECTION OF MORPHINE INTO THE PERIAQUEDUCTAL GRAY (PAG) Fred H. Gage, III James J. Valdes*, and Roy G. Thompson, Dept. of Psychology, Chemistry of Behavior Program, Texas Christian University, Fort Worth, Texas 76129.

Lesions to the septal nuclei of rats initiate a transient hyperreactivity to somatosensory stimuli which lasts approximately two weeks. This increase in responsiveness has been previously described as an increase in aggression, irritability, emotionality, or rage; the common element of the syndrome reflecting an increase in affective responding to stimuli. We have previously demonstrated the possible involvement of both norepinephrine and serotonin in modulating this hyperreactivity. Opioid narcotics have similarly been suggested to modulate the affective component of pain via interactions with the biogenic amines, particularly serotonin. The purpose of this study was to determine whether an opioid narcotic (morphine) could selectively attenuate the increase in affective responding following septal lesions. Rats with sham or septal lesions received 1ul injections of either saline or morphine (5ug/ul or 15ug/ul) into the rostral PAG, an area involved in analgesia and through which aminergic projections to the limbic forebrain pass, within one day following the lesion when the hyperreactivity was maximal. The animals were behaviorally tested for reactivity at 10 and 60 minutes after injection, and at 2, 4, 8, 16, and 30 days following surgery. Magnitude of response to electric foot shock was quantitatively assessed using a calibrated force transducer and polygraph recorder. A standard rating scale of septal hyperreactivity was employed along with additional measures of motor and sensory abilities. Immediately and for 24 hours following injection, morphine completely eliminated the increased affective responding induced by the septal lesion on all measures, without debilitating motor or sensory components. The selective attenuation of affective responding was immediately reversed by a 1ul injection of naloxone (40ug/ul) given 10 minutes after the morphine injection. These results demonstrate a selective, naloxone reversible, morphine attenuation of septal lesion-induced hyperreactivity. The effect appears specific to the affective component of the septal syndrome as neither motor nor sensory abilities were impaired, and suggests the involvement of brain mechanisms of pain perception in the syndrome.

685 AMYGDALOID LESIONS DISRUPT INCENTIVE-MOTIVATION IN THE RAT. Michael G. Gaston* (SPON: K. M. Gregory). California State Univ., Los Angeles, CA 90032

A series of three experiments provided data consistent with the view that the amygdala is involved with mechanisms of incentive-motivation. Rats with electrolytic lesions aimed at the basolateral amygdala were less responsive than Controls under conditions that afforded reward, but more responsive when reward was not obtainable. The performance of lesioned animals was also relatively unaffected by differences or shifts in amount of reward. In Experiment I, subjects were trained on a go-no-go successive discrimination problem. They found food at the end of a black (white) straight-alley and no food in a white (black) alley. Amygdalectomized rats ran significantly slower than Controls in the positive alley, but significantly faster in the negative alley. These subjects were subsequently tested in a T-maze, one arm of which was black and the other white. Lesioned rats entered the positive arm significantly less often than Controls, suggesting that amygdaloid lesions disrupt the extent to which a stimulus paired with primary reward acquires secondary reinforcing and/or incentive-motivational properties. In Experiment II, subjects found a large quantity of food at the end of a black or white alley and a small amount of food in the other alley. Control rats ran much faster in the high reward alley than in the low. For amygdala lesioned animals, however, there was little difference between the speeds with which they ran to get large and small amounts of food. Anticipatory attempts to push through a top-hinged startbox door were automatically recorded. Lesioned rats showed less overall anticipatory responding than Controls. Further, amygdaloid animals, unlike Controls, made almost as many anticipatory responses before entering the low reward alley as before entering the high reward alley. In Experiment III, separate groups of subjects ran a gray straight-alley for either a large or a small amount of food reward. Subsequently, half the high (low) reward rats were shifted to low (high) and half the animals continued to run for the same reward quantity. Differences in running speed as a function of reward amount were significantly less for lesioned subjects than for Controls. Lesioned rats shifted down in reward amount showed less of a negative contrast effect, and those shifted up displayed less of a positive contrast effect, than did Controls. Taken together, the findings reported here suggest that amygdaloid lesions produce deficits in the development of appropriate excitatory or inhibitory motivational tendencies in situations involving changes in the parameters of reward.

686 CORTICAL PROJECTIONS OF THE THALAMIC MEDIODORSAL NUCLEUS IN THE OPOSSUM. Gregory T. Golden, Jan C. Jackson* and Robert M. Benjamin. Dept. of Neurophysiology, University of Wisconsin Medical School, Madison, Wisconsin 53706.

The cortical projection field of the thalamic mediodorsal nucleus (MD) in the opossum was mapped with retrograde horseradish peroxidase and anterograde tritiated proline techniques. The results of several large proline injections placed in MD indicate that MD projection cortex extends to all four surfaces of the frontal lobe. The labeled area begins at the frontal pole and includes most of the medial wall rostral to the tip of the fissura intercalata. It widens on the dorsal surface to reach a posterior limit at the caudal limb of the orbital fissure. On the lateral and ventral cortical surfaces the area widens again to produce a 'tail' which extends along the rhinal sulcus for a short distance behind the orbital fissure and ends ventral to taste cortex as defined electrophysiologically.

Cortical HRP injections corroborated the major findings obtained with proline and added detail concerning the topography of the MD projection. In general, lateral portions of the MD project to the dorsal cortical surface and within this field anterior lateral MD projects to anterior dorsal cortex while posterior lateral MD projects to posterior dorsal cortex. Medial portions of MD project to medial and lateral walls of cortex: anterior medial MD to the medial wall and posterior medial MD to the lateral wall.

The location and the topographic arrangement of projections from medial MD are similar to those previously described for the rabbit. However, the position of the lateral MD projection field is strikingly different in the two species.

687 DESTRUCTION OF GRANULE CELLS IN THE DENTATE GYRUS AND CEREBELLAR CORTEX OF ADULT RATS FOLLOWING INJECTIONS OF COLCHICINE. Richard Goldschmidt* and Oswald Steward (SPON: S.S.Winans). Depts of Neurosurgery, Anatomy and Physiology, University of Virginia School of Medicine, Charlottesville, VA 22901.

In experiments attempting to use colchicine to block axonal transport in the dentate gyrus (DG), a seemingly selective cytotoxicity of colchicine for DG granule cells was observed. Forty-eight hours after injections of 25 or 2.5µg of colchicine in 0.5µl of distilled water, affected granule cells were shrunken and of irregular shape and small densely stained particles of cell debris were present in the granular layer. Massive invasion of the affected area by microglial cells was also observed. A variety of longer survival times were investigated, and by 50 days after the injection only glial cells remained in the injected area of the DG. Pyramidal cells in the hilus of the DG were also destroyed, but other nearby pyramidal cells seemed unaffected. Fink-Heimer staining of cases 3 and 4 days after injection revealed degeneration in the mossy fiber and commissural systems within the hippocampus.

Injections of 25µg of colchicine into the vermis of the cerebellar cortex were made to investigate the generality of this colchicine effect on granule cells. Gross behavioral abnormality appeared within hours after the injections. Forth-eight hours after injection microglial invasion and cell debris in the granular layer of the vermis was apparent around the injection site. Purkinje cells nearby appeared paler staining and misaligned while the cytoarchitecture of more lateral regions of cerebellar cortex appeared normal.

Since control injections of colcemid or saline do not produce this effect, the cytotoxic action of colchicine on granule cells seems to be unrelated to its effects on microtubules. A toxic effect on membrane nucleoside transport mechanisms might explain this cytotoxic action. Why this effect is relatively specific for granule cells remains uncertain.

(Supported by NIH Grant #5 RO1 NS12333 to O.S.)

688 SUPPRESSION OF EMOTIONAL BEHAVIOR IN CATS BY STIMULATION OF VENTRAL TEGMENTAL AREA AND NUCLEUS ACCUMBENS. Jeffrey M. Goldstein* and Jerome Siegel, Institute for Neuroscience and Behavior and School of Life and Health Sciences, University of Delaware, Newark, DE 19711.

Previous studies have shown that the mesolimbic dopamine system may subserve a functional role in emotional behavior. The present study investigated the effects of concomittant electrical stimulation of the ventral tegmental area (VTA) or nucleus accumbens (NA) on lateral hypothalamic-induced attack behavior in cats. Low frequency (6pps) stimulation of VTA or NA suppressed hypothalamically-induced attack without affecting the autonomic or non-directed somatic components of the attack reaction. High frequency (60pps) stimulation either failed to suppress attack (VTA) or produced less suppression of attack compared to low frequency stimulation (NA). To rule out the possibility that low frequency stimulation per se would disrupt ongoing behavior, natural (light flashes) and artificial (lateral geniculate) stimulation were tested against hypothalamically-induced attack. Both forms of sensory stimulation failed to alter the attack reaction. The results of this study suggest that a function of the mesolimbic dopamine system is to inhibit emotional behavior.

689 LESION OF HIPPOCAMPAL CA3 PYRAMIDAL CELLS WITH KAINIC ACID IMPAIRS SPATIAL MEMORY IN RATS. Gail E. Handelmann*, Joseph T. Coyle, and David S. Olton. (SPON: B.L. Bird) Dept. Psychol., and Dept. Pharmacol., Sch. Med., The Johns Hopkins Univ., Baltimore, Md. 21218.

Numerous studies have suggested that the subfields of the hippocampus mediate different behaviors. Due to the complex anatomy of the structure, however, destruction of a single subfield without interrupting efferent or afferent fibers of other areas has been difficult. In the present experiment, selective destruction of subfield CA3 was produced by chemical means, making it possible to study the behavioral function of this subfield alone.

Bilateral injections of kainic acid, each containing 160 nanograms, were made directly into the CA3 subfield, causing pyramidal cell death without damage to fibers of passage. Three groups of rats were tested. The first received an injection in only the dorsal hippocampus, the second in only the ventral hippocampus, and the third received injections in both sites. The injections of kainic acid were found to produce damage only at the sites of injection. Therefore, the rats who received injections in only the dorsal or ventral hippocampus had damage in only these areas, but the group that received both injections showed damage to the entire extent of CA3.

All rats were tested for acquisition of a spatial memory task, the eight-arm radial maze. Controls performed identically to normals. Rats with total CA3 damage were impaired on the task and tended to perseverate their choice of arms. Rats with only partial damage acquired the task faster than the impaired group but slower than controls. Rats were also observed for emotional reactivity to aversive stimulation. All lesioned animals were hyperreactive compared to controls, and rats with damage to either the dorsal or ventral CA3 alone displayed the greatest reactivity.

These data demonstrate that selective damage to a group of anatomically similar cells within the hippocampus, the CA3 pyramidal cells, produces identifiable changes in spatial and emotional behavior. The findings on spatial memory are consistent with data from total hippocampus ablations, but those on emotionality are not.

690 DEMONSTRATION OF THE HABENULO-INTERPEDUNCULAR FIBER SYSTEM BY [^{14}C]-2-DEOXY-GLUCOSE. Miles Herkenham. Laboratory of Neurophysiology, NIMH, Bethesda, MD 20014.

The habenular complex is composed of two anatomically distinct parts, the medial and lateral nuclei, differing in morphology and input-output connections. As an extension of an ongoing study of their anatomical differences, an attempt was made to use a functional marker to select out one of the habenular mechanisms. The medial habenula is the source of all habenulo-interpeduncular tract fibers that terminate in the interpeduncular nucleus. It issues thin, myelinated axons that comprise the central core of the fasciculus retroflexus. The 2-deoxy-D-[^{14}C]-glucose method for determining regional brain glucose consumption was used to examine this neural system. The autoradiographic maps of glucose consumption in awake rats show that 1) the medial habenula consumes slightly less glucose than does the lateral habenula, 2) the habenulo-interpeduncular tract cannot be distinguished from surrounding neuropil, and 3) the interpeduncular nucleus consumes more glucose than does the adjacent tegmentum. However, when rats are anaesthetized with chloral hydrate, a very different pattern emerges: glucose consumption is generally reduced throughout the brain, but the medial habenula, the habenulo-interpeduncular tract and the interpeduncular nucleus all consume much more glucose than the surrounding tissue and appear by contrast as very dark spots on the autoradiograms. The result is not specific to chloral hydrate since the pathway can also be visualized with pentobarbital or ether anaesthesia. Since anaesthesia (barbiturate) is known to reduce brain glucose consumption, part of the effect must have been due to a drop in glucose consumption in the surrounding tissue. Quantitative methods will be needed to determine whether there may have been an actual increase in glucose utilization within the habenulo-interpeduncular system.

This demonstrates that anaesthesia can selectively spare metabolism in an anatomically defined neuronal fiber system including the regions containing the cells of origin, the *myelinated fiber tract* and the terminal field. The actual subcellular compartments responsible for the altered glucose consumption in each component of the pathway cannot be determined at the present time. The high amount of glucose consumption in the habenulo-interpeduncular tract is quite puzzling since 1) white matter generally has less glucose consumption than does gray matter and 2) anaesthesia generally reduces consumption in both gray and white matter. The greater glucose utilization by this tract than by the surrounding gray matter under conditions of anaesthesia suggests that the habenulo-interpeduncular tract has unique metabolic and functional properties.

691 SLEEP EEG RECORDING FROM THE CAT AMYGDALA. J. Eric Holmes and Judith F. Stern.* Dept. of Neurology, USC School of Medicine, Los Angeles, CA 90033.

Chronically implanted electrodes were used to monitor sleep, arousal, and paradoxical sleep in unrestrained cats. A 30-40 cycle per second (Hz) sinusoidal burst of waves characterizes the EEG from the cat amygdala during arousal. During slow wave sleep this activity disappears from the records. During paradoxical sleep there is a slower, approximately 20 Hz sinusoidal wave form occurring periodically in the amygdala. Paradoxical sleep was identified by recordings of cortical EEG, geniculate spikes, hippocampal theta rhythms or eye movements. The slower, paradoxical sleep bursts of the amygdala are not obviously correlated with respiration. Preliminary results from chronically implanted microelectrodes indicate a decrease in firing of amygdala neurons during paradoxical sleep.

692 SUBCORTICAL PROJECTIONS TO THE ORBITAL REGION OF THE FRONTAL LOBE. S. Jacobson, N. Butters*, and H. Kowalski*. Depts. Anat. and Neurosurg., Tufts Univ. Sch. Med., Boston, MA 02111, and Dept. Psychol., BU Sch. Med., Boston, MA 02118.

In a previous study (Jacobson, Butters, and Tovsky; Brain Res., 1978) on subcortical relationships of the cortex forming the wall of the principal sulcus, we have demonstrated projections from thalamus and hypothalamus to the convexity of the frontal lobe. In this study we have investigated the subcortical projections into the orbitofrontal cortex of the monkey to determine if they are similar to those into the convexity.

Five young adult male Macaca nemestrina monkeys were used for this study. The orbital surface was divided into 5 behaviorally important zones: gyrus rectus, posterior orbital, anterior orbital, lateral orbital, and central orbital. One animal was used for each division. The zone under investigation was injected with a "cocktail" of horseradish peroxidase (HRP: Boehringer Mannheim, Grad I) and tritiated amino acids. The animals were perfused 2-4 days postoperative, frozen sections were reacted with diaminobenzidine (DAB) to demonstrate the presence of HRP positive cells.

The thalamic afferents to the orbital region were primarily from the medial dorsal nucleus. Many cells were also seen in the anterior medial, ventral anterior, and medial pulvinar nuclei. Some cells were also seen in the intralaminar and posterior group. A few cells were also seen in the lateral hypothalamic area, lateral mamillary nucleus and dorsomedial nucleus. The observations from this study will be discussed in relationship to the behavioral studies on these same regions.

693 SELECTIVE HIPPOCAMPAL LESIONS AND SPATIAL DISCRIMINATION IN THE RAT. Leonard E. Jarrard. Dept. Psychol., Washington & Lee Univ., Lexington, VA 24450.

Recent research demonstrating different behavioral effects resulting from selective damage to either hippocampal cell fields or hippocampal projections, together with studies implicating the hippocampus in spatial memory, prompted the present investigation of the effects of selective hippocampal lesions on spatial discrimination in the rat. The apparatus consisted of an 8-arm radial maze. The animals were trained preoperatively to choose 4 of the 8 arms for food (Problem A) and then underwent reversal training where the opposite 4 arms were baited (Problem B). The rats were then divided into two control groups (operated and unoperated) and 3 groups that received bilateral lesions to either the fimbria, posterior CA1 cell field and alveus, or hippocampus (including damage to all cell fields, dentate gyrus, and fimbria). Following recovery the animals were retrained to approach the last 4 arms that had been learned (Problem B).

Analysis of the data indicated that animals with extensive damage to hippocampus and those with fimbrial lesions were impaired in postoperative testing; performance of animals with CA1-alveus lesions and those in the operated and unoperated control groups was similar. Further analysis of the error data revealed that the performance impairment in complete hippocampals, and to a less extent in fimbrials, was due to the animals reentering correct (baited) arms that had already been visited on that trial rather than entering incorrect (unbaited) arms. These results indicate that the difficulty encountered in performing the complex spatial task was not in remembering which of the arms were correct from trial to trial (reference memory) but rather in remembering which of the correct arms had been visited during a trial (working memory).

(Supported by NSF Grant BNS 75-18160.)

694 ATTENUATION OF LATERAL HYPOTHALAMIC SELF-STIMULATION BY MICROINJECTION OF LIORESAL INTO THE VENTRAL ANTERIOR NUCLEUS OF THE THALAMUS. James A. Johnsen and Ernest W. Kent, Dept. Psch., UICC, Chicago, IL 60680

Male Sprague-Dawley rats were implanted with 70μ twisted wire stainless steel monopolar electrodes directed at the far lateral aspect of the lateral hypothalamus (Pellegrino and Cushman coordinates: AP +5.4, H -2.1, L 1.8). In addition, 27 guage injection cannulae were directed bilaterally at the ventroanterior nucleus of the thalamus (VAT).

The rats were trained to press for constant current, square wave form trains of current (train duration, 250 msec; frequency, 100 pulses/sec; pulse width, 30 msec) at intensities sufficiently strong to maintain stable rates (usually 60 to 80μA). Rats pressed at rates averaging 200 presses per minute. 126 minute trials consisted of an initial 20 minutes to establish daily baseline rates, followed by intracranial injection of the drug and replacement of the animal in the stimulation chamber. Doses of Lioresal as low as 90 nanograms (in .5μl saline) were seen to attenuate self-stimulation rates. Effects were usually seen within 10-20 minutes following the injection. 90 ng injections were followed by a gradual decay to a much reduced rate, or zero. In some cases this was followed by full or partial resumption. Injections of 180 ng, however, were less likely to be followed by a return to the lever. During the period when the effect was seen, the animals appeared neither hypoactive nor sedated. Priming at this time elicited sniffing and exploratory behavior, often inducing a return to the lever. However, as soon as priming ceased, the animals no longer attended to the lever.

As Lioresal is reported to be a GABA agonist and substance P antagonist, the possible role of these substances in the thalamic control of self-stimulation requires further examination. It is noteworthy that the VAT receives projections from the substantia nigra and striatum. These areas support self-stimulation and are reported to contain GABA and substance P cell bodies.

695 EFFERENTS OF THE "VOMERONASAL AMYGDALA" TO PROJECTION FIELDS OF THE ACCESSORY OLFACTORY BULB. Golda A. Kevetter and Sarah S. Winans. Neuroscience Prog. and Dept. Anatomy; Univ. of Michigan; Ann Arbor, MI. 48109. USA

The projections of the "vomeronasal amygdala", i.e., those nuclei which receive direct projections from the accessory olfactory bulb, were traced autoradiographically. Tritiated proline (1 - 5 μCi in 20 - 250 nl) was stereotaxically injected via a 1 or 5 μl Hamilton syringe into the amygdala of 25 male hamsters. After survival times ranging from 6 hours to 6 days (average survival time was 27 hours), animals were perfused through the left ventricle with isotonic saline followed by phosphate-buffered 10% formalin, 20 micron frozen sections were mounted on slides and coated with Kodak NTB2 emulsion and exposed for 2 - 6 weeks in the dark. Slides were developed with Kodak D - 19 developer, counterstained with cresyl violet and observed under darkfield light microscopy.

Efferents of the "vomeronasal amygdala" include projections to other primary projection fields of the accessory olfactory bulb (AOB). Specifically, the posteromedial cortical nucleus, C3, projects to the ipsilateral Nucleus of the Accessory Olfactory Tract (NAOT), ipsilateral AOB, ipsilateral medial nucleus (M), ipsilateral and contralateral Bed Nucleus of the Stria Terminalis (BNST), and the contralateral C3. Efferents of the medial nucleus terminate in the ipsilateral NAOT, ipsilateral C3, and the ipsilateral and contralateral BNST. The projections of C3 and M terminate in different layers of these structures than the efferents of AOB.

Additionally, M projects to the anterior hypothalamic-medial preoptic area, ventromedial nucleus of the hypothalamus, premammillary nucleus, and the subiculum.

696 **AFFERENTS TO AREA 8 AND OTHER PREFRONTAL AREAS IN THE MONKEY AND BABOON: AN HRP STUDY.** **George R. Leichnetz and Juan A. Astruc, Department of Anatomy, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Virginia 23298.**

The large expanse of granular cortex rostral to the agranular motor areas of the frontal lobe distinguishes primates from other mammals. The prefrontal cortex has been shown to be involved in many seemingly divergent functions: learning and memory, attention-focusing mechanisms, eye movement, sensory convergence, and limbic mechanisms. Its cytoarchitecture is also varied in several respects. On this basis, Walker subdivided the prefrontal cortex into a number of sectors. Our previous work has focused on prefrontal efferent systems in primates, and this report represents preliminary findings in our study of its afferents in the general context of our efforts to better understand prefrontal function through a more complete definition of its connectivity.

Large horseradish peroxidase injections (3-5ul, 50% sol. in sterile saline, Sigma Type VI) or solid HRP pellets were introduced into arcuate cortex (Area 8), dorsal convexity or medial prefrontal cortex in three macaque monkeys, two cebus monkeys and three baboons. After survival periods of 1-6 days, the animals were perfused transcardially with normal saline followed by a mixed aldehyde fixative (1%Paraformaldehyde, 1.25% Glutaraldehyde) in a 0.1M phosphate buffer. The brains were removed, blocked, and immersed in a 10% sucrose/buffer solution overnight in the refrigerator prior to cutting. The brains were sectioned on a freezing microtome at 40 micra, and were reacted according to the Mesulam (1976) blue-reaction protocol.

Area 8 (arcuate, "frontal eye field") placements resulted in labelled cells in Lamina III of the ipsilateral inferior parietal cortex (Area PG of Von Bonin and Bailey) and caudal cingulate cortex, and Area PE, nucleus basalis in the basal forebrain, and the thalamic intralaminar complex (paracentral and central lateral nuclei). The mediodorsal nucleus of the thalamus contained a few back-filled cells. The intralaminar complex was characterized by both retrogradely and orthogradely-transported HRP.

Dorsal convexity and medial prefrontal placements resulted in labelled cells in MDpc, ventral cingulate cortex (Areas 24 and 25) basal forebrain, lateral hypothalamus, ventral tegmental area, dorsal raphe and superior central nuclei of the midbrain.

The differences in these afferent projections to the prefrontal cortex may account in part for its many divergent functional roles which will be discussed.

697 LONG-TERM LIMBIC STIMULATION WITHOUT KINDLING OF SEIZURES. Henry Lesse and Jeremiah Collins. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, Calif. 90024.

Cats were trained to use electrical stimulation of limbic sites (amygdala, hippocampus, septal region) as a discriminative stimulus for a food-reinforced bar-pressing response. Current required to elicit focal afterdischarges (AD) was determined initially for each site and intensities 80% below this level were then employed in training sessions. Behavioral and electrophysiological responses of subjects stimulated repeatedly in a series of conditioning experiments extending over 1 to 4 years were analyzed for evidence of kindling.

Neither generalized convulsions nor behavioral automatisms characteristic of limbic seizures were observed in any subject even following thousands of focal stimulations. There was no decrement in discriminative performance associated with the repeated brain stimulations and subjects continued to respond to limbic excitation with appropriate bar pressing and milk drinking. Concomitant electrophysiological recordings disclosed no abnormalities (i.e., epileptoid afteridscharges, interictal spikes). Detection thresholds (the minimal current required to elicit the conditioned response) remained stable over extended periods. When initial AD thresholds were retested following long series of training stimulations, there were no progressive declines in threshold and neither increases in AD duration, nor propagation of the localized AD to other brain sites. Behavioral reactions to these induced ADs remained limited to automatisms and did not progress to generalized convulsions despite numerous intervening conditioning stimulations.

These results indicate that limbic sites can be excited repeatedly at intensities that elicit conditioned behavioral responses without engendering kindling effects or inducing progressive changes in the excitability of the stimulated structure. Although kindling procedures that result in repeated induction of seizure afterdischarges and other abnormalities provide a useful model for studying epileptogenesis, caution is indicated in generalizing conclusions based on kindling phenomena to other brain stimulation situations.
(Supported by NIDA DA-1351 and NIH S07 RR05756.)

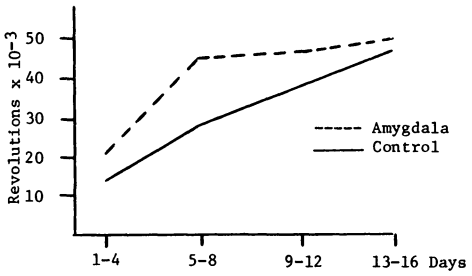
698 EVOKED POTENTIALS IN HIPPOCAMPAL CA1 IN ANESTHETIZED AND WAKING RATS. L. Stan Leung. Dept. Physiol.-Anat., UC Berkeley, CA 94720.

Three inputs -- the posterior alveus (PA), the anterior alveus (AA) and the Schaffer collaterals (Sch) -- were studied in rats anesthetized with sodium pentobarbital, using near-threshold electrical stimuli. Averaged evoked potentials (AEPs) were mapped in order to calculate the current source-links over a coronal plane. By correlating AEPs with unitary post-stimulus time histograms, a field generated by excitatory postsynaptic potentials (EPSPs) in pyramidal and interneurons and another generated by inhibitory postsynaptic potentials (IPSPs) in pyramidal cells could be inferred for each stimuli. The sink for the EPSP field components evoked by the alvear stimuli (AA,PA) was at stratum oriens and that evoked by Sch at stratum radiatum. The source of the IPSP component included strata pyramidale and radiatum. The sinks of the EPSP fields and the source of the IPSP field were the active synaptic sites for the respective events. By recording simultaneously from a 64-electrode array, anterior stimuli evoked a broad surface distribution of AEP which suggested significant spatial divergence of the orthodromic inputs in contrast to the sharp projection of the caudally directed efferent fibers in PA.

In implanted, awake animals, AEPs showed signifcant changes. For AA and PA stimulations, the late component inferred as the IPSP field became smaller while the early EPSP field was enhanced. For Sch stimulations, the AEPs showed multiple peaks and valleys or oscillations (at 20 to 50 Hz) when the rat was moving. These oscillations were likely manifested by the recurrent-inhibitory or negative-feedback interactions between pyramidal cells and interneurons either in CA1 or in CA3. The significance of these results will be discussed. (Supported by MH 06686).

699 AMYGDALOID MODULATION OF WHEEL RUNNING ACQUISITION. Irwin N. Lourie*, Michael M. Krieger* (SPON: N. S. Thampi). Research Laboratory, Norristown State Hospital, Norristown, PA 19401

Using females from an inbred strain of Sprague Dawley derived rats selectively bred for high activity wheel levels for 18 generations, the following paradigm was carried out. Subjects starting at 68 days of age were allowed to acquire a wheel running pattern through free access to an activity wheel over a period of 20 days. Bilateral lesions to both basolateral and corticomedial structures were produced by radiofrequency technique in 4 subjects (A). An equal number of sham operated controls (C) were prepared. After a period of 16 days to allow for recovery and partial extinction of the activity pattern, reacquisition of wheel running was measured over a period of 16 days. In addition to measurement of circadian patterns, hourly activity determinations were taken. The results of the acquisition are shown in the accompanying graph. (A) shows a 57% increase in initial activity (days 1-4). The acquisition rate for both (A) and (C) are the same for the first 8 days, both showing a doubling of activity. From day 9 to 16 a compensatory mechanism becomes evident and the activity levels are essentially the same in the last block (13-16). The averaged hourly data taken during the last block also indicate a tendency to rhythmic lability of response in (A) with peak activity occurring at hours 2, 5 and 9, (C) showing one major peak at 2 hrs with a gradual decline. The observed disinhibitory effect in (A) does not appear to be related to a concommitant deprivation state due to food or water uptake. The results suggest that the amygdala, receiving input from all sensory modalities, functions as a scanning and gating organ which determines 'physiological readiness' in behavioral states.



700 LIMBIC INPUT INTO THE VENTROMEDIAL NUCLEUS OF THE RABBIT. J.E. Marchand, J.F. DeFrance, and J.C. Stanley. Department of Neurobiology and Anatomy, The University of Texas Medical School at Houston, P.O. Box 20708, Houston, Texas 77025.

Field and extracellular potentials were recorded in the Ventromedial Nucleus of the Hypothalamus (VMH) to stimulation in the fimbria and stria terminalis at the level of the dorsal hippocampus. Recording and stimulating electrodes were glass micropipettes filled with 2M NaCl and fast green for later localization of tip position (Thomas and Wilson, 1965). Animals were anesthetized with urethane (1g/kg, i.p.). In order to better visualize recording and stimulating electrode placements, the overlying cortex was removed by aspiration and the exposed structures were covered with warm mineral oil. Recording positions were localized to the VMH using field responses to stria terminalis stimulation (Renaud, 1976), and later verified by histology.

The field response to lateral fimbria stimulation was dominated by a positivity with a peak latency of approximately 15 msec. Responses to stria terminalis were of two types: a negativity with peak latency of 8 msec, and a positivity with peak latency of 25 msec. Histology of stimulating locations indicated that the 8 msec negativity occurred when the electrode tip (25-50μm) was in the ventral component of the stria terminalis, and that the 25 msec positivity occurred when the electrode tip was in the dorsal component of the stria. This corresponds well with the anatomical studies of de Olmos (1972). All three responses were sharply localized to the VMH and in depth profiles, their appearance and disappearance were parallel to each other.

Paired stimulus testing was used to evaluate the functional characteristics of these pathways, in particular with regard to inhibition. Most notable was a long-term suppression of the negativity to stria stimulation with interstimulus intervals of up to 3 seconds.

Anatomical studies indicate that the fibers of the lateral fimbria originate in the ventral subiculum of the hippocampal formation, and as they curve ventral and caudal, they make up the medial cortico-hypothalamic tract (Raisman, 1966). The stria terminalis consists of fibers arising largely from the amygdala. This study, then, indicates a strong and apparently complete overlap of amygdala and subicular inputs to the VMH. Such overlap suggests strong functional interaction of these two limbic centers in control of the VMH.

Supported by NSF GB 35532 and NIH IF 32 NS05874-01.

701 AFFERENTS TO THE INTERPEDUNCULAR NUCLEUS OF THE RAT. R. Marchand, J. N. Riley, and R. Y. Moore. Dept. of Neuroscience School of Medicine, Univ. Calif., San Diego, LaJolla, CA 92093.

The interpeduncular nucleus (IPN) receives its major input from the habenular nuclei. Recent studies indicate that fibers of other afferents may undergo sprouting in the IPN, following lesioning of the habenular nuclei. It seemed worthwhile, therefore, to further characterize the afferent connections of the IPN in order to determine candidate structures for this observed morphological and biochemical plasticity.

Horseradish peroxidase (HRP) was deposited in the IPN of adult female Sprague-Dawley rats by microelectrophoresis. Following intracardiac perfusion, frozen sections were prepared and processed for a blue reaction product. To date, brains from 20 animals have been examined.

Reaction product was observed in neurons of the medial habenular nuclei, raphe nuclei (nucleus central superior and nucleus dorsalis), dorsal and probably ventral tegmental nuclei, parabigeminal nucleus and the locus coeruleus. In addition, neurons of the trigeminal principal sensory nucleus were labeled following injections of caudal IPN, probably reflecting uptake into fibers of passage. While our analysis of caudal brainstem structures is incomplete, there appear to be no major afferents to IPN from this region.

Afferents from the habenular nuclei appear to arise exclusively from the medial cell group. Following injections restricted to the IPN, only cells in the medial nucleus were labeled. The number of labeled cells in the lateral habenular nuclei appears directly related to the degree of spread of HRP into the ventral tegmental area. The projection of the medial habenular nuclei appears to be bilateral, with a tendency for the ipsilateral side to predominate.

The input from the raphe nuclei appears to be topographically organized. Deposits of HRP restricted to ventral IPN only occasionally labeled cells in the nucleus central superior. Cells in raphe dorsalis are labeled only after injections of the entire or lateral portions of the IPN.

Dorsal tegmental nucleus labeling is restricted to its medial and dorsal portions; few labeled cells appear in the central portion. Labeled cells were observed lateral to the dorsal tegmental nucleus. The ventral tegmental nucleus is filled with a dense fiber plexus making interpretation of labeled cells difficult.

Based on the number of cells demonstrating HRP reaction product, the major afferents to the IPN appear to be the medial habenular nucleus and restricted portions of the dorsal tegmental nucleus.

Supported by USPHS Grants NS-12080, NS-05732.

702 DISSOCIATIONS BETWEEN HYPOTHALAMIC KNIFE CUTS AFFECTING SEXUAL BEHAVIOR, PUP RETRIEVAL, AND OBJECT RETRIEVAL IN FEMALE HAMSTERS. David M. Marquest, Charles W. Malsbury§, and Joanne T. Daood*§. †Dept. Psychol., U. of Pittsburgh, and §Depts. Psychiatry and Psychol., Western Psychiat. Inst. and Clinic, U. of Pittsburgh Sch. Med., Pittsburgh, PA 15260

This study explores the roles of the lateral connections of the medial hypothalamus in hamster pup retrieval, pup cannibalism, nest building, sexual behavior, and object retrieval. Sagittal knife cuts lateral to the medial preoptic-anterior hypothalamic area disrupt pup retrieval and nest building but not sexual behavior in female rats (Numan, JCPP, 1974, 87:746). Sagittal knife cuts lateral to this area do not disrupt female mating behavior (lordosis) in the hamster, either, but more posterior cuts do (Malsbury and Daood, sub. for pub.). In the present study, female hamsters were pretested for their response to pups as virgins, then received sagittal cuts either lateral to the anterior hypothalamic area (anterior cuts) or lateral to the medial anterior hypothalamic-ventromedial nucleus (posterior cuts). Postoperatively females were tested a) as virgins, for changes in pup retrieval and cannibalism, b) for mating behavior, c) for maternal care and cannibalism of their own pups, and d) for object retrieval.

On first encountering hamster pups, virgin female hamsters typically either retrieve pups to their nest or cannibalize them. When mated, nearly all female hamsters cannibalize part of their litters, yet retrieve all pups in retrieval tests. Anterior cuts converted retrieving virgins to cannibalistic ones and reduced nest building. Almost all females with anterior cuts cannibalized their entire litters within a few days of parturition. Half of these females, however, did retrieve all pups in retrieval tests during the first three days post partum, and most of them retrieved pieces of wood and food. In contrast, posterior cuts reduced pup retrieval and cannibalism in virgins, and disrupted object retrieval. Posterior cuts did not reduce postpartum pup retrieval, but did reduce postpartum cannibalism. Anterior cuts did not affect lordosis; posterior cuts greatly reduced lordosis without eliminating ovulation. Anterior cuts are interpreted as having a somewhat selective effect of increasing cannibalism, and posterior cuts as having a more general disruptive effect on behavior.

Supported by NIMH Training Grant No. MH 14634 to A.E.Fisher and Grant No. MH 28440-02 to C.W.M.

703 INCREASED ACOUSTIC STARTLE FOLLOWING DORSAL RAPHE OR FORNIX LESIONS. C. M. Miezejeski, L. W. Hamilton, and C. R. Timmons. N.Y.S. Inst. Bas. Res. Ment. Retardation, 1050 Forest Hill Rd., Staten Island, N.Y. 10314 and Rutgers University, New Brunswick, N.J.

Contrary to most other reports, Miezejeski (EPA, 1977) demonstrated that dorsal raphe lesions produce a form of hyperreactivity that is distinguishable from that produced by median raphe lesions. While the latter appear to cause indiscriminate hyperreactivity, the dorsal lesion produces heightened reactivity to distinctive forms of stimulation. Using a form of stimulation that offers a very high signal/noise ratio, i.e. a startle eliciting auditory stimulus, the first experiment examined the effect of a dorsal raphe lesion on the acoustic startle response.

A special apparatus that could record the rapid ballistic movement of the rat's muscular response to the stimulus was developed. The startle-eliciting stimulus was the click of a heavy duty 24VDC solenoid at 50 cm from the test cage. The startle apparatus was interfaced with a Nova-2 computer equipped with an A-D converter. After a 50 msec post-stimulus delay, the computer cumulated the voltage output of the apparatus during the next successive 450msec. Adult male Sprague-Dawley rats, 8 with dorsal raphe lesions and 8 operated controls, were each tested during one 90 min session in the startle apparatus. A session consisted of 15 min of adaptation during which the computer sampled baseline body movement once every 30 sec and 75 min during which the startle-eliciting stimulus was presented every 30 sec (150 trials). Rats with dorsal raphe lesions showed greater startle amplitudes than did the controls ($< .05$).

Recent findings indicate that surgical disruption of either the forebrain septo-hippocampal pathway of the fornix or the midbrain dorsal raphe produces similar increases in behavioral reactivity (Capobianco & Hamilton, Physiol. Behav. 17:65, 1976; Miezejeski, EPA, 1977; Miezejeski, Life Sci. 20:2087, 1977; Ross, Grossman, & Grossman, J. comp. physiol. Psychol 89:5, 1975). Consequently, in a second experiment identical to the first, 10 rats with fornix lesions and 10 operated controls were each examined once in the startle apparatus. Again, the lesioned rats showed greater startle amplitudes ($< .001$).

These results, combined with the above mentioned previous reports of similar changes after either dorsal raphe or fornix lesions, can be contrasted with other findings obtained following either median raphe or hippocampal lesions (Jacobs, Trimbach, Eubanks, & Trulson, Brain Res. 94:253, 1975). Such comparisons suggest that there may exist two functionally different ascending midbrain raphe inhibitory circuits: 1)a direct monosynaptic median raphe-hippocampal pathway and 2)an indirect polysynaptic dorsal raphe-septo-hippocampal pathway.

704 FIMBRIA-FORNIX LESIONS EFFECT THE RESPONSE OF HIPPOCAMPAL UNITS TO SPATIAL CUES IN THE RAT. Virginia M. Miller* and Phillip J. Best. Dept. Psychol., Univ. of Va., Charlottesville, Va. 22901.

Hippocampal unit activity (HUA) is correlated with an animal's location in space while performing spatial discrimination tasks. Intact animals trained to sample each arm of an 8 arm radial maze for food rely on distal spatial cues in making arm selections. Fimbria-fornix (FF) lesions substantially debilitate performance on this task. Furthermore, following axial rotation of the maze, intact animals do not avoid the physical arm from which food has previously been removed, but rather avoid the directional orientation that the arm had occupied.

The present study considers two problems: does the spatial correlate of HUA persist after axial rotation of a radial maze, and how do FF lesions effect the spatial behavior of hippocampal units?

All 15 units recorded in intact rats showed spatial correlates. Typically, the unit showed substantial increase in activity on one of the arms. After 90° rotation of the maze, 13 of the 15 ($p<.004$) maintained increased activity in the original directional orientation. Nine of the 13 units studied in FF rats showed spatial correlates similar to those seen in intact rats. However, only 2 of these 9 ($p>.98$) maintained increased activity in respect to the original directional orientation after rotation of the maze.

Of the units which did not show maintained directional orientation, the activity of 3 units (1 intact, 2 FF) followed the physical arm. The activity of the remaining 6 (1 intact, 5 FF) showed no relationship to the physical arm or the original directional orientation.

The directional orientation of HUA is maintained following axial rotation of the maze. Since 9 of 13 units in FF lesioned animals showed spatial responses prior to maze rotation, the deficit in performance of FF lesioned rats must be due in part to disturbance of hippocampal efferents. However, since most of the units (7 of 9) did not show the maintained spatial response seen in intact animals following maze rotation, afferent information entering the hippocampus by way of the FF must also play a role. (Supported by Sloan Foundation Fellowship to VMM and NIMH Grant #16578 to PJB).

705 CROSS CORRELATION ANALYSIS OF 40 Hz ACTIVITY IN THE SIGMOID GYRUS, NUCLEUS ACCUMBENS AND AMYGDALA. R. John Morgan, Colorado State Univ., Ft. Collins, CO 80523; Calvin C. Turbes, Gerald T. Schneider* and J. Marc Simard*. Dept. Anat., Sch. Med., Creighton University, Omaha, NE 68178

Cross correlation analysis is carried out on electrical activity in the sigmoid gyrus, nucleus accumbens and amygdala. The studies are on eight cats with chronic electrode implants. Recordings are made using radiotelemetry and hardwire procedures.

Correlation analysis shows the direction of signal flow (40 Hz) between the sigmoid gyrus and nucleus accumbens and amygdala. With correlation analysis feedback pathways as from sigmoid gyrus to the amygdala or amygdala to nucleus accumbens and vice versa can be investigated. This aspect of the analysis is important and interesting since it is possible to study functional oscillating or reverberating circuits in relation to hormonal and/or behavioral influences.

Time delay, or, tau (τ), values indicate direction of electrophysiologic transmission of information from one recording site to another. Tau values for amygdala to sigmoid gyrus ranges from 20.13 to 22.43 milliseconds. Neurotransmission between sigmoid gyrus to amygdala shows one pathway ranging from 612 μs to 7.21 ms. There is another pathway with (τ) value of 24.31 ms to 26.70 ms; another animal showed a sigmoid gyrus to amygdala transmission of 6.82 ms with an average of 1000 samples.

In three animals amygdala to sigmoid gyrus transmission ranged between 10.35 ms to 14.51 ms. Another slower transmission pathway was apparent in these data at 38.21 ms to 42.43 ms. Transmission from amygdala to nucleus accumbens ranged between 12.06 ms to 13.40 ms in two animals. Accumbens to amygdala transmission ranged between 4.80 ms to 5.32 ms. In this data there was a second path with values that ranged between 10.34 ms to 11.78 ms.

706 PROJECTIONS TO MESENCEPHALIC CENTRAL GREY RELATED TO ESTROGENIC CONTROL OF REPRODUCTIVE BEHAVIORS. J. I. Morrell, L. M. Greenberger*, D. W. Pfaff. The Rockefeller Univ., New York, NY 10021.

Many specific cell groups in the forebrain contain cells which concentrate sex steroids; the neuroanatomical connections of these cell groups are of particular interest with regard to mediation of reproductive behavior and neuroendocrine events (Krieger et al., 1977, Brain-Endocrine Interaction III, Scott et al., eds., Karger). Several such cell groups in the hypothalamus have been shown to have connections with the central grey, which itself has been implicated by neurophysiological data in the control of sex behavior and pain mechanisms. To study its afferent connections, horseradish peroxidase (HRP) was applied in and around the central grey of 41 rats. The animals were allowed to survive 2 days, and after appropriate preparation, the sections were reacted in a DAB (diaminobenzidine) procedure. The brains from 14 animals were fully analyzed for cells with regular brown granules in the somatic cytoplasm and processes (defined as an HRP labeled cell). The ventromedial nucleus (VMN) of the hypothalamus contained the largest number of HRP labeled cells, mostly in the rostral half of the nucleus, and scattered in the ventrolateral portion, ipsilateral to application site. A smaller number of HRP labeled cells were seen contralateral to the application site. Zona incerta also contained many labeled cells. In addition, labeled cells were seen, mostly ipsilateral, in the anterior and the lateral hypothalamic areas, periventricular area, arcuate nucleus and paraventricular nucleus. A small number of labeled cells were found in the lumbar and sacral spinal cord contralateral and mostly in laminae III-V. Control applications of HRP just outside the central grey resulted in very few labeled cells in the hypothalamus. Injections into the mesencephalic ventricle produced no HRP labeled cells or fibers. These results are consistent with previous reports on the efferent projections of hypothalamic cell groups, specifically the VMN (Krieger et al., 1978, J.C.N. in press). The connections are a route by which hypothalamic cell groups which contain sex steroid concentrating cells could effect activity in behaviorally-relevant brainstem circuits.

Supported by NIH Grant HD-10655.

707 CYTOPATHOLOGICAL CHANGES IN HIPPOCAMPUS AFTER INTRAVENTRICULAR INJECTION OF KAINIC ACID. J. Victor Nadler, Bruce W. Perry*, Christine Gentry* and Carl W. Cotman. Dept. Psychobiol., Univ. Calif., Irvine, CA 92717.

Kainic acid preferentially destroys hippocampal pyramidal cells when the drug is injected intraventricularly. We have characterized the hippocampal cytopathology by light and electron microscopy. In these studies 2.5 nmol of kainic acid was injected into each lateral ventricle over a period of 30 min. Pathological changes in area CA3, the most sensitive hippocampal region, could be detected by electron microscopy as soon as 1 h after kainic acid administration, a time when convulsive symptoms were already prominent. The earliest changes included astrocytic hypertrophy, irregularities in the shape of pyramidal cell nuclei and some increase in the number of lipofuscin granules within the pyramidal cell cytoplasm. There followed dilation of cisternae of the endoplasmic reticulum, disaggregation of polyribosomes, dissolution of chromatin and dendritic swelling. Within 4 h the pyramidal cells atrophied, assumed an irregular shape and became electron dense. Similar changes could be recognized at 4-h survival in light microscopic preparations stained with cresyl violet. Breakdown of cytoplasmic organelles and glial phagocytosis were well advanced 24 h after treatment. Some neurons in area CA3 appeared completely unaffected, but it is not clear what proportion of these were pyramidal cells and what proportion interneurons.

When the CA3 pyramidal cells had degenerated, mossy fiber boutons were less frequently encountered. Very few of them appeared to degenerate, however. Mossy fiber boutons present 6-8 wk after treatment were nearly always found to be making synapses on the remaining neuronal population. These afferents therefore did not seem to have been directly or permanently injured by the drug treatment.

Bilateral kainic acid administration considerably reduced the synaptic density of the molecular layer of the fascia dentata and of stratum radiatum of area CA1. The synaptic loss in the dentate molecular layer was attributable in part to degeneration of associational and commissural boutons within its inner third and in part to a decrease in the incidence of small dendritic spines throughout the layer. In stratum radiatum of area CA1 up to 90% of the presynaptic elements were found to be degenerating 1-7 d after treatment, due to destruction of Schaffer collateral and commissural afferents. Both denervated zones were largely reinnervated by 6-8 wk after treatment. (Supported by NSF grant BNS 76-09973 and NIH grant NS 08597).

708 IMMUNOHISTOCHEMICAL LOCALIZATION OF N-ACETYLSEROTONIN IN RAT HIPPOCAMPUS. Aldis V. Porietis, Gregory M. Brown and Lee J.Grota Dept. Neurosciences, McMaster University, Hamilton, Ontario, Canada, L8S 4J9.

In order to investigate the function of indolealkylamines, in particular N-Acetylserotonin(NAS), in brain, we have developed several antibodies to these compounds. Antiserum against both NAS and melatonin was produced in rabbits using NAS coupled to bovine serum albumin(BSA) via the Mannich reaction, (Br.Res.81, 233,1974). Antibody to melatonin only was produced using the antigen melatonin-BSA, coupled by the Mannich reaction (Can.J. Bioch.52,196,1974). Antiserum to NAS was produced using, as an antigen, NAS coupled to BSA via a paracarboxybenzyl bridge attached at the indole nitrogen (unpubl.). The first two antibodies have been used in the past studies to differentiate, histologically, between NAS and melatonin (Br.Res.118,417,1976). We decided to use this technique as well as the more recent, specific anti-NAS antibody to extend an earlier observation which suggested the presence of N-Acetylindolealkylamines (NAI's) in the hippocampus.

All three antibodies were used in parallel preparations in a double-antibody fluorescence technique. Controls consisted of saturating the primary antibody with the appropriate amine,either NAS or melatonin, in a small volume of ethanol. The non-absorbed sera contained an identical concentration of ethanol. Results using the anti-NAI antibody confirmed the presence of either or both NAS and Melatonin in the hippocampus of rats. This staining was localized to the polymorphic cell layer of the dentate gyrus, and Layer III of the hippocampus proper. An identical distribution of staining was seen with the antibody directed against NAS. The antibody directed against melatonin, however, did not stain the hippocampus in any detectable fashion, leading to the conclusions that the staining seen in the hippocampus is largely, if now exclusively, NAS. Present data suggest that the visualized NAS is located in smaller structures, perhaps nerve fibres, rather than in cell bodies of the hippocampal formation. Studies are presently under way to improve the resolution of the technique to the point where the precise localization within cells is possible.

It is concluded that NAS is specifically localized in the hippocampus of the rat. Further physiological and anatomical studies will be necessary to determine the possible function of this amine as a neurotransmitter or neuromodulator in this area of brain.

709 NEOCORTICAL AND HIPPOCAMPAL ELECTRICAL ACTIVITY IN THE DOPAMINE DEPLETED RAT: RELATIONS TO BEHAVIOR AND EFFECTS OF ATROPINE. T.E. Robinson*, I.Q. Whishaw* and T. Schallert* (SPON.: C.H. Vanderwolf). Dept. of Psychology, Univ. of Lethbridge, Lethbridge, Alberta Canada.

Previous studies have shown that dopamine(DA)-depleted rats are impaired in initiating movements such as rearing and walking ('voluntary' or Type I movements), movements which are accompanied by neocortical low voltage fast activity (LVF) and hippocampal rhythmical slow activity (RSA; theta; see Vanderwolf, JCPP 38: 300, 1975). This study examined the possibility that DA is also involved in producing these slow wave (EEG) waveforms. EEG records were obtained from unrestrained rats which were depleted of 97-100% of brain DA by the use of multiple intraventricular injections of 6-hydroxydopamine (6-OHDA), following pretreatment with pargyline. Neocortex: For the first 1-5 days following 6-OHDA large amplitude slow waves were common in the neocortical record during behavioral immobility. The presence of these immobility-related slow waves decreased from 91% of the sample time on day 1 post-op to 3% on day 5 and 0.1% on day 10. Although the 6-OHDA treated rats were severely akinetic, whenever they did move spontaneously, or were induced to struggle, LVF appeared (even on day 1). Hippocampus: On days 1 through 30 post-op RSA with normal amplitude and frequency accompanied any spontaneous Type I movements, and induced struggling. After day 5 post-op low frequency RSA was occasionally observed during immobility, in 6-OHDA treated rats only. Atropine: After treatment with atropine sulfate (50mg/kg, i.p.) both RSA and LVF continued to accompany any Type I movements, as in controls. On days 15 and 30 post-op atropine caused 6-OHDA rats to walk vigorously with short abbreviated steps, and RSA and LVF accompanied this behavior. Behavior: Video-recordings (24 hr) of behavior revealed that 6-OHDA rats did not initiate movements such as walking or rearing, did initiate grooming as often as controls, and continuously made postural shifts reminiscent of the akathisia of some Parkinson patients.

In conclusion, DA is probably not critically important for the production of RSA or LVF. However, DA may be indirectly involved in controlling the probability of occurence of LVF and RSA by controlling the probability of occurence of behaviors such as walking and rearing (Type I behaviors). In addition, comparison of the effects of 6-OHDA treatment and hypothalamic lesions on neocortical and hippocampal electrical activity suggests that the effects of hypothalamic lesions on the EEG are not due only to DA depletion.

710 FURTHER OBSERVATIONS ON GRANULE CELL DETERIORATION AFTER ANODAL DIRECT CURRENT LESIONS. Russell E. Ruth, Scott Cain,* Anthony DiGianfilippo* and Aryeh Routtenberg. Cresap Neuroscience Laboratory, Northwestern University, Evanston, ILL 60201.

We recently described extensive granule cell deterioration (GCD) following small, electrophysiologically-guided anodal direct current (a-DC; 60 μA) lesions of rat dentate gyrus (Ruth and Routtenberg, Brain Research, in press). It was found that (i) a-DC lesions caused the spread of GCD far beyond the confines of the primary lesion (defined as central cavity plus surrounding acellular region); (ii) GCD appeared as a beam of degenerated cells oriented roughly in the lamellar plane of Blackstad et al. (J. comp. Neur., 103, 1970, 433-450); (iii) this reaction was detectable light-microscopically between 1½ - 3 hrs post-lesion; (iv) neither radio-frequency (heat) nor microknife lesions provoked GCD outside the lesion boundary.

Several additional observations have been made. 5 μA a-DC is near threshold for provoking GCD. At 7 μA (applied for 2 min) extensive GCD can be observed. The severity of GCD appears greater in younger animals (25 days old). Spontaneous discharge rate of granule cells recorded approximately 1 mm from the lesion site shows a rapid decrease following small a-DC lesions.

Of particular interest is the failure of cathodal direct current lesions to provoke GCD even though the primary lesion is at least as large as in the case of anodal lesions. This observation, coupled with the failure of radio-frequency or microknife lesions in producing GCD, emphasizes that a lesion per se is not sufficient to produce GCD. The specificity of response of granule cells to a-DC may be indicative of an endogenous sensitivity to DC gradients, which are known to exist in dentate gyrus (Gloor et al., Electroenceph. Clin. Neurophysiol., 15, 1963, 353-378). (Supported by MH 25281 and NSF 19388 to A. R.)

711 ELECTROPHYSIOLOGICAL ACTION OF KAINIC ACID ON HIPPOCAMPAL SLICES. John Ryan* and Carl W. Cotman. Dept. Psychobiology, Univ. Calif., Irvine, CA 92717.

Intraventricular kainic acid (KA) destroys rat hippocampal pyramidal cells with great potency. CA3 pyramidal cells are most sensitive to KA, followed by CA1 pyramidal cells, and dentate granule cells are virtually resistant to the drug. Since it is believed that the neurotoxic action of KA is linked to its neuroexcitatory properties, we have investigated its electrophysiological actions on hippocampal neurons in a slice preparation. KA (47 μM) similarly altered the response of CA3 pyramidal cells to mossy fiber stimulation, CA1 pyramidal cells to Schaffer-commissural stimulation and dentate granule cells to perforant path stimulation. First, a population spike appeared on the extracellularly recorded EPSP, then the amplitude of the population spike increased and its latency decreased, and finally the population spike broadened and subsided as all postsynaptic response was lost. This sequence occurred rapidly in area CA3 and relatively slowly in the dentate gyrus. The loss of the orthodromic response recorded in area CA3 is coincident with loss of the antidromic response to the same region. Prolonged washing with drug-free solution often restored the evoked response to perforant path stimulation, but only seldom to Schaffer-commissural stimulation and never to mossy fiber stimulation.

All hippocampal neuronal types responded to KA (47 nM-47 μM) with a large burst of firing, usually followed by an abrupt decline in spontaneous activity while still in KA. CA3 pyramidal cells were most sensitive and dentate gyrus granule cells least sensitive to KA. Bursting cells responded similarly to non-bursting cells of the same region. In contrast to the sensitivity of extracellular EPSPs and single unit activity to KA, the fiber potential of the mossy fibers was unaffected.

In the absence of Ca^{2+} only a slight increase in spontaneous unit firing preceded the cessation of activity in pyramidal cells. The offset of activity was typically more gradual than in Ca^{2+} media; it partially returned in some cases when KA was removed. No spontaneous activity of granule cells was observed. KA also reversibly abolished activation of all cells by antidromic stimulation in Ca^{2+} free media and, for CA3, in normal media. Thus Ca^{2+} deficient medium essentially dissociated the excitatory effect of KA on hippocampal neurons from the ultimate depression. These findings suggest that the excitatory action of KA depends on a Ca^{2+}-dependent process, but its depressant effect does not. (Supported by NIMH grant MH19691).

712 THE DISTRIBUTION OF CELLULAR DEGENERATION FOLLOWING SYSTEMIC OR INTRACEREBRAL INJECTION OF KAINIC ACID. J.E. Schwob*, T. Fuller*, and J.L. Price, (SPON: W.T. Thach, Jr.). Depts. Anat. & Neurobiol., and Psychiat., Wash. Univ. Sch. Med., St. Louis, MO 63110

The intracerebral or systemic injection of kainic acid (KA) or other analogues of glutamate causes neuronal cell death; this toxicity has been suggested to be related to the direct excitatory action of glutamate and its analogues (Olney et al., '74). We have surveyed the effects of systemic or intracerebral injections of KA on the forebrain of rats using frozen sections stained with the Nissl method or the cupric-silver method of De Olmos to reveal areas of cellular damage and agyrophilia.

Systemic administration of KA has effects on widespread regions on the forebrain although the extent of the lesion can vary even among animals that received the same dosage of KA. Agyrophilic neurons and/or cellular debris and gliosis are seen especially in parts of the hippocampal formation, septum, olfactory cortex, amygdaloid complex, mediodorsal and medioventral nuclei of the thalamus and several related neocortical areas, as well as in other parts of the neocortex, thalamus and hypothalamus. More severe lesions have an increased degree of degeneration within the listed areas as well as damage that extends to other areas of neocortex and thalamus.

Direct intracerebral injections will destroy neurons near the injection in virtually all forebrain regions and may also cause cell damage at more distant sites. These patterns of cellular degeneration vary with the injection site, and may be different from those seen with systemic injections. For example, field CA3 of the hippocampus is more sensitive than field CA1 to systemic or direct intracerebral injections near the dorsal hippocampus. However, intracerebral injections of KA into the posterior piriform cortex (PC)/entorhinal area (EA) affect CA1 more severely than CA3. Damage to CA1 is correlated with degeneration of the cells in layer III of the EA and in the ventral subiculum. Also, injections that cause cellular degeneration in some parts of neocortex can induce cell death in the corresponding thalamic nuclei (e.g. the taste cortex and VM, pars basalis of the thalamus may be affected concurrently although they are relatively undamaged following systemic injections which destroy adjacent neocortical areas). Furthermore, injections in the rostral PC cause cellular damage extending to the posterior limit of PC ipsilateral and, in some cases, contralateral to the injection. These results are difficult to explain on the basis of diffusion and/or differential sensitivity and suggest that the pattern of damage may be related to axonal connections between the affected areas. (Supported by NIH grants NS09518, GM07200, ES07066)

713 REGIONAL ORGANIZATION OF INPUT AND OUTPUT PATHWAYS OF THE NUCLEUS ACCUMBENS SEPTI. R.W. Sikes, J.F. DeFrance, R.B. Chronister, J.E. Marchand, and J.C. Stanley. Department of Neurobiology and Anatomy, The University of Texas Medical School at Houston, P.O. Box 20708, Houston, Texas 77025, and the Department of Anatomy, University of South Alabama, Mobile, Alabama 36688.

Combined electrophysiological and horseradish peroxidase (HRP) techniques were used to explore regional differences within the nucleus accumbens septi (nAcS), with respect to efferent and afferent pathways. Glass micropipettes, filled with fast green (Thomas and Wilson, 1965), were used both for stimulation and recording in acutely prepared rabbits. Field and unitary potentials were recorded in the nucleus accumbens septi following stimulation of the following regions or structures: fimbria (IFIM), stria terminalis (ST), lateral septal nucleus (LSN), lateral preoptic area (lPOA), and mesencephalic cell group A10. HRP injections were made in the nAcS and in A10.

The results can be summarized as follows: IFIM, ST, and LSN input terminate, for the most part, in the nucleus accumbens septi, proper of Koikegami et al (1967). The IFIM and ST are restricted to the more caudal aspects of the nucleus. The lPOA input is heaviest in the portion of the accumbens nucleus below the proper nucleus accumbens septi; i.e., nucleus accumbens pars medialis. The major efferent projection of the nucleus accumbens appears to arise from pars medialis.

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Supported by NSF #GB35532 and NIH #1 F32 NS05874-01.

714 POTENTIATION CHARACTERISTICS OF HIPPOCAMPAL RESPONSES IN THE ACUTE RABBIT. J. C. Stanley, J. F. DeFrance, and J. E. Marchand. Dept. Neurobiol. and Anat., Univ. of Texas, Houston, TX 77025.

A variety of studies have shown that the hippocampus (HC) displays considerable plasticity of response. Stimulation of HC afferents using low frequency pulse trains causes marked potentiation of responses during and after the stimulus train. Facilitation during the train, called tetanic potentiation (TP), can reach several hundred percent of pre-tetanic levels. The potentiation can outlast the stimulus train for seconds or hours; this effect is post-tetanic potentiation (PTP). Potentiation has been investigated using a number of HC afferent systems, both in vivo and in vitro. Afferents from the septum have not been well studied, however. We deal here with the potentiation characteristics of septo-hippocampal and HC commissural afferents to field CA1 of the acutely prepared rabbit.

Adult, male, New Zealand rabbits were anesthetized with urethane. Following craniotomy, the cortex overlying the septum and HC was removed by suction and the exposed tissue was covered with warm paraffin oil. Micropipettes filled with 2M NaCl (1-10 MOhm) were used for both stimulating and recording. Recording was carried out in the stratum radiatum layer of field CA1 of the dorsal HC, where both septal and commissural afferents terminate. Stimulation of contralateral CA3 (cCA3) and of a fiber bundle arising from the ipsilateral medial septal region (MSR) gave rise to slow negativities, indicative of population EPSPs, and spikes, indicative of the simultaneous discharge of pyramidal cells. Brief trains (8 or 16 stimuli) of low frequency (1 to 20 Hz) were used. To study TP, the responses were averaged. In the study of PTP, individual responses elicited following the tetanus train were recorded for study.

Slow-wave responses to both cCA3 and MSR stimulation showed TP that commenced at 2Hz and reached a plateau at 8-12Hz. Both slow-wave responses displayed PTP which decreased rapidly in the first two seconds following the tetanus and decayed more slowly thereafter; baselines were generally reached after 8-10 seconds. The population spike exhibited TP in the range of 2-6Hz, but was suppressed at higher frequencies. The spike also showed PTP lasting several seconds. cCA3 test responses were enhanced 10-20% following a tetanus train applied to the MSR. Our results show that responses in the acute rabbit to both MSR and cCA3 stimulation display TP in the range of frequencies of the rabbit theta rhythm. Both responses displayed PTP lasting several seconds. Facilitation of the MSR response led to a small but consistent increase in the cCA3 response, in a process of heterosynaptic facilitation.

Supported by NIH #1 F32 NS05874-01 to JCS and NSF #GB 35532 to JDF.

715 ORGANIZATION AND EFFERENT PROJECTIONS OF THE INTERPEDUNCULAR COMPLEX IN THE CAT. W.D. Stofer* and S.B. Edwards. Dept. Anat., Sch. Med., Univ. of Va., Charlottesville, Va. 22901

The cytoarchitecture and efferent projections of the interpeduncular complex (IPC) were studied using Nissl-stained normal material and autoradiographic and horseradish peroxidase (HRP) tracing techniques.

Observations of normal material show that the IPC can be divided into rostral, central, and caudal thirds. Within each third are two or three cytologically distinct subgroups. A total of eight paired and unpaired subgroups are identifiable.

The autoradiographic tracing method revealed that IPC efferents are organized into two main fiber bundles. One main bundle ascends and then splits in the rostral midbrain into two divisions. One division follows the fasciculus retroflexus and terminates largely in the dorsomedial nucleus of the thalamus and not, as previously thought, in the lateral habenula. The other division follows the medial forebrain bundle to the diagonal band of Broca with en passage terminations in the lateral hypothalamus. Other fibers in the medial forebrain bundle turn dorsally and terminate in a triangular-shaped region of the thalamus situated ventrolateral to the central medial nucleus. Still other fibers leave the medial forebrain bundle and pass into medial and lateral septal nuclei and region CA3 of the ventral hippocampus. The other main efferent bundle from the IPC descends to the nucleus centralis superior, ventral central grey matter, dorsal and ventral tegmental nuclei of Gudden, and nucleus raphe dorsalis. Autoradiographic and HRP methods were used to determine whether these various efferent connections arise from specific IPC subgroups. Tritiated leucine injections restricted to the central third of the IPC produce labeling mainly of descending fibers. Single HRP injections in the lateral hypothalamus, septum, or raphe dorsalis consistently produce reaction product filled neurons restricted to some, but not all, of the subgroups of the IPC. Thus, at least some of the subgroups of the IPC maintain selective projections within the total pattern of IPC efferent projections.

These results reveal IPC efferent projections that are new or different from previous reports. New projections are those to the thalamus, hippocampus, and raphe dorsalis. The results further indicate that a high degree of specificity exists within the efferent connections of the different subgroups of the IPC.

Supported by NIH Grant NS 11254.

716 VALIUM: A SUPPRESSOR OF HIPPOCAMPAL PYRAMIDAL CELL EXCITABILITY. K. Taber, J.F. DeFrance, J.C. Stanley, J.E. Marchand, P. Divakaran and Y. Clement-Cormier. Department of Neurobiology and Anatomy and the Department of Pharmacology, The University of Texas Medical School at Houston, P.O. Box 20708, Houston, Texas 77025.

Diazepam (Valium) was studied electrophysiologically and biochemically in the hippocampus of acutely prepared rabbits. For the electrophysiological analysis microstimulation electrodes (1-2 megohms) were placed in the medial septal region (MSR) and in the contralateral hippocampal field CA3. Microelectrodes were used to record monosynaptic field responses in hippocampal field CA1. Power and paired-stimulus testing, along with trains of tetanically potentiating stimuli, were used to characterize field responses and to assess the drug effect. The results were: (1) Iontophoretically applied diazepam increased the threshold for the appearance of the population spike to single stimuli. The corresponding population EPSPs were unaffected, except at high dosages given either i.v. or i.p. Also, there was a reliable increase in the amplitude of the population spike at supramaximal stimulus intensities. (2) Diazepam attenuated test response facilitation in paired-stimulus testing paradigms. (3) Diazepam attenuated the post-tetanic potentiation seen following the presentation of high-frequency trains. (4) Diazepam prevented the normal increase in cyclic GMP levels following tetanic stimulation.

The data indicate that diazepam decreases hippocampal pyramidal cells excitability, and may do so by suppressing cyclic GMP mechanisms.

Supported by NSF GB35532 and Hoffman-LaRoche, Nutley, N.J.

717 AFFERENTS TO THE MACAQUE AMYGDALA BY WAY OF THE ANTERIOR COMMISSURE. Blair H. Turner. Howard University, Washington, D.C. 20059.

A study was made to determine if the primate amygdala receives afferents from the contralateral telencephalon by way of the anterior commissure. In the control monkey, an aspiration lesion was made in that part of the body of the corpus callosum through which the anterior commissure can be approached. In the two experimental animals, the same part of the corpus callosum was cut, and the anterior commissure visualized and sectioned. Following a survival period of six days, the animals were perfused and their brains prepared with the Fink-Heimer technique. No evidence of terminal degeneration was found in the amygdala of the control brain. In the experimental brains, degeneration was observed in layers I and III of the temporal prepiriform cortex, and in layers I and III of the medial amygdaloid nucleus. Of the deep amygdaloid nuclei, only the lateral nucleus received contralateral afferents via the anterior commissure. Moderately dense degeneration was seen in this nucleus in its entire anterior-posterior and dorso-ventral extent. In the medio-lateral direction, degeneration was heavier in the lateral two-thirds than in the medial third of the lateral nucleus.

Besides containing fibers en route to the amygdala, the anterior commissure is known to include axons connected with temporal neocortex. In a previous study, unilateral lesions had been made in all of the cytoarchitectural divisions of temporal neocortex, and these brains revealed no evidence of projections to the contralateral amygdala. Based on these results, it may be hypothesized that the cells of origin of the projections reported here following section of the anterior commissure are located in the contralateral amygdala.

Supported by NIMH grant MH 25495.

718 CORTICAL PROJECTIONS TO THE LATERAL ENTORHINAL AREA IN THE RHESUS MONKEY. Gary W. Van Hoesen, Douglas L. Rosene and Marek-Marsel Mesulam. Depts. Anat. and Neurol., Univ. Iowa, Iowa City, IA 52242, and Harvard Neurol. Unit, Beth Israel Hosp., Boston, MA 02215.

Previous silver impregnation studies in the monkey have demonstrated that the hippocampal formation is linked to cortical association areas via the entorhinal cortex. More recently, autoradiographic studies have demonstrated reciprocal connections between the subicular area of the hippocampal formation and several cortical areas of the temporal lobe. Although these studies establish a strong hippocampal-cortical relationship in the primate, much more remains to be learned about this linkage. In this study cortical projections to the entorhinal cortex were investigated in the rhesus monkey using the autoradiographic method. All major architectonic subdivisions of the temporal lobe were sampled with the exception of the primary auditory cortices.

Four areas have been identified which send direct, powerful projections to the entorhinal area. Individually, these correspond to Brodmann's area 35 and Bonin and Bailey's areas TF, TG and TH. Projections from areas TG and TH are directed primarily toward the lateral entorhinal area and a sizable zone previously called the intermediate entorhinal area. Projections from area 35 are distributed widely within the entorhinal area, but are especially heavy in the lateral and intermediate entorhinal areas. These projections as well as those from areas TG and TH terminate primarily in layers I-III. Projections from area TF are directed primarily toward the medial and intermediate entorhinal areas where evidence of termination is observed over layers I-II. In addition to these projections, areas TF, TG, TH and also area TE send projections to area 35 (perirhinal cortex) immediately lateral to the rhinal sulcus, and to the prorhinal cortex immediately medial to the rhinal sulcus.

These results demonstrate that the lateral and intermediate portions of the entorhinal area in the rhesus monkey receive several direct and powerful cortical projections. It would seem of considerable interest that in the human brain entorhinal areas greatly resembling these in terms of architecture are massive, and account for a majority of the cortex forming the hippocampal gyrus. Moreover, the temporal areas which project to the lateral and intermediate entorhinal areas have themselves been implicated in memory-related processes in both non-human primates and humans.

Supported by NIH grant NS 09211, and the Benevolent Foundation of Scottish Rite Freemasonry, Northern Jurisdiction, USA.

719 HISTOCHEMICAL DISTRIBUTION OF ACETYLCHOLINESTERASE IN THE HIPPOCAMPAL REGION OF THE RHESUS MONKEY (Macaca mulatta). Vijaya K. Vijayan* (SPON: Richard C. Carlsen). Department of Human Anatomy, University of California, Davis, California, 95616.

The localization of cholinergic neurotransmitter enzyme acetylcholinesterase (AChE, EC 3.1.1.7) in the hippocampus (H) and the dentate gyrus (DG) of the adult rhesus monkey was examined using a histochemical procedure. The supragranular zone of the molecular layer of DG exhibited the most intense AChE staining in the form of a band of reaction product occupying the lower one-third of that layer. Moderate enzyme reaction was present in the hilus of DG, in the neuropil as well as intracellularly in a small percentage of the polymorphic neurons located there. The AChE activity in the H appeared to be relatively greater in subdivisions CA3 and CA4 than in CA2 and CA1. The alveus and the fimbria exhibited a small percentage of stained fibers intermixed with a major number of unstained ones. The stratum oriens and the stratum radiatum demonstrated moderately AChE-reactive neuropil with occasional spindle shaped, AChE-positive neurons in the former zone. The mossy fiber zone was devoid of AChE staining with the exception of few enzyme-reactive processes running across it.

The distribution of AChE in the H and DG of the adult monkey was compared to that in neonatal animals and also to the previously described pattern of enzyme localization in the hippocampal region of the mouse (Vijayan, Anat. Rec. 187: 738, 1977).

This research was supported in part by PHS grants RR00169 and DA 00135.

720 DIFFERENTIAL EFFECTS OF LIMBIC SYSTEM LESIONS ON LONG-TERM AND SHORT-TERM SPATIAL MEMORY IN THE RAT. John A. Walker* (Spon: W. S. Stark) Department of Psychology, Johns Hopkins Univ., Baltimore, MD 21218

The limbic system in rats has recently been proposed to have a critical role in all types of spatial processing. However, damage to the limbic system, particularly hippocampus and its connection often results in no deficit in relatively simple tasks, but severe deficits in more complicated tasks requiring flexibility of memory. The present experiment was conducted to determine if the memory requirements of spatial tasks influences the retention of those tasks following limbic system damage.

Two tasks were developed, requiring either memory for only one goal within a test (a long-term memory task) or memory for each of two goals within a test (a short-term memory task). Each rat was assigned to one of the two tasks. Both tasks were conducted on an elevated spatial maze. In both tasks, rats were presented with a choice between two goals. In the long-term memory task, only one goal was rewarded, the same goal on each test, so that the rat was required to remember which goal was the rewarded goal. In the short-term memory task, both goals were rewarded, but only on the first choice of each goal, so that within a test the rat was required to remember which of the two goals had already been chosen in order to choose the other goal (rewarded alternation). Care was taken to prevent simple position habits from providing a solution to the tasks. Rats in both groups were tested preoperatively. Rats were then given either bilateral fornix lesions, disconnecting the hippocampal connections to subcortical structures, or sham operations. Testing was resumed after a week of recovery.

After surgery, sham operated rats in both groups continued to choose as accurately as in the preoperative period. In contrast, rats with fornix lesions in both groups showed a deficit in performance immediately after surgery. For rats with fornix lesions in the long-term memory task, performance improved over postoperative sessions with a time course similar to that of preoperative acquisition. For rats with fornix lesions in the short-term memory task, performance did not recover even when tested for 250 postoperative sessions, remaining at levels expected by chance.

The recovery of accurate performance by the rats in the long-term memory condition suggests that the fornix lesions abolished the memory of the preoperatively learned task, but did not prevent relearning the task postoperatively. The lack of recovery of accurate performance by rats in the short-term memory condition suggests that the fornix lesions permanantly abolished the ability to form short-term memories. These data indicate that performance in spatial tasks following limbic system damage is a function of the memory requirements of the task.

721 FUNCTIONAL ANATOMICAL MAPPING OF THE BASAL FOREBRAIN IN THE RAT AND CAT: A (^{14}C)-2-DEOXYGLUCOSE STUDY. R. Watson,* R. Troiano,* A. Siegel, R. Basri, R. D'Ascoli, C. Block* and J. Kerr.* (SPON: J. McArdle). Departments of Neurosciences and Physiology, New Jersey Medical School, Newark, New Jersey 07103.

When a specific brain region is electrically stimulated in order to evoke a specific behavior in an awake animal, it is difficult to determine the sum total of the functional subsystems which are activated or inhibited during the time period relative to that stimulation. We have utilized the technique of (^{14}C)-2-deoxyglucose radioautography to analyze the pattern of brain activity in the rat and cat following electrical stimulation of sites in the basal forebrain and limbic system. Most of the preliminary experiments have been performed in rats lightly anesthetized with ketamine and xylazine.

The results obtained to date indicate that the pattern of distribution of brain activity corresponds closely to the known anatomical pathways related to the stimulated sites. Specifically, orthodromic activation of efferent connections appear to predominate over other possibilities such as antidromic activation or recurrent inhibition. Multisynaptic activity has been difficult to identify under these experimental conditions.

When the lateral hypothalamus was stimulated a wide extent of the ipsilateral septal area, lateral habenular nucleus, ventral and central tegmental regions, and central gray were diffusely activated. Similarly, stimulation of the region of the mamillary bodies, dorsal hypothalamus, ventromedial nucleus, preoptic region and substantia innominata resulted in the activation of the known anatomical target neurons associated with these respective structures. Current experiments are attempting to compare the patterns of brain activity in unanesthetized, awake animals manifesting stimulus-bound behaviors with those obtained in the anesthetized preparations.

(Supported by NIH Grant NS 07941-09)

722 ULTRASTRUCTURAL EVIDENCE FOR CHANGES IN THE X-IRRADIATED RAT HIPPOCAMPUS. Walter I. Weiss*, Robert B. Wallace (SPON: N. Kirkland) Dept. of Psych., Univ. of Hartford, CT 06117.

Previous studies (Wallace, Kaplan & Werboff, 1976, Exp. Brain Res.; Neuroscience Abstracts, 1977) have reported the behavioral effects of focal hippocampal X-irradiation on neonatal rats. These studies included morphological evidence by light microscopy of dentate granule cell layer population depletions of about 80%. The following study was carried out to assess ultrastructural changes as a result of the X-irradiation.

Timed pregnant Long-Evans female rats were obtained from the Blue Spruce Breeding Farms. Ten pups served as experimental animals, and were exposed to 200 rads per day on days two and three, then 150 rads per day on alternate days 5 through 15 post partum. An additional 10 pups served as controls. All animals were weaned at 30 days and caged separately, with food and water available ad libitum.

At 90 days of age, 8 animals from each group were perfused according to the method of Cole, Matter & Karnovsky (J. Mol. & Exp. Path., 1971). Brains were removed and the dentate gyrus and area CA3 dissected bilaterally. Blocks were post-fixed in 1% Osmium Textroxide, stained en block in uranyl acetate, and embedded in Epon-Araldite. Toluidine blue lu sections were examined to facilitate orientation and localization. Gold sections were mounted on copper grids, stained with lead citrate, and examined on a Zeiss EM9S-2. The remaining 2 animals in each group were perfused, via the aorta, with 10% buffered formalin, post-fixed in Bouin's solution, and cleared for three weeks with daily changes of buffered formalin. They were then blocked, embedded in Paraplast + cut at 6u and stained H & E.

Light studies of the experimental animals showed 80% reduction in dentate granule cells. The EM studies revealed granule cell nuclear shrinkage, as well as other ultrastructural changes. Axo-somatic synapses on area CA3 were also significantly reduced. The significance of these results will be discussed.

723 THE ORGANIZATION OF THE FIMBRIA, DORSAL FORNIX AND VENTRAL HIPPOCAMPAL COMMISSURE IN THE RAT. J.M. Wyss. Dept. of Anat., Washington U. Sch. Med., St. Louis, MO 63110.

The morphological organization of the fimbria, dorsal fornix and ventral hippocampal commissure has been studied in a large number of rats, each of which was injected with a small amount of a ^{3}H-labeled amino acid in one or another cytoarchitectonic field of either Ammon's horn (fields CA_1-CA_4) or the subicular complex (subiculum, presubiculum and parasubiculum). Efferent fibers from the septal parts of the subiculum and field CA_1 project through the dorsal fornix; all the remaining rostrally directed efferents from the hippocampal formation are distributed by way of the fimbria, and the fibers arising within each cytoarchitectonic field are confined, for the most part, to a discrete portion of the fimbria. Thus axons arising within the hilus of the dentate gyrus (fields CA_4 and CA_{3c}) are distributed, in an ordered manner, along the ventral (or pial) surface of the fimbria; fibers from the rest of fields CA_3, CA_2 and CA_1 occupy its mid-portion, and fibers from the subicular complex are located along the ependymal (or dorsal) surface. Similarly, fibers from the temporal part of the hippocampal formation are located near the tip of the fimbria (close to the stria terminalis) whereas those from septal levels are located close to the stratum oriens beneath field CA_3. Septo-hippocampal fibers are confined to the tip of the fimbria. Commissural fibers from the temporal parts of fields CA_3 and CA_4 cross the midline in the rostral part of the ventral hippocampal commissure, while those from septal levels cross in the most caudal part of the commissure. Near its rostral pole the fimbria contains about 900,000 fibers. Of these 700,000 (76%) are myelinated with internal diameters ranging from 0.2-2.7 µm (mean 0.76 µm), and the remainder are unmyelinated with diameters between 0.1 and 0.6 µm (mean 0.25 µm).

Supported by USPHS Grant NS 05683 and by a grant from the Sloan Foundation.

724 AUTORADIOGRAPHIC MAPPING OF BRAIN REGIONS ACTIVATED DURING SELF-STIMULATION USING ^{14}C-2-DEOXY-D-GLUCOSE. John S. Yeomans*, Charles R. Gallistel and Martin Reivich (SPON: Harvey Grill). U. Pennsylvania, Phila., PA 19174.

Seven self-stimulating rats were injected via intracardiac catheters with ^{14}C-2-deoxy-D-glucose, a glucose analog taken up by active neurons. The rats were awake and freely behaving, delivering current through electrode tips placed in the lateral hypothalamus, substantia nigra or diagonal band of Broca. In all cases intense unilateral uptake of the isotope was found around the electrode tip, as compared to a contralateral control electrode, even when bar pressing was irregular. The uptake around the tip spread roughly 1 mm per 500 µA of current using 0.1 msec duration cathodal pulses, but the shape of the region of uptake was sometimes irregular.

For all rewarding placements increased uptake was found throughout the medial forebrain bundle (MFB) from medial septum to ventral tegmental area and substantia nigra, zona compacta. The intensity of of MFB uptake correlated poorly with the absolute current intensity, but correlated well with how far the stimulating current exceeded the threshold current for bar pressing. At currents considerably above threshold, unilateral uptake was also found in midbrain reticular formation and central gray, and in the medial frontal cortex immediately anterior to the septum.

In only one rat was unilateral activity clearly found in locus coeruleus, and unilateral uptake was not apparent in striatum. Uptake in these catecholaminergic areas, then, was not found to correlate well with reward.

(Supported by NSF grant 524186 and NIMH training grant IT32 MH15092.)

MEMBRANE BIOPHYSICS

725 A MECHANISM FOR CUMULATIVE INACTIVATION OF OUTWARD CURRENT IN MOLLUSCAN SOMATA. R. W. Aldrich Jr., S. H. Thompson*, P. A. Getting. Department of Biological Sciences, Stanford University, Stanford, CA 94305.

During prolonged or repetitive low frequency voltage-clamp steps, the outward current recorded from molluscan somata shows marked inactivation. A prominent feature of this inactivation is that the peak outward current during successive voltage clamp pulses at low frequency (e.g. 1 Hz) is less than the minimum current at the end of the preceding pulse. We have termed this phenomenon cumulative inactivation. To account for the apparent increase in inactivation during the inter-pulse interval, Eckert and Lux (Science, 197, 472-475, 1977) postulated that cumulative inactivation in Helix neurons results from an intracellular accumulation of calcium ions.

In voltage clamp studies of dorid somata, we have found that cumulative inactivation can be attributed to the delayed, voltage-dependent component of outward current termed K-current and that inactivation is independent of calcium influx. The kinetics of cumulative inactivation are voltage and time dependent. At 10°C, the time course of inactivation can be approximated by the sum of two exponentials having time constants of about 0.5 sec. and 3.5 sec. The time constant for recovery from inactivation is very much slower, about 30 seconds.

The mechanism of cumulative inactivation has two contributing factors. The first is the large difference between the onset and recovery time courses of inactivation. Slow recovery means that most of the channels inactivated by one pulse are not available for activation by the next pulse. The second contributing factor is competition between the activation and inactivation rate constants. This means that, at a given voltage, the number of open channels can never equal the total number of available channels. Provided that the amount of recovery during the interval is less than the rapid inactivation occurring at the onset of the second pulse, the peak current on one pulse will be less than the minimum current of the preceding pulse. Inactivation will, therefore accumulate until it saturates.

726 ACTIONS OF MANGANESE AT THE MEMBRANES OF MYOEPITHELIAL CELLS. Margaret Anderson. Dept. of Biol. Sci., Smith College, Northampton, MA 01063.

Current pulses (30 - 200 ms duration and 0.7 - 6.0 x 10^{-6}A) applied directly to the myoepithelial cells of the proventriculus of the marine polychaete worm Syllis spongiphila elicited overshooting, regenerative responses which were associated with contractions. Responses elicited in control artificial sea water (ASW) were 72 - 118 mV in amplitude and 60 - 680 ms in duration. Several results suggest that the regenerative responses result from an influx of calcium ions. The responses were abolished in calcium-free ASW (Ca^{++} replaced by equimolar Mg^{++}) and in calcium-free ASW containing 1 mM EGTA. They were maintained in solutions containing $\leq 10^{-5}$ M TTX. Their amplitudes remained at control levels in solutions in which 80, 90 or 95% of the sodium was replaced by sucrose. Mn^{++} can replace Ca^{++} in the generation of the regenerative spike. The amplitudes of responses elicited in calcium-free ASW containing Mn^{++} increased as the $[Mn]_o$ (these responses were not associated with contractions). The mean slope for a 10-fold change in $[Mn]_o$ in calcium-free ASW was 22 mV (15 preparations): amplitudes (in mV) in 5 mM Mn, 93 ± 3 (S.E., n = 13); in 10 mM Mn, 98 ± 2 (S.E., n = 27); in 25 mM Mn, 107 ± 2 (S.E., n = 11); in 50 mM Mn, 115 ± 2 (S.E., n = 19). These data indicate a dependence of the regenerative responses on Mn^{++}. The increase in amplitudes with increasing $[Mn]_o$ was less in calcium-containing ASW than in calcium-free ASW. The mean slope for a 10-fold change in $[Mn]_o$ in ASW containing 10 mM Ca^{++} was 5 mV (6 preparations): amplitudes (in mV) in 5 mM Mn, 105 ± 3 (S.E., n = 5); in 10 mM Mn, 107 ± 3 (S.E., n = 10); in 25 mM Mn, 107 ± 4 (S.E., n = 6); in 50 mM Mn, 111 ± 4 (S.E., n = 5). These data indicate a competition between Ca^{++} and Mn^{++}, and they suggest that Mn^{++} ions permeate calcium channels of the myoepithelial cell membrane. Although variable, durations of responses in manganese-containing solutions were consistently longer than those in control ASW, often exceeding 10 s. However, at any given $[Mn]_o$, the durations of responses elicited in the presence of Ca^{++} were consistently shorter than those in the absence of Ca^{++}. In addition to permeating calcium channels, Mn^{++} ions may also affect the general condition of leakiness of the myoepithelial cell membrane. Strength-duration curves indicate that at any given duration of applied current pulse, the intensity required to elicit a threshold response decreased as the $[Mn]_o$ increased. Further, current-voltage relationships of electrotonic responses elicited in solutions of different manganese concentrations indicate that, for a given intensity current pulse, the amplitude of the responses increased as the $[Mn]_o$. Supported by USPHS Grant # NS12196.

727 BLOCKAGE OF Na^+-Ca^{++} EXCHANGE SLOWS OUTWARD TAIL CURRENTS. M. E. Barish. Hopkins Marine Station, Stanford Univ., Pacific Grove, CA 93950.

The potassium conductance of molluscan neurons is partially dependent on intracellular free Ca^{++} ions for its activation (Meech & Standen, 1975, J. Physiol. 249: 211-239). According to this hypothesis, the kinetics of Ca^{++}-dependent K^+ current should depend on the rate of removal of free Ca^{++} ions from the vicinity of the membrane. After a transient load, Gorman & Thomas (1978, J. Physiol. 275: 357-376) showed that a slow component of K^+ tail current followed a time course similar to changes in arsenazo absorption. This suggests that the decay of outward current reflects a decline in free Ca^{++} concentration. Treatments that will change the time course of free Ca^{++} decline should therefore result in parallel changes in the relaxation of outward current. One important component of Ca^{++} removal from cytoplasm in squid axon is the Na^+-Ca^{++} exchange pump (Baker, 1972, Prog. Biophys. Mol. Biol. 24: 177-223). This process can be substantially slowed by removal of extracellular Na^+.

Microsurgically isolated dorid central ganglion somata were studied with two microelectrode voltage clamp. Tail currents were measured between 0.5 and 7 seconds following repolarization to a holding voltage of -40mV from a step to 0mV. Substitution of Tris for Na^+ in artificial sea water (ASW) significantly slowed the relaxation of tail current. During this time period, as a first approximation, the time course of the decay of tail current can be decomposed into two summed exponentials with time constants (TCs) in normal ASW of 0.74 and 4.18 seconds. In Tris (0 Na) ASW, the faster component remained essentially unchanged (TC: 0.72 sec.) while the slower time constant was increased (TC: 5.71 sec.).

The inhibition of Na^+-Ca^{++} exchange should increase the concentration of free Ca^{++} at the membrane. These experiments suggest the time course of decay of the Ca^{++}-dependent K^+ current is dependent on the rate of cellular Ca^{++} removal, binding and sequestration. Further, they point towards an important contribution of the Na^+-Ca^{++} exchange pump in the removal of free Ca^{++} following Ca^{++} influx.

728 CALCIUM BINDING TO INTACT AND SUBCELLULAR FRACTIONS OF CRAB NERVE. Jesse Baumgold* and Susumu Terakawa* (SPON: Robert H. Wurtz), Lab of Neurobiology, NIMH, Bethesda, Maryland 20014

The mechanism of action of calcium in nerve conduction is still quite controversial. In an attempt at elucidating this mechanism, we have studied the binding of calcium-45 both to intact crab nerves and to subcellular fractions of crab nerves. The subcellular fractionation procedure used was essentially that of Balerma et al. (FEBS Lett. 21: 335, 1975), yielding a membrane fraction with very little mitochondrial contamination. Calcium-45 binding to this fraction was studied by incubating the membranes for 9 min in 10 mM Tris buffer (pH 7.4) containing either 170 mM NaCl or 170 mM KCl, and the appropriate amount of calcium-45. The mixture was then filtered through a Millipore filter and, after washing the filter, the radioactivity was measured in a scintillation counter. By plotting the data according to the Scatchard formulation, two components of binding were detected. In the NaCl containing buffer, one component had a dissociation constant of about 2 x 10^{-5} M with a binding capacity of about 4 nmoles of calcium per mg protein, and the other component had a dissociation constant of about 2 x 10^{-4} M with a binding capacity of about 45 nmole of calcium per mg protein. By replacing the NaCl in the incubation buffer with KCl, the binding capacity of the higher affinity site was doubled. The binding under study is not an energy dependent process since the addition of ATP to the incubation medium did not enhance the calcium binding. Since neither tetrodotoxin, veratridine nor diphenylhydantoin had any effect on the calcium binding, the calcium binding characteristics reported in this work represent the binding to the membrane as a whole rather than to the excitable sites.

The binding of calcium-45 to intact desheathed crab nerve was studied as follows. After being labeled in calcium-45 containing sea water, the nerve was inserted into a chamber made by drilling a hole through a cylindrical piece of plexiglass. By passing artificial sea water through this chamber and collecting it as it dripped out, the level of calcium-45 desorbed from the nerve could be studied. After a prolonged washing period, the nerve could be electrically stimulated through two pairs of electrodes in the chamber, resulting in an increase in the level of calcium-45 desorbed from the nerve. Electrical stimulation has previously been shown to increase the rate of desorption of calcium-45 from muscle and ganglion cells. This finding suggests that calcium ions bound to the excitable sites are immobile in the resting state of the membrane and that they become more mobile during excitation.

729 SYNAPTIC VESICLE REUTILIZATION AT ACTIVE SITES: THEORETICAL CONSIDERATIONS. Alan F. Boyne and Michael Mento* Dept. Pharm., Northwestern Medical School, Chicago, Ill. 60611.

Divalent cations facilitate the fusion (adhesion) of synaptic vesicles to the nerve terminal membrane. Neurosecretion apparently requires a subsequent fission or tearing open of the fused region. One hypothesis for the fission event is that the local surface tension rises in the fusion site and exceeds allowable levels so that a tear forms. Such a local rise in surface tension would be transient because lateral diffusion of lipid molecules from adjacent regions would relieve the tension. This simple model has two obvious implications which seem to correlate with the experimental facts of neurotransmission:

(1) The rate of rise of tension in the fused region must exceed the compensating lateral diffusion of lipid into the area, otherwise the tension cannot reach threshold and fission cannot occur. This suggestion is consistent with the phenomenal speed of the Ca-triggered physiological fusion + fission events (200 microsec; Llinas et al., PNAS 73 2918 (1976)). On the other hand, published micrographs of Ca-induced vesicle fusions to the nerve terminal membranes in aldehyde treated tissues (Boyne et al., J Cell Biol. 63 780 (1974)) may represent fusions forming too slowly to fission.

(2) A fission opening may be reversible if lateral lipid diffusion can occur before the vesicle flattens into the membrane plane.

It has previously been noted that the removal of free Ca from the active site region would tend to retard or reverse flattening (Hall and Simon B & B Acta 436 613 (1976) and Boyne, Life Sci. 22 (1978)). A second retarding influence can now be suggested. Electron microscopy of the apparent active sites in fast-frozen, freeze substituted Torpedine ray electric organ shows that clusters of synaptic vesicles are aligned against the terminal membrane. While this clustering probably serves to provide a pool of releasable quanta, a second likely consequence is that the entry of Ca will fuse vesicles both to the terminal membrane and to each other. Such additional adherences may retard the flattening of those vesicles opening to the synaptic cleft. As indicated above, lateral lipid diffusion may then reseal the fission site and permit local vesicle reutilization. In this tissue there is strong evidence that during 0.1 Hz stimulation, there is no vesicle loss but rather that vesicles are immediately reutilized (e.g. to release newly synthesized acetylcholine) (Zimmerman and Denston, Neurosci. 2 695 (1977)).

We are considering possible mechanisms for an increase in surface tension at the fusion site and hope to be able to relate the kinetics of fusion and fission to the rate of lateral lipid diffusion.

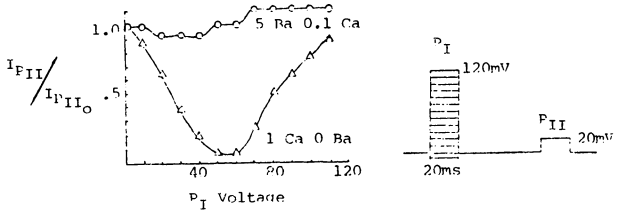
730 CA-DEPENDENT INACTIVATION OF CALCIUM CONDUCTANCE IN PARAMECIUM. Paul Brehm* and Roger Eckert (SPON: Douglas Junge). Biology Dept. UCLA, Los Angeles, CA 90024.

Inactivation of early inward calcium current (I_{Ca}) was examined in voltage-clamped P. caudatum. In 1.0 mM Ca, depolarizations of 5 to 25 mV from rest (which averaged -25 mV) activated an early inward current which reached maximum within 3 ms and showed nearly complete inactivation within another 5 ms. There was no sign of late voltage-activated outward current over this potential range.

The inactivation seen during one pulse (PI) persists so that there is suppression of inward current recorded during a subsequent pulse (PII) presented after a 60 ms interval (See Fig.). The inactivation of the Ca channel was shown to depend on the entry of Ca as follows. PI amplitudes progressing from 0 to 60 mV resulted in more complete inactivation of I_{Ca} during PII. Further increase of PI amplitude toward the calculated E_{Ca} (ca. +150 mV) resulted in a return of the early I_{Ca} during PII to 90% of its control value. The outward current during PII was independent of PI amplitude indicating no substantial persistent-Ca activated I_K. Substitution of Ca by Ba decreased inactivation of the early inward current. In 0.1 mM Ca, 5 mM Ba there was essentially no PII inactivation (See Fig.). Injection of EGTA slows inactivation of I_{Ca} and also increases peak I_{Ca}.

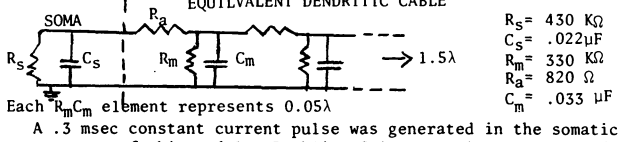
During a 100 ms, 20 mV depolarization the inward current shows substantial time-dependent inactivation in a 5 Ba, 0.1 Ca solution. This inactivation may result from either Ba influx, Ca influx, or direct voltage effects on the channel. A 120 mV, 100 ms pre-pulse was followed without interval by a 20 mV depolarization. The inward current with and without the 100 ms prepulse was nearly identical indicating no significant voltage-dependent inactivation.

These results indicate that inactivation of the Ca channel in Paramecium depends on entry of Ca ions. Interference with Ca entry by competition from Ba or by voltages approaching E_{Ca} eliminates or diminishes the inactivation. In contrast, there appears to be little or no H-H voltage-dependent inactivation of the Ca channel with depolarization. Supported by NSF BNS 77-19161.



731 MODELLING THE EFFECTS OF TONIC CONDUCTANCE CHANGES ON EPSP'S AND SHORT INTRASOMATIC CONSTANT CURRENT PULSES - WAYS TO ESTIMATE THE LOCATION AND EXTENT OF NEURONAL CONDUCTANCE CHANGES DUE TO LONG-LASTING POSTSYNAPTIC POTENTIALS OR DRUGS. Peter L. Carlen and W.A. Corrigall, Neurobiology Lab, Addiction Research Foundation, Toronto, Canada, and University of Toronto.

Tonic conductance (G) changes which result from temporally summated or long-lasting PSP's, or from drugs, play an important role in central neuronal integration. Using the short intrasomatic constant current pulse technique developed by Redman and colleagues (Jack & Redman, J. Physiol. 1971, 215, 321-352) it is possible to calculate neuronal membrane parameters including τ_m (neuron time constant), τ_1 (first peeled exponential), L (electrotonic length of neuron) and $\rho\left(\frac{G\ dendrites}{G\ soma}\right)$. This technique also permits estimation of the location and extent of a tonic G change in a neuron under certain circumstances. A simple analogue soma-dendritic compartmental neuronal model modified from Walmsley, B. (Ph.D. thesis, 1975, Dept. Electrical Engineering, Monash University, Australia) with a time constant of 12 msec, and an electrotonic length of 1.5 λ (time constant) was used.

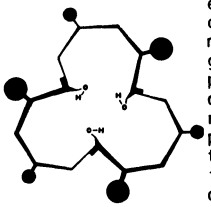


A .3 msec constant current pulse was generated in the somatic compartment of this model. Doubling G in a proximal as opposed to a more peripheral compartment of the dendritic cable caused a more rapid voltage decay of the short pulse with an earlier onset of decay. The final τ_m as estimated from the semilogarithmic decay of the intrasomatic short pulse differed little with relatively small peripheral or proximal G increase, whereas the τ_1 was smaller with more proximal G increase. The opposite effect occurred with decreased compartmental G. Electrotonic EPSPs generated in the dendritic tree and measured in the soma were diminished more by a somatic G increase as opposed to the same G increase in the compartment wherein the EPSP was generated, except for the most distally generated EPSPs. Equal but opposite effects were noted for compartmental G decreases. Using this simple analogue neuronal model facilitates interpretation of physiological and pharmacological conductance changes measured from intracellular neuronal recordings.

Supported by MRC grant MA-6019.

732 THE TRIPLE HELIX: A POSSIBLE STRUCTURE FOR EXCITABLE MEMBRANE CHANNELS. H. Robert Guy* (Spon: D.O. Carpenter). Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Although excitable membrane channels differ in ion selectivities and gating mechanisms, some drugs (e.g. local anesthetics, barbiturates, and strychnine) apparently block a large number of these channels. This suggests that the structure of the ion conducting portion of these channels is similar. I have explored the possibility that this structure is a beta helix. β helices are perhaps the simplest family of peptide structures which form ion permeable channels in membranes. Of many β helices that I have analyzed, a triple stranded left handed beta helix with slightly more than 15 residues per turn ($^3L_{\beta^{15}}$ helix) is most consistent with electrophysiological and pharmacological data obtained from 5 biological channels (the Na^+ and K^+ channels underlying the action potential, the fast i.p.s.p. Cl^- and e.p.s.p. Na^+ channels of Aplysia neurons, and the end plate channels of frog skeletal muscle). The polypeptide (Ser-Gly-Apl-Gly-Apl)$_n$, where Apl is a residue with an apolar side chain, should form a $^3L_{\beta^{15}}$ channel (see figure) which has a high conductance, is relatively nonselective, and is blocked by drugs which block all five of the biological channels. Differences between the ion selectivities of the biological channels may be due to a more ion selective segment of a $^3L_{\beta^{15}}$ helix which is in series with this nonselective segment. Sequences which form selectivity filters for the respective channels consistent with experimental data are: (Ser-Ala-Pro-Ala-Pro) for end plate, (Asp-Ala-Pro-Ala-Pro) for a.p. Na^+, (Arg-Ala-Pro-Ala-Pro) for i.p.s.p. Cl^-, and (Gln-Ala-Pro-Gly-Apl) for a.p. K^+. Some drugs which affect only one channel type can bind at the selectivity filter; these include tetrodotoxin and saxitoxin for the a.p. Na^+ channel, tetraethylammonium and its derivatives for the a.p. K^+ channel, and pentylenetetrazol, penicillin G, bicuculline and picrotoxin for the i.p.s.p. channel. The nonselective conformation should be able to undergo a conformational change to a closed state. Large molecules which enter the open channel should inhibit this conformational change. A number of molecules, e.g. batrachotoxin, aconitine, grayanotoxin, veratridine, and DTT, may prolong the open conformation of a.p. Na^+ channels by only partially blocking the nonselective segment of the channel while preventing its closing. This theory can be tested by synthesizing the polypeptides, incorporating them into membranes, and comparing the pharmacology and ion selectivities of the synthetic channels with those of biological channels.



733 VOLTAGE CONTROLLED GAP JUNCTIONS BETWEEN EMBRYONIC CELLS: A VOLTAGE CLAMP STUDY. A.L.Harris*, D.C.Spray, M.V.L.Bennett, & R.B. Hanna*, Dept. Neurosci., Einstein Coll. Med., Bronx, N.Y. and Ctr. Intrastructural Studies, SUNY, Syracuse, N.Y.

Current clamp studies reveal voltage dependence of electrical coupling between blastomeres of early axolotl (Ambystoma mexicanum). To study the junctional conductance under voltage-clamp, each blastomere of a coupled pair was clamped near the resting potential with a two microelectrode circuit. Voltage in either cell was stepped to various potentials and current flowing across the junction was measured as current provided by the clamp on the other cell. This new method allowed us to directly measure junctional current and to study its voltage dependence and kinetics. Consistent with current clamp data, junctional conductance is highly dependent, decreasing as a function of the voltage difference between the cells. Typically, junctional conductance (1-4 μmho) drops to less than 10% of its resting level with a 20mV step, but a small voltage insensitive conductance persists, even at much greater polarizations. Voltage steps of either polarity in either cell produce similar conductance changes with respect to voltage sensitivity and kinetics. Large polarizations of both cells simultaneously have little or no effect on junctional resistance. The decrease in junctional current is stable and does not reverse over the longest voltage steps given (50 sec). The time course of the conductance decrease can be fit with a single exponential and is voltage dependent. The relaxation of the junction to the conducting state when the cells are returned to zero potential difference can be fit with a single exponential with a time constant of 350 ms and is slightly voltage-dependent. Junctional conductance shows only partial transient recovery when transjunctional voltage is stepped between voltages of opposite sign, each of which is sufficient to cause low conductance. We believe that the conductance elements which underlie this phenomenon are the gap junction particles which we have visualized in freeze-fracture electron micrographs. Unlike iontophores of nerve and muscle, they have conductance symmetry around zero potential and first order kinetics. The precise nature of the control of their conductance is unclear, but it is likely to be more complex than that of channels which are sensitive to voltage differences across only one membrane.

DCS is a McKnight Scholar.

734 ACTION OF 4-AMINOPYRIDINE ON VOLTAGE-AND CALCIUM-DEPENDENT POTASSIUM CURRENTS OF MOLLUSCAN PACEMAKER NEURONS. Anton Hermann* and A.L.F. Gorman, Dept. of Physiology, Boston U. Sch. of Med., Boston, MA 02118.

Neuronal soma membranes exhibit both, a voltage-dependent potassium-current, $I_{K(V)}$ and a calcium-dependent potassium-current, $I_{K(Ca)}$. These current components in Aplysia pacemaker neurons were separated as reported previously (Hermann and Gorman, J. Biophys. 21, 178a, 1978) and the external and internal effects of 4-Amino-pyridine (4-AP) investigated. External application of 4-AP reduced $I_{K(V)}$ (activated by voltage steps of 200 msec duration in zero Ca^{2+} solution) in a dosage dependent manner. The blockage was voltage dependent. No blockage of $I_{K(Ca)}$ (activated by intracellular iontophoretic injection of Ca^{2+}), however was observed; rather with higher concentrations of 4-AP this current component was increased. These results are consistent with the observation of the effect of 4-AP on the total outward current obtained with step depolarizations in artificial seawater (ASW). 4-AP reduced the N-shaped I-V-relation in the voltage range between +80 and +120 mV where $I_{K(V)}$ is predominant, while the outward current was increased in the voltage range between -20 and +80 mV where $I_{K(Ca)}$ is predominant. Maximal blocking effects were observed after about 5 minutes; recovery from blockage had a similar time course. The effect of internal 4-AP was qualitatively similar to external 4-AP. The results suggest that with external or internal application the drug acts at the same site near the internal surface of the membrane. 4-AP appears to constitute a useful means to separate pharmacologically voltage- and calcium dependent potassium currents.
(Supported by NIH grant NS11429)

735 NOREPINEPHRINE ANTAGONIZES CALCIUM-DEPENDENT POTENTIALS IN RAT SYMPATHETIC NEURONS. John P. Horn and Donald A. McAfee, Dept. of Physiol. & Biophys., U. of Miami Schl. of Med., Miami, FL 33152, and Div. of Neurosciences, City of Hope Natl. Med. Ctr., Duarte, CA 91010.

Depolarization of the rat sympathetic postganglionic neuron results in a hyperpolarizing afterpotential (HAP). Yarowsky and McAfee (Neurosci. Abstr. 3:25, 1977) have demonstrated that the HAP is caused in part by an increased gK^+ triggered by Ca^{++} influx during the depolarization. We now report that norepinephrine inhibits this process.

Superior cervical sympathetic ganglia were isolated from mature rats and superfused with oxygenated Locke's solution (24° C) for up to 12 hrs. Drugs and ionic changes in the extracellular fluid were introduced via modified superfusates. A single-barreled microelectrode was used for both transmembrane current passage and voltage recording from impaled postganglionic neurons.

Norepinephrine (NE) reduced the amplitude and duration of the HAP in a dose-dependent manner (EC_{50} = 1-2 μM) with little or no effect on resting membrane potential. This reversible effect of NE was quite reproducible, occurred within 2 min., and did not desensitize during a 15-min. exposure. The response to NE appeared to be mediated by an α-receptor. Dopamine and isoproterenol antagonized the HAP but were less potent and less efficacious than NE. Phentolamine (5 μM), an α-adrenergic antagonist, reduced the potency of NE about twofold. While NE did not affect the resting input resistance, the transient conductance increase during the inhibited HAP was reduced. This observation suggests that the decrease of the HAP in NE occurred by some mechanism which reduced gK^+ rather than by a mechanism which increased conductances to other ions.

The mechanism of NE action appears to be related to Ca^{++}-dependent processes. The magnitude of the HAP is substantially reduced in low (0.1 mM) extracellular Ca^{++}. NE had no effect on the remaining Ca^{++}-independent portion of the HAP. A Ca^{++} action potential followed by a HAP can be evoked in postganglionic neurons treated with tetrodotoxin (1 μM) and tetraethylammonium (5 mM) to block gNa^+ and gK^+. NE reversibly inhibited this Ca^{++} action potential and the subsequent HAP in a dose-dependent manner (EC_{50} = 1-2 μM). We conclude that NE inhibits Ca^{++} influx during depolarization and thus the Ca^{++}-dependent portion of the HAP.

Supported by USPHS NS-05820 and the Scottish Rite Schizophrenia Research Program.

736 INTRACELLULAR RECORDING FROM THE BRAINS OF CONSCIOUS BEHAVING CATS ACHIEVED BY A NEW METHOD OF FLOATING THE PIPETTE TIP. Gregory L. King* and James E. Skinner. Dept. Physiol. and Sect. Neurophys., Dept. Neurol., Baylor College of Medicine, Houston, Texas 77030, U.S.A.

A difficulty inherent in recording transmembrane potentials from the higher vertebrate brain is the prevention of movement of the neurons to be recorded relative to the penetrating pipette tip. These movements are generally thought to be caused by cardiovascular pulsations and respiratory cycles and can be minimized in anesthetized animals by placing pressor foots on or in the tissue and draining the cerebrospinal fluid that couples the thoracic pressures to the cranial cavity. However, spontaneous or evoked limb movements produce small shock waves that travel through the gelatinous brain tissue and also cause micromovements of the cells relative to the rigid penetrating micropipette tip. This latter difficulty makes transmembrane recording from the conscious animal brain practically impossible since such recordings are infrequently achieved and can be maintained, at most, for only 5-10 min. To deal with these problems we have minimized the cardiovascular- and respiratory-induced movements by a closed-cranium method utilizing agar and solved the limb movement problem by "floating" the micropipette tip in the brain so that it moves with the tissue, thus eliminating the problem of the relative motion.

We have successfully and frequently recorded intracellular potentials from moving animals with the times of holding the cell ranging from 20 to 55 min. We employ filled micropipette tips that are electrically coupled to the amplified by a 25-micron diameter wire and mechanically coupled to the hydraulic drive unit by -2°C ice. The temperature of the ice is maintained by a circulating alcohol coolant and direct current heater. A slight increase in the heater current causes a rapid melting of the ice, thus allowing the small, almost massless micropipette tip to float freely in the brain. We believe that this method provides a breakthrough in intracellular recording from the conscious vertebrate brain and will enable us to investigate the membrane properties of neurons during higher cerebral processes.

737 DIFFERENTIAL PROLONGATION OF ACTION POTENTIAL DURATION BY SPARTEINE AND ITS POSSIBLE RELATION TO Ca-DEPENDENT MEMBRANE RESPONSES IN FOUR IDENTIFIED LEECH NEURONS. Anna L. Kleinhaus. Dept. Neurol. Yale Med. Sch., New Haven, CT. 06510.

Previous work from this laboratory showed that tetraethylammonium chloride (TEA) prolonged the action potentials of Retzius and N, P and T sensory leech neurons probably by blocking a K current which normally repolarizes them. As a consequence, a late divalent cation conductance was unmasked which dominated membrane behavior during excitation. The order of responsiveness of the neurons to TEA was R>N>P>T which paralleled their ability to sustain active membrane responses in Na-free solutions. These observations led us to postulate that the differential responses of the cells could be explained by variations among them in the number of Ca conductance channels available to affect membrane behavior when outward K currents were blocked (J. Physiol. 246: 351, 1975; J. Physiol. 270: 181, 1977).

The present work was undertaken to test this hypothesis with Sparteine (SPT) which is known to block K currents in other excitable membranes (Ohta and Narahashi, J. Pharm. exp. Therap.,1973; Schauf, et al, J. Pharm. exp. Therap., 1976).

SPT, 0.1-1.0 mM, applied extracellularly in the bath produced a dose-dependent, reversible prolongation of the action potential of Retzius and N, P and T sensory leech neurons. After application of 0.5 mM SPT, the average value for the increase above control of action potential duration was 2700% for R cells, 245% for N cells, 200% for P cells and 130% for T cells. The prolongation of the R and N cell's action potentials by SPT was seen in normal Ringer containing 2.0 mM Ca or when 2.0 mM Sr replaced Ca, but was partially reversed by Mn. These findings suggest that a large portion of the prolongation was due to current going through the divalent cation channel known to dominate R and N cell electrical behavior when the K current,which normally counteracts it, is blocked. Further confirmation of this interpretation was obtained by experiments in Na-free solutions in which R and N cells were capable of sustaining active membrane responses induced by SPT. The responses to stimulation of the neurons in Na-free, SPT solutions were dependent on extracellular Ca. Sr was capable of substituting for Ca as a current carrier, while Mn, known to block divalent cation responses in leech R and N cells, abolished them. Intracellular injection of SPT by pressure had essentially no effect in R, P and T cells, in contrast to intracellular TEA injection. However, preliminary results with pH changes indicate that SPT is more active at pH 8.0 than at pH 7.4 which might in part explain its low intracellular potency.

Pharmacological block of K current may provide a useful method for the study of Ca channels in individual neurons and further our understanding of their responses to drugs.

738 THE ACTION OF ANTHOPLEURIN A ON CRAYFISH AXON. Phillip A. Low*, Chau H. Wu and Toshio Narahashi. Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

The effects of anthopleurin A (AP-A) isolated from Anthopleura xanthogrammica on resting and action potentials and membrane currents were studied on crayfish giant axons using the intracellular microelectrode and voltage clamp techniques. At low concentrations of AP-A (10^{-8}M) the resting potential underwent cycles of spontaneous depolarization followed by repolarization, producing an undulating pattern. Repetitive firing occurred both spontaneously and in response to a single stimulus. The rate of rise, amplitude and initial falling phase of the action potential were unaffected but the later falling phase was replaced by a long plateau. At higher concentrations of AP-A (10^{-7} - 10^{-6}M), depolarizations of 10-40mV were seen, which were not followed by repolarizations. Both the depolarization and undulating pattern were antagonized by each of the following modified van Harreveld (VH) solutions: 1) VH + 300nM TTX; 2) VH + 10-20mM procaine; and 3) VH containing 1mM sodium. This indicated that the ionic channel responsible for these perturbations was that of sodium.

In contrast to the effects of neurotoxin II of Anemonia sulcata (ATX II), prior treatment with TTX or procaine did not affect subsequent interaction of AP-A with its receptor. As the TTX effect was reversed on washing, the action potential returned and had a marked plateau typical of AP-A effect. Identical findings were made with the procaine protocol.

Membrane currents were measured on the axons in which potassium channels were blocked with 2mM 4-aminopyridine. Sodium activation appeared normal but inactivation was markedly slowed in the presence of 10^{-7}M AP-A. The peak current was slightly reduced after AP-A application and the reversal potential was unaffected. However, steady-state currents reflecting mainly altered sodium channels were greatly increased following AP-A treatment.

A potent and specific action of this toxin on sodium inactivation by external application provides another useful tool in the pharmacologic dissection of ionic channels. The close structural similarity between ATX II and AP-A and their identical actions on membrane currents and action potentials are contrasted by their quite different interactions with TTX. This difference, when explored, may provide insights into the physical and molecular relationship of TTX receptor and the sodium channel. Supported by NIH grant 14144.

739 INTRACELLULAR pH REGULATION IN CRAYFISH SLOW MUSCLE FIBERS: EFFECTS OF CHANGES IN INTERNAL pH ON CALCIUM ELECTROGENESIS. W. Moody, Jr. Dept. Physiology, UCLA, Los Angeles, CA 90024.

I previously reported that crayfish slow flexor muscle fibers, which normally generate only small graded responses when depolarized with constant current pulses, become capable of generating all-or-none Ca^{++} action potentials after prolonged anoxia or treatment with uncouplers of oxidative phosphorylation (J. Comp. Physiol., in press). The possibility that changes in intracellular pH might in part mediate the effects of alterations in cell energy metabolism on membrane calcium electrogenesis has been investigated. Internal pH was measured using recessed-tip pH microelectrodes (Thomas, J. Physiol. 1974. 238:159). Lowering the external pH from 7.4 to 5.6 had little effect on internal pH or fiber electrical properties. Exposure to 100% CO_2-10mM HCO_3 at pH 5.6 caused a drop in internal pH from the normal 6.9-7.3 to between 6.15 and 6.4. During CO_2 exposure, the small graded electrical responses of the fibers were converted into overshooting action potentials. Effects of CO_2 on internal pH and electrical properties were readily reversible upon perfusion with CO_2-free pH 5.6 Ringer's. Linearization of steady-state constant-current I-V plots obtained in Ca^{++}-free, Mn^{++} Ringer's upon CO_2 exposure suggests that the decrease in internal pH blocks delayed rectification. This suggests that internal H^+ may block a voltage-dependent potassium current which normally shunts the inward Ca^{++} current and limits the size of the depolarizing response in these fibers. Effects of internal pH on the inward current were not studied. Another, preferable, means of producing internal acidification while the fiber is exposed to the normal external solution is by removal of external NH_4Cl after brief exposure. The internal pH of the slow flexor fibers increased 0.5-0.8 units upon exposure to 30-50mM NH_4Cl at pH 7.4. Removal of NH_4^+ after 15-35min exposure caused a prolonged drop in internal pH below the pre-NH_4^+ value by 0.5-1.0 units. Preliminary results indicate that as this NH^+ rebound acidification develops, a gradual potentiation of Ca^{++} responses occurs, with all-or-none spikes appearing at internal pH values below about 6.3. Evidence relating the electrophysiological changes during NH_4^+ rebound to the internal pH changes, as well as the significance of these results to calcium electrogenesis in other cells, will be discussed. Supported by USPHS 09012 to S. Hagiwara and a Helen Hay Whitney Foundation postdoctoral fellowship to the author.

740 TETRA-ALKYL AMMONIUM IONS AS PROBES OF BRAIN CELL MICROENVIRONMENT. J.M. Phillips* and C. Nicholson. Dept. Physiol. & Biophys., N.Y. Univ. Med. Ctr., 550 First Ave., New York NY 10016

In order to better define the physical properties of the extracellular space of the brain, and provide a reference for the movement of endogenous cations within the brain cell microenvironment, the migration of tetra-alkyl ammonium ions (TAA) was examined in the rat cerebellar cortex. Tetra-alkyl ammonium ions were chosen as probes because their spherical symmetry and single charge facilitates theoretical analysis, and allows direct comparison with K^+.

Halide salts of tetramethylammonium, tetraethylammonium and tetrapropylammonium ions at 1 mM/L concentration in Ringer solution were superfused over the cerebellar cortex for 1-3 hr. Locally evoked field potentials remained unchanged during the TAA superfusion, thus showing that neuronal function was unaffected by the concentration of TAA used. Concentration profiles of the TAA ions were measured to a depth of 500 µm using ion-selective micropipettes containing Corning 477317 ion exchanger. Although this exchanger is nominally a K^+-sensor, its primary sensitivity is to quaternary ammonium ions (selectivity 250:1 for TAA^+: K^+).

$[TAA^+]_0$ as a function of depth was highly consistent with Fickian diffusion in a semi-infinite, homogeneous medium. In some cases, however, the profile was more consistent with diffusion through a 2-component, semi-infinite composite medium, indicating the presence of a superficial barrier. Values of the apparent diffusion coefficient, D*, were computed. For TAA ions, D* decreased monotonically with increasing ionic radius, and was about 20-25 times smaller than their theoretical diffusion coefficients in water. In contrast, D* for K^+ in the cerebellar cortex was only 8-10 times less than that in water.

When current is passed normal to the brain surface, $[K^+]_0$ decreased for current into the brain, or increases for current out of the brain, due to K^+ penetration of brain cell membranes (Gardner-Medwin and Nicholson, J. Physiol. 275: 66-67P, 1978). In contrast, all $[TAA^+]_0$ behaved in the opposite fashion. Interpreted on the basis of electrodiffusion theory, these results, together with the lack of influence on evoked potentials, confirm that the TAA ions remain in the extracellular space.

TAA ions thus serve as microenvironment probes, confined to the extracellular space and contrast with the behavior of K^+, whose movement through the brain is not so restricted, apparently. (Supported by Public Health Service, Grant NS-13742).

741 VOLTAGE-SENSITIVE Ca-CHANNELS AND Ba ION IN PARAMECIUM TETRAURELIA. Youko Satow. Lab. Molecular Biology, Univ. of Wisconsin, Madison, WI 53706.

Transient inward current (I_{in}) in Paramecium tetraurelia recorded upon a step depolarization with a voltage clamp is normally carried by Ca^{++} through a Ca-channel. The maximal inward current (I_{max}) is smaller when the membrane is held at a depolarized or a hyperpolarized level. In order to maximize the resolution of the Ca-channel activity, the membrane is held at or near the resting potentials registered in various test solutions.

Maximal inward current (6.8±0.7 nA) of paramecia bathed in a Ca-solution (1.5 mM Ca, 1 mM citric acid, 1.3 mM Tris, pH 7.2; $[Ca^{++}]_o$ 0.91 mM) is reduced by the addition of Ba (up to 2 mM) (2.7±0.9 nA in 1.5 mM Ca-2 mM Ba and 2.8±0.8 nA in 1.5 mM Ca - 4 mM Ba). The remaining I_{in} appears to be insensitive to further addition of Ba in terms of I_{max}. The 1.5 mM Ca-solution (or over 1.5 mM Ca) gives the highest I_{max} in Paramecium. The I_{max} is smaller in a Ca-solution with 0.75 mM Ca ($[Ca^{++}]_o$ 0.38 mM). The addition of Ba to this 0.75 mM Ca-solution reduces the I_{max}. It is not possible to study the membrane of paramecium in Ca-free solutions, since it disintegrates in such solutions.

The addition of Ba shifts the V-I_{peak} curve in the direction of more sensitive to voltage. The processes of activation and inactivation of the Ca-channels are slowed down in the presence of Ba. Ca competes with Ba in terms of their effects in sensitivity and the time courses of activation and inactivation. Addition of Ca shifts the V-I_{peak} curve in the direction of less sensitivity and speeds up activation and inactivation. Peak times (T_{max}) of the inward current are 2.1±0.3, 4.1±0.3, 3.9±0.7 and 2.4±0.1 msec in 1.5 mM Ca, 1.5 mM Ca - 4 mM Ba, 0.75 Ca - 2 mM Ba and 3.5 mM Ca - 2 mM Ba solutions, respectively.

Ionophoretically injected Ba^{++} reduces the I_{max}. On the other hand, such internally applied Ba^{++} has no effect on the voltage sensitivity and the time courses of activation and inactivation.

These results show that Ba inhibits rather than enhances the maximal inward transient in Paramecium. External but not internal Ba^{++} changes the behavior of Ca-channels or its surrounding leading to a change in their voltage sensitivity and the time course of their activation and inactivation.

Supported by NSF grant BNS77-20440 to C. Kung.

742 EFFECTS OF EXTERNAL CALCIUM AND CALCIUM BLOCKING AGENTS ON THE PHOTORESPONSE IN LIMULUS VENTRAL PHOTORECEPTORS. Jeffrey A. Schmidt* and Alan Fein* (SPON: Ernest M. Wright). Marine Biological Laboratory, Woods Hole, MA 02543.

The effects of Ca-blocking agents and changes in extracellular calcium, $[Ca]_o$, on the light-evoked discrete wave responses (bumps) were measured. Ca-blocking agents $CdCl_2$ (2 mM), or D-600 (10^{-4}M) in the presence of 10 mM Ca did not significantly alter bump depolarization amplitude or response latency, while reduction in $[Ca]_o$ reversibly increased both parameters. A slowing of the bump repolarization occurred with these blocking agents but not with reduced $[Ca]_o$. The hyperpolarization which follows the depolarizing transient becomes larger under steady depolarization. The amplitude of the hyperpolarization was correlated with amplitude of the depolarizing transient on top of steady depolarization of +10mV, and was eliminated when the membrane was hyperpolarized. The hyperpolarization was smaller in low $[Ca]_o$ when normalized to the depolarization amplitude, and was eliminated by the Ca-blocking agents and by the K-blocking agent quinidine (10^{-4}M).

The magnitude of outward tail currents measured under voltage clamp following depolarization depend on duration and magnitude of the depolarization. The outward currents appeared maximal at depolarizing pulse durations approximating bump durations. D-600 and quinidine reversibly suppress the tail currents as well as the outward current during the depolarization. Reversal of the tail current occurred at a potential negative relative to resting potential.

Thus, elimination of the hyperpolarization by Ca-blocking agents and reversal of the outward current at a potential somewhat negative to the resting potential suggests that the hyperpolarization results from a Ca-dependent rise in K conductance that may serve to actively repolarize the photoresponse in the unclamped receptor.

Superfusion of IBMX (5mM), a phosphodiesterase inhibitor, qualitatively mimicked the effect of these two blocking agents on both tail currents and outward current during depolarization. Thus, cyclic nucleotide metabolism may play a role in generation of the Ca-dependent outward current.

Waveshapes of light-evoked bumps and photoresponses elicited by dim flashes could be mimicked by intracellular current injection. The hyperpolarizing phase of these responses was reversibly reduced in low Ca and eliminated by the Ca-blocking agents. A late outward current was absent following the light-evoked inward current at all steady clamped voltages. These results indicate that the Ca entry that activates a late K conductance requires depolarization and is not produced in direct response to light.

(Partially supported by a Grass Fellowship given to J.A.S.)

743 A PERSISTENT INWARD CURRENT PROBABLY CARRIED BY CA^{++} IS ACTIVATED OVER A LARGE RANGE OF MEMBRANE POTENTIALS IN CAT SPINAL MOTONEURONS. Peter C. Schwindt* and Wayne E. Crill. VA Hosp. and Depts. Physiol. Biophys. and Med., Univ. Wash., Seattle, WA 98118.

Voltage clamp analysis has revealed that motoneurons may exhibit two regions of negative resistance in their steady current-voltage relations. A previously described negative resistance occuring ca. 10-30 mV above rest is caused by a persistent inward current component. The time course and voltage dependence of this current component, as well as its resistance to intracellular injection of the lidocaine derivative QX314 and its occurrance in some cells with inactivated sodium suggest this inward current component is carried by ions other than Na^+. A second region of negative resistance, seen ca. 100-150 mV above rest, also results from activation of a persistent inward current component which achieves relative dominance over the outward K^+ currents at these potentials. Furthermore, the behavior of tail currents upon repolarization from intermediate potentials in normal cells and in those in which the K^+ current is partially blocked by agents such as tetraethylammonium (TEA), suggests that a persistent inward current component also underlies the more dominant K^+ currents over this range of membrane potentials. The behavior is qualitatively similar to that of certain mollusk neurons. It is concluded that depolarization over the range 10-150 mV above rest activates one or more components of persistent inward current probably carried by Ca^{++} in addition to the transient Na^+ and steady K^+ currents.

(Supported by V.A. research grant MRIS 1610.)

744 BLOCKAGE OF PEAK AND STEADY STATE SODIUM CURRENTS IN SQUID AXON BY TETRAETHYLAMMONIUM IONS. Jonathan J. Shoukimas* and Robert J. French* (SPON: D. C. Eaton). Lab. of Biophysics, IRP, NINCDS, NIH, Marine Biological Laboratory, Woods Hole, MA 02543.

In squid axons perfused with K-free NaF solutions, the sodium conductance does not inactivate completely (Chandler & Meves, 1970. J. Physiol. 211: 623). We have performed experiments on axons perfused with high Cs, low K solutions, without the use of any proteolytic enzymes, and on intact axons with and without 4-aminopyridine to reduce K conductance. For the intact axons, sodium currents were determined as the difference between records taken in the absence and presence of tetrodotoxin (TTX). In no case was sodium inactivation complete. Rather, the current approached a non-zero steady state plateau at long times. Thus, incomplete inactivation appears to be a general property of the squid axon sodium conductance. Measured in perfused axons, the ratio of the amplitude of the plateau current to the amplitude of the peak current rose from <0.1 for E_m (inside minus outside) = -40 mV to 0.2-0.25 at E_m = +80 mV.

We have studied the effect of tetraethylammonium (TEA) ions on both peak and plateau sodium currents. Although TEA is widely used to block potassium channels and facilitate the study of other conductances in excitable cells, there are contradictory reports of its effect on sodium channels. Armstrong and Binstock (1965. J. Gen. Physiol. 48: 859) noted about 50% reduction of sodium currents in TEA-injected axons, whereas Rojas and Rudy (1976. J. Physiol. 262: 501) state that TEA has no effect on sodium conductance unless inactivation has been removed by prior perfusion with pronase. In our experiments, axons with normal sodium inactivation showed decreased sodium currents with TEA present internally. About 40 mM TEA produced a 50% reduction in the currents when there was 50 mM internal Na. The TEA reduced both TTX-sensitive peak and steady state currents by approximately equal proportions. Both inward and outward currents were blocked by TEA, but the block was somewhat greater at more positive potentials. We also observed an increase in time to peak of sodium currents in axons perfused with TEA.

745 DOES CALCIUM INFLUX FACILITATE DURING REPETITIVE VOLTAGE-CLAMP DEPOLARIZATIONS OF MOLLUSCAN NEURAL SOMATA? Stephen J. Smith* and Robert S. Zucker. Dept. Physiol.-Anat., Univ. Calif., Berkeley, CA 94720.

It has been suggested that membrane calcium channels undergo facilitation during repetitive membrane depolarizations. This has been inferred from 1) studies using potassium-sensitive microelectrodes to resolve voltage-clamp currents into potassium and calcium components, and 2) studies using the calcium-sensitive photoprotein aequorin as an indicator of calcium influx.

We have studied this facilitation using voltage-clamp and aequorin methods in identified neurons in Aplysia abdominal ganglia. In TEA-substituted zero-sodium medium, we record a sustained inward calcium current which does not facilitate to successive depolarizing pulses. In normal sea water, we record a rapidly decaying inward tail current following depolarizing pulses. This inward tail current may be isolated by the use of a postpulse to the potassium equilibrium potential. The inward tail vanishes in cobalt-substituted zero-calcium sea water. These calcium inward tail currents do not facilitate.

In normal sea water, successive depolarizations elicit increasing aequorin emissions and are accompanied by a cumulatively inactivating outward potassium current. Due to the series resistance, this leads to increasing membrane depolarizations in a train of identical command pulses. Elimination of this artefact by electronic series resistance compensation or by the use of TEA to block outward currents removes the portion of the aequorin emission facilitation due to the series resistance artefact.

The remainder of the facilitation of aequorin emissions may be due to the power law and calcium detection threshold properties of the calcium-aequorin reaction. This possibility is now under study. (Supported by NSF grant BNS 75-20288; SJS is a Miller Research Fellow and RSZ is an Alfred P. Sloan Research Fellow.)

746 VOLTAGE CONTROLLED RESISTANCE AND PERMEABILITY OF GAP JUNCTIONS BETWEEN EMBRYONIC CELLS. D.C.Spray, A.L.Harris*, M.V.L.Bennett, & P.G.Model, Dept. Neurosci., A.Einstein Coll. Med., Bronx, N.Y.

Blastomeres (32-cell stage to morula) of the axolotl Ambystoma mexicanum are electrotonically coupled and are joined by gap junctions. Each blastomere of a coupled pair was doubly impaled for current delivery and potential measurement; junctional and cell resistances were calculated by the π-T transformation. Moderate polarization of either cell in either direction resulted in increased input resistance of both injected and recipient cells and a decrease in coupling coefficient from 0.8 or more to 0.1 or less. The increase in input resistance developed in a sigmoid manner; its onset was more rapid with larger pulses and it completely reversed in less than a second after the pulse was terminated. The non-junctional membrane rectifies only slightly over the same voltage range, and junctional resistance increases as a function of transjunctional voltage over 10-20mV in either direction. The changes are insensitive to absolute membrane potential over the range of ±30mV. The increase in junctional resistance is sufficiently sensitive to voltage for there to be negative slope regions in both quadrants of the I-V relation for currents applied in either cell. As a consequence regenerative increases in input resistance can be produced by brief pulses superimposed on longer ones of the same polarity. The increase in junctional resistance is accompanied by a decrease in permeability to moderately sized molecules. Long hyperpolarizing current pulses through an electrode containing Lucifer Yellow CH (MW 443) simultaneously held pairs of cells uncoupled and injected dye into one cell. After 5 min of iontophoresis with short and weak pulses that did not uncouple, dye passage between cells was always observed. Even when dye was injected for 20 min with pulses that uncoupled the cells, there was little transjunctional dye passage. After either iontophoresis ended or the second cell was similarly hyperpolarized, the cells recoupled, and dye soon crossed to the second cell. Thus, for the embryonic gap junctions both resistance and permeability to information carrying molecules may be controllable by voltage, and changes in resting potential in different regions of an embryo could rapidly interrupt communication to allow independent development.

DCS is a McKnight Scholar.

747 REPETITIVE MINIATURE RESPONSES EVOKED BY CHEMICAL STIMULATION OF SQUID GIANT AXONS. I. Tasaki, Lab of Neurobiology, NIMH, Bethesda, Maryland 20014.

Periodic electric signals of 1 to 30 μV (peak-to-peak) across the squid axon membrane were recorded by using an intracellular electrode connected to a real-time spectrum analyzer (purchased from Nicolet Scientific Cor.). Various chemical stimulants, including scorpion venoms, 4-dimethylaminopyridine, low calcium, etc. were employed to evoke these "periodic miniature responses". The frequency spectra of these responses were always broad, extending usually from 100 to 200 Hz at room temperature (19°C). The frequency of response maximum (usually about 150 Hz) was decreased by lowering of the temperature, as well as by intracellular injection of tetraethylammonium (TEA). Tetrodotoxin (TTX) was found to suppress the miniature responses completely.

The spectra of the miniature responses evoked by scorpion venoms were found to be very irregular. There is little doubt that this irregularity is caused by the difference in the frequency of periodic responses at different membrane sites. As the effect of the chemical stimulant advanced, there was a gradual enhancement of the response amplitude, indicating that the number of membrane sites involved in production of miniature responses was increased. When the response amplitude exceeded about 100 μV, there was a strong tendency toward synchronization of periodic responses, resulting in a decrease in the band-width of the spectrum. Eventually, the axon was thrown into a state of repetitive firing of full-sized action potentials. With a given chemical stimulant, the frequency of repetitively fired full-sized action potentialswas found to be very close to the peak frequency of the miniature responses.

The resting membrane potential was measured during the course of the development of miniature responses. With scorpion venoms, 4-dimethylaminopyridine, low calcium, etc., the production of miniature responses was not preceded by a fall in the membrane potential. This finding strongly suggests that these responses are not produced by potential-dependent changes in the membrane conductance. The origin of these responses is discussed. A portion of the results reported here has been published in Japan. J. Physiol. 28: 89, 1978.

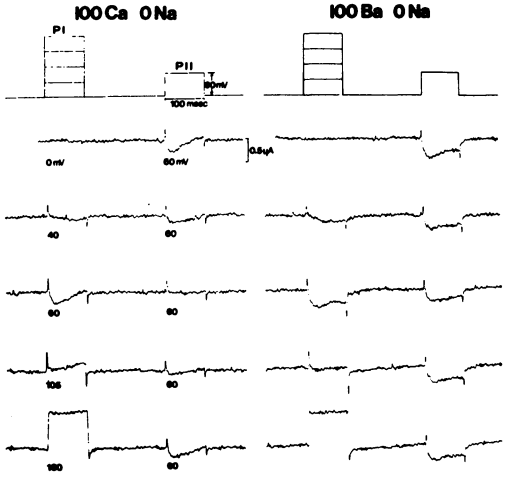
748 A CAUTION REGARDING THE USE OF TEA AND COBALT TO SEPARATE VOLTAGE-DEPENDENT AND CA-DEPENDENT OUTWARD CURRENTS. S. H. Thompson*, R. W. Aldrich Jr., P. A. Getting. Department of Biological Sciences, Stanford University, Stanford, CA 94305.

Cobalt and TEA have been used to separate the delayed outward currents in molluscan neurons into two components; one, called K-current, which is voltage dependent, and another, called C-current, which is Ca^{++} dependent. The use of these two blocking agents results in a variety of current patterns in different cells. Using voltage clamp of dorid somata we found that this variability results from 1) incomplete block of either component by these agents, and 2) differences in the ratio of C-current to K-current (I_c/I_k) in different identified cells. Analysis of tail currents, which allows temporal separation of the components independent of the pharmacological techniques, indicate that 100 mM TEA blocks 90% of K-current and 10% of C-current. Ca-free Co (10mM) saline blocks 84% of C-current with minimal effect on K-current.

Due to incomplete block, the applicability of these agents for the study of current components depends on the C-current to K-current ratio. If I_c/I_k is small, then Ca-free-Co saline will have little effect on the outward current pattern. Under these conditions the properties of K-current can be studied because contamination from residual C-current will be very small. Isolated in this way, K-current shows time and voltage dependent inactivation. When I_c/I_k is large, TEA can be used to isolate C-current since residual K-current is comparatively small. C-current activates more slowly and does not inactivate during depolarization. If, however, I_c/I_k is small, TEA cannot be used to isolate C-current since the resulting current pattern will be strongly contaminated by K-current. At intermediate values of the I_c/I_k ratio, outward currents do not truly reflect the properties of either component.

749 CALCIUM CURRENT INACTIVATION DEPENDENT ON CALCIUM ENTRY IN APLYSIA NEURONS. Douglas Tillotson.* (SPON: Roger Eckert) Dept. Biol., UCLA, Los Angeles CA 90024

Inactivation of calcium current (I_{Ca}) is revealed when interference by other ionic currents is eliminated. I_{Ca} in Aplysia neurons was observed directly under voltage clamp by substituting the impermeant ion Cs for nearly all internal K with aid of the antibiotic nystatin. This eliminates effects due to changes in both the voltage- and calcium-activated K channels. Further, all test solutions contained no Na or K. The Figure shows representative data from a double-pulse experiment on R-15 recorded with a holding potential of -40mV in 100mM Ca bathing solution. Two 100msec pulses were separated by 200 msec. The voltage of the first pulse (PI) was varied while the voltage of the second pulse (PII) was held constant at +20mV. Inactivation of I_{Ca} of PII increases in the PI voltage range -20 to +20mV; is completely inactivated from +20 to +50mV; and returns to 80% of control (without PI) as PI voltage increases toward putative E_{Ca} (ca +140mV). When barium was substituted for bathing Ca far less inactivation was seen. These data indicate that it is the calcium entry associated with PI rather than the voltage of PI that accounts for the major portion of inactivation of I_{Ca} during PII. NIH NS8364 and S07RR07009.



750 CALCIUM ACTION POTENTIALS IN SINGLE, FRESHLY ISOLATED SMOOTH MUSCLE CELLS. John V. Walsh, Jr. and Joshua J. Singer*. Dept. Physiol., Univ. Mass. Med. Sch., Worcester, MA 01605.

The use of single, isolated cells makes it possible to study the electrical properties of smooth muscle without such complications as low resistance intercellular coupling or the presence of neural elements found in tissue. Tissue strips of the stomach muscularis of the toad Bufo marinus were digested with trypsin and collagenase to yield single, isolated smooth muscle cells which were employed for experiments on the same day as isolation to avoid long term changes which might occur in culture. Cells were impaled with one or two high resistance (100-200 MΩ) micropipettes.

When external calcium was elevated to 10mM or greater, an action potential could be elicited with a depolarizing step of current or at the offset of a large hyperpolarizing step. Spontaneous activity was not observed. At 2lmM calcium the action potential duration at half-maximum amplitude typically ranged about 20 msec with a maximum rate of rise of about 5V/sec or less and an approximately equal maximum rate of repolarization; these parameters are of the same order of magnitude as seen in a variety of smooth muscle tissue preparations. In the presence of TEA (≥ 18mM), the action potential duration was markedly prolonged with a "plateau" in the range of 100 msec preceding the phase of maximum repolarization; prolonged repetitive discharge was common. The following data indicate that calcium ions carry the inward current of these action potentials: 1) Action potentials with overshoots were readily obtained at sodium concentrations as low as 3mM. A comparison of overshoot amplitude in 12 vs. 100mM Na^+ solutions (choline substitution for Na^+ with Ca^{++} constant at 21 mM) disclosed a mean increase of only 5mv which is significantly different ($p < 0.001$) from the 54mv change to be expected of a Na^+ electrode. 2) In the presence of 18mM TEA, the mean overshoot amplitude was 17mv greater at 50mM Ca^{++} than at 15mM ($p < 0.001$, n = 10), in good agreement with the expected difference of 15mv for a calcium electrode. 3) At high concentrations of Ba^{++} or Sr^{++} (64-74mM), but low concentrations of Na^+ (12mM), Ca^{++}(0.16mM) and Mg^{++} (0.09mM), overshooting spikes could be elicited; Sr^{++} spikes displaying overshoots required TEA (18mM) to be elicited. Barium spikes were extremely prolonged with no phase of rapid repolarization.

The steady-state current-voltage relationship of the cells always showed outward going rectification. Input resistance increased as $[Ca^{++}]_o$ was raised and often exceeded 500MΩ, ranging as high as 3000MΩ; specific resistance often exceeded $30 \cdot 10^3 \Omega\text{-cm}^2$ and ranged as high as $150 \cdot 10^3 \Omega\text{-cm}^2$. Specific membrane capacity was 1.32±0.33 μF/cm² (mean±S.D.). Supported by Nat'l. Fdn. March of Dimes and by NIH HL #14523.

751 PENICILLIN BLOCKS INHIBITORY CONTROL OF DENDRITIC BURST GENERATION IN HIPPOCAMPAL NEURONS. R.K.S. Wong and D.A. Prince, Dept. Neurology, Stanford University Med. Ctr., Stanford, CA 94305.

Previous studies have shown that epileptiform field potentials occur spontaneously in CA1 and CA3 regions of hippocampal slices exposed to penicillin in vitro. Intrasomatic recordings show that neurons in these areas generate depolarization shifts (DSs) and spike bursts coincident with spontaneous or orthodromically evoked epileptiform field potentials. Data suggest that DSs are intrinsically generated in dendrites. In contrast, CA1 neurons from slices bathed in normal medium generate neither spontaneous nor orthodromically evoked bursts. In order to determine how penicillin alters the behavior of CA1 cells rendering them susceptible to burst generation, we obtained intracellular recordings from somata and dendrites of CA1 cells in hippocampal slices bathed in normal and penicillin containing media. Dendritic recording sites were confirmed by intracellular injection of horseradish peroxidase. In normal medium, orthodromic stimulation in stratum radiatum produced EPSP-IPSP sequences in dendrites. EPSPs could reach amplitudes of up to 30 millivolts. The magnitude and duration of the conductance change associated with dendritic IPSPs was comparable to that recorded in the soma suggesting that these IPSPs were generated on dendritic membrane. Short duration, intracellularly injected depolarizing current pulses regularly triggered intrinsic burst responses in the dendrites. Supramaximal orthodromic input however could only evoke single spikes postsynaptically. This could be due to the shunting effect of IPSPs during orthodromic activation. Following exposure to penicillin (2000 units/ml) orthodromic stimuli became effective in generating dendritic bursts. This change was due to a gradual attenuation of the IPSP and associated conductance change. The EPSP was not obviously increased in amplitude after penicillin however it was significantly prolonged (> 50%) due to the attenuation of the following IPSP. Penicillin produced no significant effect on passive membrane properties. Simultaneous recordings from dendrite and soma of CA3 neurons revealed that dendritically evoked bursts could trigger bursts in the soma and thus affect the output of the soma. We conclude that penicillin in this system leads to generation of spike bursts by interfering with the inhibitory mechanism on dendrites which normally serves to block the occurrence of dendritic burst generation during orthodromic activation of these neurons. (Supported by NIH Grant NS06477).

752 TEMPERATURE ACCLIMATION ALTERS MEMBRANE PROPERTIES OF IDENTIFIED NEURON IN THE LAND SNAIL, HELIX. Dejan Zecevic* and Herbert Levitan. Dept. Zool., Univ. Maryland, College Park, MD. 20742

The physiological and biochemical properties of neurons are in general very sensitive to temperature change. Even though Helix neurons are affected in this way the snails exhibit a similar behavioral repertoire at 5° and 20°C when acclimated to these environmental temperature extremes. In order to explore the neuronal correlates for thermal acclimation we examined the effects of temperature acclimation on several properties of a neuron from H. aspersa. The large, easily identifiable bursting neuron in the isolated right parietal ganglion of snails acclimated for at least two weeks at either 5° or 20°C was penetrated with two independent microelectrodes filled with 3M KCl. Membrane potential (RMP) was monitored with one electrode and current passed through the other allowed determination of effective membrane resistance (R_m). A third electrode filled with 3M NaCl was used to inject Na^+ ions to test the activity of the electrogenic pump. Cooling neurons from warm (20°C) acclimated snails (active or dormant) to 5°C generally caused 1) cessation of spontaneous activity, 2) decrease in excitability, 3) increase in R_m, 4) prolongation and slight increase in amplitude of individual action potentials, 5) 70% decrease in calculated electrogenic pump current, 6) decrease in absolute passive Na-permeability, 7) change in cation permselectivity such that P_{Na}/P_K decreased 75%, but P_{Cs}/P_K and P_{Rb}/P_K both increased about 20%, and 8) no change in RMP. Some but not all of these characteristics changed further with acclimation to 5°C. For example, neurons from animals acclimated to 5°C were quite excitable and spontaneously active when tested at 5°C, their permselectivity properties more closely resembled those of warm acclimated neurons at 20°C than at 5°C, and the passive Na-permeability of the cell membrane increased. Acclimation appeared to have little effect however, on the RMP, R_m, action potential duration, or activity of the electrogenic Na-K pump. These membrane characteristics were very similar at a given temperature (5 to 20°C), independent of the animal's thermal history. Warming to 20°C a neuron from an animal acclimated to 5°C caused a greatly increased frequency of spontaneous activity, and the permselectivity changed such that P_{Na}/P_K was 90% greater, and P_{Cs}/P_K and P_{Rb}/P_K 50% and 15% less than in cells acclimated to 20°C. In sum, the thermal acclimation of snails is reflected at the level of a single neuron by changes in specific physiological properties, and these in turn are the result of changes in membrane biophysical properties such as permselectivity to different ions.

MEMBRANE STRUCTURE AND FUNCTION

753 ASSOCIATION OF TWO RAPIDLY-LABELED MEMBRANE GLYCOPROTEINS WITH SPECIFIC SUBCELLULAR ORGANELLES IN R2, THE GIANT NEURON OF *APLYSIA*. Richard T. Ambron, Ariel A. Sherbany*, and James H. Schwartz. Depts. of Anatomy and Physiol., Div. of Neurobiol. and Behav., Columbia Coll. Phys. & Surg., New York, N.Y. 10032.

Giant neurons are ideal for studying the synthesis of membrane glycoproteins and their subsequent assembly into specific organelles. Injection of ^{3}H-L-fucose or ^{3}H-N-acetylgalactosamine directly into the cell body of R2 rapidly labels a small number of membrane glycoproteins. Analyses of electron-microscope radioautographs indicate that these ^{3}H-glycoproteins are first synthesized on membranes of the reticula and Golgi and later become associated with other somatic organelles. Can individual glycoprotein components be assigned to specific organelles? We have begun to answer this question by isolating the external membrane and vesicles from R2's cell body after removing it from the abdominal ganglion. The labeled external membrane was separated from the cytoplasm and nucleus by manual dissection. Gel electrophoresis in SDS shows the external membrane to be greatly enriched in a glycoprotein with M.W. 120,000. A component of similar mobility was labeled when the isolated cell body was treated with galactose oxidase and $NaB^{3}H_{4}$. Treatment of R2 *in situ* with 0.01% trypsin 24 h after injection releases ^{3}H-glycopeptides into the bath, indicating that glycosyl moieties extend outward from R2's surface. R2's resting potential was normal throughout this mild digestion.

A different labeled somatic glycoprotein appears to be associated with vesicles. It was previously shown that only glycoprotein-I (apparent M.W. = 180,000) continues to be glycosylated in the presence of anisomycin, an inhibitor of protein synthesis. Thus, in cells injected under these conditions almost all of the incorporated radioactivity is present in this glycoprotein. Analyses of radioautographs showed a marked increase in the proportion of silver grains over vesicles in cells exposed to the inhibitor compared with untreated cells. To demonstrate the association of this component with vesicles, the cytoplasm from an injected R2 treated with anisomycin was fractionated by differential centrifugation, glass bead column filtration, and discontinuous sucrose-Ficoll gradient centrifugation. ^{3}H-glycoprotein-I and marker vesicles containing ^{3}H-serotonin co-purified and were found in a vesicle fraction characterized by electron microscopy. When cytoplasm from untreated cells was fractionated in this way, ^{3}H-glycoprotein-I was slightly enriched in the vesicle fraction. Thus it is likely that this glycoprotein is a constituent of vesicles, but it probably is not the only glycoprotein component. Further fractionation is necessary to determine whether specific types of vesicles contain unique glycoproteins.

754 FILIPIN TREATMENT GIVES EVIDENCE FOR CHOLESTEROL IN PARTICLE-FREE PATCHES OF MOUSE ROD OUTER SEGMENTS. Lary Andrews* and Adolph I. Cohen. Dept. Ophth., Sch. Med., Washington U., St. Louis, Mo. 63110

Particle-free patches have been reported in freeze-fracture replicas of mouse and bovine ROS plasma membrane (Jan and Revel, J. Cell Biol. 62: 257-273, 1974; Krebs and Kühn, Exp. Eye Res. 25: 511-526, 1977) and have been seen by us in mouse, rat, and goldfish acclimated to about 20° C. Persistance of these patches in mouse ROS incubated at temperatures from 5 to 80 °C raised doubts that they were due to phase separation in membrane phospholipids. Patches were seen in unfixed, uncryoprotected retinas as well. Filipin has been found to produce characteristic membrane lesions observable by freeze-fracture (Tillack and Kinsky, BBA 323: 43-54, 1973; Verkleij et al., BBA 291: 577-581, 1973) as a result of interaction with sterols present in exposed membranes (Kinsky, Ann. Rev. Physiol. 10: 119-142, 1970). Hubbel's observation that incorporation of cholesterol into artificial membranes containing rhodopsin produces particle-free patches not present before its addition (Accounts Chem. Res. 8: 85-91, 1975) suggested to us that the particle-free patches found in mouse ROS plasma membrane could be due to cholesterol. Treatment of mouse retinas with filipin was used here to explore this possibility. In mouse ROS plasma membrane, filipin-induced pits were found in particle-free patches but not in particulate regions. Particle-free patches are also observed in the basal discs of mouse, rat, goldfish, and frog ROS. In mice, filipin-induced pits were found confined to these patches also. Mature discs have no patches or pits. The plasma membrane of the sclerad portion of the inner segment is densely covered with pits, involving areas containing many particles. These observations suggest that cholesterol in mouse ROS is largely confined to particle-free patches in the plasma membrane, and that a relatively large concentration of cholesterol is found in the nearby inner segment plasma membrane.

755 NEUTRAL AMINO ACID TRANSPORT INTO ISOLATED BRAIN CAPILLARIES: EVIDENCE FOR POLARITY OF THE BLOOD-BRAIN BARRIER. A. Lorris Betz* and Gary W. Goldstein. Depts. of Neurology and Pediatrics, Univ. of California, San Francisco, CA 94143.

Transport of neutral amino acids into cells occurs by at least two distinct systems, a Na^{+}-dependent A-system and a Na^{+}-independent L-system. The neutral amino acid analogue α-(methyl-amino)isobutyric acid (αMeAIB), serves as a specific substrate for the A-system, while L-leucine is a good substrate for the L-system (Fed. Proc. 32: 19, 1973). In this investigation, we studied the transport of ^{14}C-αMeAIB and ^{14}C-L-leucine into capillary endothelial cells isolated from rat brain.

Capillaries were prepared from a rat brain homogenate by albumin flotation and glass bead filtration as previously described (J. Neurochem. 25: 715, 1975). Suspensions of capillary segments were incubated at 37° for various periods of time in the presence of labelled amino acid and the reaction was terminated by filtration through glass fiber filters.

Uptake of αMeAIB by brain capillaries required the presence of Na^{+} and occurred against a concentration gradient. However, when the transcellular Na^{+} gradient was eliminated by preincubation with ouabain, uptake of αMeAIB was equilibrative rather than concentrative. In contrast, L-leucine uptake was unaffected by the presence or absence of Na^{+}. There was minimal accumulation of L-leucine against a concentration gradient and this could be eliminated by preincubation with ouabain. The uptake of these two amino acids was differentially inhibited by a number of other neutral amino acids. The pattern of this inhibition was similar to that observed for the A- and L-systems in other cells.

These results indicate that both an A-system and an L-system for neutral amino acid transport are present in brain capillaries. Since numerous in vivo studies demonstrate that the A-system carrier is not present on the luminal (blood side) of the brain capillary, we conclude that this transport system is located on the antiluminal (brain side). Thus, brain capillary endothelial cells demonstrate a functional polarity for neutral amino acid transport.

756 EXPERIMENTAL BASIS FOR AN AUTORADIOGRAPHIC TECHNIQUE TO MEASURE THE PERMEABILITY OF NORMAL AND ABNORMAL BRAIN CAPILLARIES. Ronald G. Blasberg*, Clifford S. Patlak* and Joseph D. Fenstermacher. NCI, NIMH, NIH, Bethesda, Maryland 20014.

An autoradiographic technique has been developed to measure the blood to brain transfer of a small neutral amino acid, α-aminoisobutyric acid (AIB) (Neurol. 28:363,1978). The technique is based on very slow transport of AIB across normal brain capillaries coupled with rapid transport into brain cells. A three compartment model (blood, brain extracellular fluid (ECF), and brain cells) separated by two unit membranes (capillary and cell wall) was constructed. The rate constants for AIB flux across both the capillary and the cell wall have been determined by independent studies. The capillary rate constant for blood to brain ECF transport (K_1) was determined from experiments during which the plasma concentration of AIB was maintained constant and during which the plasma level was falling. The values for K_1 obtained for rat cerebrum and rhesus monkey caudate nucleus and corpus callosum were 1.8×10^{-3}, 3.4×10^{-4}, and 2.6×10^{-4} min^{-1}, respectively. The capillary rate constant for AIB transport in the opposite direction (K_o) - brain ECF to blood - was estimated from ventricutocisternal perfusion (VC-P) studies with serial periventricular tissue sampling in the rhesus monkey. K_o was found to be less than 1×10^{-3} min^{-1}. The brain cell rate constants for AIB influx and efflux were determined from the time course of brain tissue (cell) uptake. Previous in vitro studies with slices of mouse cerebrum (Blasberg and Lajtha, Biochem. Biophys. 112: 361, 1965) and recent VC-P studies in rhesus monkey were analyzed. The rate constants for AIB flux into the cells (K_1') of mouse cerebrum and monkey caudate nucleus and corpus callosum were calculated to be 1.9×10^{-1}, 8.4×10^{-2}, and 3.4×10^{-2} min^{-1}, respectively. The cell efflux rate constants (K_o') for AIB transport out of the cells of the above tissues were calculated to be 4.3×10^{-3}, 1.0×10^{-2}, and 1.4×10^{-2} min^{-1}, respectively.

On the basis of the above brain model and the experimentally determined rate constants, it is possible to measure directly up to a 100-fold increase in the permeability of brain capillaries to AIB from the autoradiograph. It is possible to localize that increase in capillary permeability to a brain region as small as 100 to 200 microns in diameter. ^{14}C-AIB autoradiographs demonstrating brain capillary disruption caused by a freeze lesion will be presented.

757 CHARACTERIZATION OF TRITIUM LABELED BATRACHOTOXIN BENZOATE BINDING TO MURINE CORTEX. George B. Brown and Stuart Tieszen*, The Neurosciences Program, University of Alabama in Birmingham, Birmingham, Al. 35294.

The specific binding of batrachotoxinin A-20α-para ^{3}H-benzoate (^{3}H-BTX-B) - specific activity 18C per mmole - to mouse cortex has been characterized with respect to the apparent dissociation constant, maximum number of binding sites, and the interaction with other batrachotoxin analogs. Using crude mouse cortex homogenates, specific binding was measured with a pellet assay as the difference in ^{3}H-BTX-B binding in the presence and absence of a 100-fold excess of unlabeled BTX-B. The results of these studies indicate that BTX-B binds to a single class of high affinity sites in mouse cortex with an apparent dissociation constant $K_d = 2 \times 10^{-8}$M. This binding is slowly reversible. The maximum number of specific binding sites is determined to be 15 pmole per gram of tissue (wet weight). Specific binding is completely inhibited by excess batrachotoxin, whereas initial experiments indicated that batrachotoxinin-A, an inactive congener of batrachotoxin, has no effect. Application of a similar pellet binding assay to red blood cell ghosts, selected as a membrane preparation lacking voltage sensitive sodium channels, showed no specific binding of ^{3}H-BTX-B.

758 AMINOPHOSPHOLIPID ASYMMETRY IN MURINE SYNAPTOSOMAL PLASMA MEMBRANE FRACTIONS. R.N. Fontaine*, R.A. Harris, and F. Schroeder* (Spon. D.G. Sherman). Dept. of Pharmacology, U. of Missouri Med. School, Columbia, MO 65212.

Vertical phospholipid asymmetry has been reported in many cellular membranes but not in the central nervous system (CNS). It is known that serum lipid components can alter membrane phospholipid composition by simple exchange processes. However, serum lipoproteins are not known to cross the blood brain barrier. Therefore, the possibility that aminophospholipid asymmetry of cell surface membranes within the CNS may be different from that of cellular membranes from outside the CNS was investigated. Synaptosomes were isolated from whole mouse brains and covalently labelled with the amino-reactive reagent, trinitrobenzenesulfonate (TNBS), under nonpenetrating conditions. The synaptosomes were then lysed. Two synaptosomal plasma membrane fractions (SPM-1 & SPM-2; fractions A and B of Gurd et al., J. Neurochem. 22:281, 1974) and two intrasynaptosomal mitochondria fractions (MITO-1 & MITO-2; Lai et al., J. Neurochem. 28:625, 1977) were isolated. The latter were used to monitor TNBS penetration into the synaptosomes. The results indicated that TNBS labelled 9.9±2.1% and 14.5±0.4% (n=6) of SPM-1 and SPM-2 phosphatidylethanolamine (PE) and 21.6±1.9% and 19.9±3.5% (n=6) of phosphatidylserine (PS) respectively. Mitochondrial fractions 1 and 2 had only 7.6% and 5.7% (n=6) of their PE labelled.. If purified synaptosomal mitochondria are labelled with TNBS under similar conditions, over 70% of the PE reacted with TNBS. These data indicate that little TNBS penetrated into the intact synaptosomes. The fatty acyl composition of labelled PE (derived from the outer monolayer of SPM-1 & SPM-2) was significantly higher in saturated fatty acids than the unlabelled PE (derived from the innter monolayer of SPM-1 and SPM-2). In conclusion, synaptosomal plasma membranes have an asymmetric distribution of both aminophospholipids and of PE acyl chains. A similar lipid assymetry has been reported for plasma membranes of murine fibroblast (LM) cells cultured in a defined medium (Fontaine and Schroeder, Fed. Proc.,in press, 1978).

759 EVIDENCE FOR COUPLING AND VARIATION IN COUPLING BETWEEN MOUSE SALIVARY GLAND ACINAR CELLS. N. Galvin* and S. B. Kater (SPON: R. Waziri). University of Iowa, Iowa City, Iowa 52242.

Coupling between cells of the mouse submaxillary gland was demonstrated by three experimental techniques. Intracellular injection of D.C. current pulses showed that electrical current could be passed from one cell to neighboring cells. The transmission of small molecules between cells was demonstrated by intracellular injection of the dye Lucifer Yellow CH, which spread from the interior of one cell to other cells within the same acinus and, occasionally, to cells of adjacent acini. Electron microscopical examination of freeze-fracture replicas of acinar cell membranes indicated the presence of gap junctions, which are the probable intercellular pathway for exchange of small ions and molecules. Utilizing these techniques, genetic variability in junctional communication between salivary acinar cells is being examined in different inbred strains of mice.

760 EFFECT OF ACETAZOLAMIDE ON THE CONCENTRATION OF ^{14}C-UREA AND ^{14}C-THIOUREA IN THE EPITHELIUM OF THE CHOROID PLEXUS. Conrad E. Johanson* (SPON: S.A. Turkanis) Dept. Pharmacology, Univ. of Utah Coll. of Med., Salt Lake City, Utah 84132

To ascertain the effect of the bulk flow of cerebrospinal fluid (CSF) on the distribution of non-electrolytes in the epithelium of the in vivo choroid plexus, the short-term uptake of radioisotopic urea and thiourea by the lateral ventricular choroid plexus (LVP) was analyzed in rats treated with acetazolamide, an inhibitor of carbonic anhydrase, which decreases CSF flow.

Sprague-Dawley rats (175-300 g) were iniected i.p. with either acetazolamide (20 mg/kg) or saline vehicle together with either ^{14}C-urea or ^{14}C-thiourea. Each animal was sacrificed one hour after the injection of drug and radioisotope; plasma, CSF, LVP, cerebral cortex, skeletal muscle and submaxillary gland (SSG) were all assayed for radioactivity. The effect of drug on the extent of uptake of radioisotope, expressed as a space (%): 100x (dpm/mg tissue ÷ dpm/μl plasma H_2O), varied considerably among the various tissues. After acetazolamide treatment, the ^{14}C-urea space in the LVP increased significantly from 29% to 39%; in contrast, in SSG (another secretory tissue containing carbonic anhydrase activity) and in skeletal muscle (a tissue without carbonic anhydrase), the ^{14}C-urea space was the same (i.e., 77-78%) in control and in drug-treated animals. Moreover, the 1-hr ^{14}C-urea space in cisternal CSF (26-28%) and in cerebral cortex (22-23%) was not significantly altered by acetazolamide. The ^{14}C-thiourea spaces are being investigated.

The significant increase in radiourea concentration in the in vivo LVP after acetazolamide treatment is presumably related to the decreased turnover of CSF by the choroid plexus subsequent to inhibition of carbonic anhydrase in the latter. Thus, it is postulated that acetazolamide affects the distribution of ^{14}C-urea in the LVP indirectly by decreasing the turnover of fluid across this tissue, thereby attenuating the previously-demonstrated sieving of urea in the basolateral membrane of the choroidal epithelium.

Supported by NIH Grant NS 13988 and RCDA # NS 00302.

761 SOUND STIMULATION INCREASES POLYPHOSPHOINOSITIDE LABELING IN AN ACOUSTIC RECEPTOR. Patricia L. Kilian and Jochen Schacht. Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109.

The possible involvement of polyphosphoinositides in hearing processes was investigated in the auditory organ of the noctuid moth, Agrotis ypsilon. This organ was selected because of its morphological and electrophysiological simplicity. The sensory tissue of this ear, the scoloparium, contains two auditory receptors along with supporting cells. Only two bioelectric events take place in these primary sensory cells: sound energy is transduced into generator potential which leads to spike activity.

Biochemical mechanisms responsible for membrane permeability changes with bioelectric events are unknown. A model has been proposed by which phosphorylation and dephosphorylation of polyphosphoinositides regulate membrane phenomena in auditory transduction (Kilian & Schacht, Neurosci. Abs. 3, 219, 1977).

This hypothesis was tested by studying the effects of acoustic stimulation on labeling of polyphosphoinositides in the scoloparium. In the first series of experiments, insects were stimulated with a pulsed tone (40 kHz) of supra-threshold intensity for 30 min after an injection of ^{32}P-orthophosphate. This stimulation led to a 50% increase of labeling of both phosphatidylinositol phosphate and diphosphate. This change seemed specific for the polyphosphoinositides, since labeling of nucleotide phosphate and of other phospholipids was not affected. It also seemed specific for the sensory tissue, since labeling in the nodular sclerite remained unchanged. The nodular sclerite is a tissue of the moth ear, but no active role in the hearing process has been ascribed to it. In a second series of experiments, a continuous tone of the same frequency and intensity was presented 30 sec before killing the moths. This stimulus did not alter polyphosphoinositide labeling in the ear tissues.

In the acoustic receptor of the moth, pulsed tones trigger both generator and action potential while continuous tones lead to adaptation of action potentials. This leads to the conclusion that the increased labeling of polyphosphoinositides is associated with spike activity in this tissue.

While a mechanism of auditory transduction remains to be established, this study demonstrates the feasibility of using the auditory receptor of the moth for such experiments. Insects may also provide suitable systems for combined biochemical and electrophysiological studies of the role of polyphosphoinositides in sensory processes other than hearing since these lipids were also demonstrated in the eye, antenna, and proboscis of the moth. (Supported by a grant from The Deafness Research Foundation and by NIH Program Project Grant No. 05785).

762 THE EFFECT OF LEAD ON MONOSACCHARIDE TRANSPORT IN CAPILLARIES ISOLATED FROM RAT BRAIN. A. R. Kolber*, P. Morell, and M. R. Krigman. Biological Sciences Research Center, Department of Biochemistry, and Department of Pathology, University of North Carolina, Chapel Hill, North Carolina 27514

Preparations of microvessels isolated from rat brain have been used as an in vitro model for the blood-brain barrier to sugars. Transport of 3-O-methylglucose (3OMG) by isolated capillaries exhibits the kinetics predicted from a carrier-mediated diffusion model. The affinity constant of 3OMG for the isolated capillary transport system (13.5 mM) is similar to that obtained from in vivo experiments.

Previous morphological studies (Toews et al., Brain Research, in press) have demonstrated that acute lead intoxication (daily intubation with 1,000 µg lead as lead acetate/gram body weight) of rats from 5 days after birth onward causes capillary vasculopathies including granular, edematous, vacuolated endothelium and distorted capillary anastamoses. These changes regress in animals which survive such acute lead intoxication for 20 days. The effect of this treatment on 3OMG transport in vitro was studied. Capillaries isolated from treated animals lost the facilitated component for 3OMG transport; the half time of uptake was not concentration-dependent and transport appeared to follow the kinetics of simple passive diffusion. The loss of the facilitated component for 3OMG transport was evident in animals treated with dosages as low as 100 µg lead/gram body weight per day. The loss of the facilitated component of transport could also be induced in capillaries isolated from normal rats by in vitro treatment at lead concentration greater than 5×10^{-7} M.

In another series of experiments capillaries isolated from normal animals were incubated with varying concentrations of lead-210. Radioactive lead was concentrated by isolated capillaries to 10-15 times the extracellular lead concentration. This accumulation of lead was not inhibited by equivalent concentrations of Ca^{++}. It is concluded that lead is tightly bound to brain capillaries, and that this association interferes with the carrier mechanism of monosaccharide transport.

Supported by U.S.P.H.S. grants ES01104, NS11615 and HD03110.

763 CHARACTERISTICS OF THE GLUTAMATE BINDING GLYCOPROTEIN FROM BRAIN SYNAPTIC MEMBRANES. Donald R. Kuonen* and Elias K. Michaelis. Dept. of Human Development, U. of Kansas, Lawrence, KS 66045.

A small molecular weight, anionic glycoprotein from brain synaptic membranes has previously been purified and found to bind L-glytamic acid with a high affinity (K_D=0.8µM) (Michaelis, Biochem. Biophys. Res. Communic. 65, 1004, 1975). Recent studies employing sucrose density centrifugation have revealed that this glutamate binding protein (GBP) tends to form multimers with molecular weights of 24,780, 40,600, 60,400 and 103,500. The specific binding activity is increased in the 40,600 and 60,400 M.W. form of the multimer. Pretreatment of this glycoprotein with 2-mercaptoethanol (10^{-4}M) reduces the binding activity of the protein and leads to an apparent decrease of the multimeric forms of the GBP. Exposure of the GBP to p-chloromercuriphenyl sulfonate (PCMPS, 10^{-4}M) does not affect the glutamate binding activity or the appearance of the various multimeric forms of the protein. SDS electrophoresis of the purified GBP yields only one protein band of 13,000 M.W.

Amino acid analysis of this glycoprotein reveals a relative enrichment in acidic and hydrophobic amino acids. Analysis of the carbohydrate make up of the protein has shown the presence of fucose, mannose, galactose, and sialic acid which represents 20-30% of the protein M.W. This carbohydrate constitution of the protein corresponds well with its demonstrated interaction with the lectin Concanavalin A. Attempts to explore the characteristics of the binding site by means of protein modification techniques are currently under way. In addition, the activity of this protein as a site for Kainic acid binding is being explored. Kainic acid is a very potent excitatory agent presumed to interact with physiologic glutamate receptors. The purified GBP can function as a binding site for [^{3}H]-kainic acid only if the non-ionic detergent Triton X-100 is dialyzed out of the system and the GBP is allowed to interact with phospholipids (phosphatidylserine). The relevance of these results in terms of the physiologic function of the glutamate receptors will be discussed. (This research has been supported by PHS grants GM 22357 from NIGMS and AA 01911 from NIAAA).

764 ANTIGENIC COMPONENTS OF NEURAL MEMBRANES. S. P. Mahadik, A. Korenovsky*, Y. Huang*, L. Graf*, and M. M. Rapport. N. Y. State Psychiatric Inst. and Columbia Univ., Coll. Phys. & Surg., New York, N. Y. 10032.

Antisera against the synaptic membrane fraction have been shown to alter several in vivo CNS functions.[1] They induce seizure activity, block passive avoidance memory consolidation, interfere with maze performance, and induce deficits during development. Since such antisera contain antibodies directed against many synaptic antigens, it is essential to separate and identify the individual antigens in order to examine the various mechanisms underlying the different biological phenomena. Immunochemical analysis of membrane antigens presents a special problem because their solubilization requires the addition of detergents (such as Triton X-100 and Berol 043), but conditions have been found that are useful in rocket immunoelectrophoresis. The antigens in membrane extracts obtained with Berol 043 (Protein:Berol, 1:4 w/w) were more stable than those obtained with Triton X-100. Extracts both of synaptic membranes and microsomes from rat brain cortex showed at least two rockets when tested with antisera to a carefully purified synaptic membrane fraction.[2] No reactions were obtained with extracts of the total membrane fraction from liver, kidney, or spleen; or with the soluble fraction from these tissues or from brain. The antigenic components were also detected by immunodiffusion (Ouchterlony). The antigens were characterized further by separation on gradient slab gels in buffers containing SDS, followed by rocket immunoelectrophoresis. Berol extracts of a synaptic membrane fraction (fraction B) and of brain microsomes[3] contained many polypeptide bands; although most were qualitatively the same in extracts of both subcellular fractions, each contained several polypeptides that were distinctive. Only 4 polypeptide bands produced rockets with the antisera. The apparent molecular weights of these bands were 56 000, 58 000, 62 000, and 66 000. These antigens were therefore different from those described by other investigators (D-1, D-2, D-3 of Jørgensen[4]; X-1, X-2 of MacPherson and Kleine[5,6]). The quantities (rocket height) of each of these antigens in extracts of synaptic membranes differed from those in extracts of microsomes. Further characterizations will be described.

Supported by an NIH grant NS 13762.

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765 LECTIN PEROXIDASE LABELING OF PIGMENT EPITHELIAL AND PHOTORECEPTOR MEMBRANES IN INHERITED RETINAL DEGENERATION. Barbara J. McLaughlin and John G. Wood. Department of Anatomy, University of Tennessee Center for the Health Sciences, Memphis, Tenn. 38163

Carbohydrate-containing macromolecules are thought to be involved in intercellular recognition. One type of intercellular recognition which occurs in the retina is that between the pigment epithelium (PE) and the discarded photoreceptor outer segments (OS) during the normal ongoing process of phagocytosis and OS membrane turnover. In the Royal College of Surgeons (RCS) strain of rat, there is a presumed breakdown in this recognition step and a failure of the PE to phagocytize OS discs. Because this breakdown may be related to changes in carbohydrate-containing macromolecules on these membranes, we have studied the localization of some sugar residues on PE and OS membranes of 15 day old RCS rats and their genetic control (RCS/rdy+) by using peroxidase-conjugated lectins from wheat germ agglutinin (WGA), castor bean (CB) and Lens culinaris (LC). Aldehyde fixed retinal slices were incubated with the lectin (either WGA, specific for n-acetylglucosamine sugars and sialic acid, or CB, specific for n-acetylgalactosamine or galactose, or LC, specific for mannose), followed by buffer rinsing, diaminobenzidine treatment, osmication and processing for EM. In both control and RCS retinas, LC, WGA and CB uniformly label the OS plasma membranes of intact photoreceptor discs but internal disc membranes are never labeled. In the controls, LC, WGA and CB also label the plasma membranes of discarded OS and WGA labels some internal disc membranes as well. In contrast, WGA and CB do not label the OS debris membranes of RCS retinas, whereas LC appears to label some of the debris membranes. The lectin labeling on PE microvilli in both control and RCS retinas differs in that WGA and LC label both proximal and distal membrane surfaces of PE microvilli whereas CB labels primarily the distal regions. In control PE cells, CB and LC label phagosome membranes whereas WGA does not. The major difference between control and RCS retinas is the absence of CB and WGA labeling on membranous debris of RCS photoreceptors and the presence of LC staining on some debris membranes. These observations may reflect some basic change in the accessibility or composition of certain cell surface sugars on photoreceptor OS membranes and PE microvilli which may be related to the breakdown in phagocytosis in RCS retinas. Supported by Fight for Sight, Inc., New York City, NS-12590 and the Sloan Foundation.

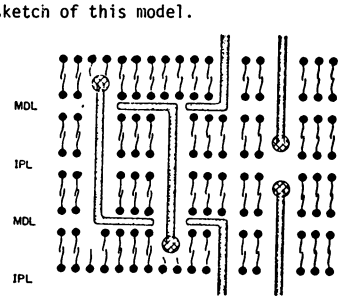
766 EFFECT OF ACETAZOLAMIDE AND FUROSEMIDE ON CSF PRODUCTION BY CAT CHOROID PLEXUS IN SITU. James M. Melby* and Donal J Reed* (SPON: J.W. Woodbury) Dept. of Pharmacology, Col. of Med., Univ. of Utah, Salt Lake City, Utah 84132

It is well known that the choroid plexus (CP) is involved in the secretion of cerebrospinal fluid (CSF). However, the magnitude of the contribution of the CP remains controversial and the mechanisms of fluid production is still obscure. In order to elucidate the role of the CP in CSF production, the present studies were conducted. The isolated CP in situ technique in the cat (Miner and Reed, J. Physiol., London 227:127-139, 1972) was used. After a control steady-state period 2-1/2 hours or more, 3-5 kg cats of either sex were given either 20 mg/kg acetazolamide or 50 mg/kg furosemide intravenously and measurements were made at 1/2 hr intervals for 2-1/2 hrs. Acetazolamide reduced flow (µl/mg CP/min) from a control value of 0.60 ± 0.05 SEM to 0.22 ± 0.09 SEM after 1/2 hr, and to 0.01 ± 0.01 SEM after 2-1/2 hr. Furosemide reduced flow from a control value of 0.52 ± 0.04 SEM to 0.31 ± 0.06 SEM after 1/2 hr and to 0.13 ± 0.06 SEM after 2-1/2 hr. After the first 30 min, the rate of reduction in CSF flow is nearly the same with both drugs, as each approached O flow. The only apparent difference in the actions of the two drugs is the magnitude of the decrease in flow during the first 30 min (the decrease produced by acetazolamide was greater than that produced by furosemide). The only statistically significant change in CSF electrolytes was a decrease in potassium, produced by both drugs. Acetazolamide lowered CSF $[K^+]$ from 3.18 ± 0.15 SEM mEq/l to 2.38 ± 0.22 SEM mEq/l ($P < 0.05$), while furosemide lowered $[K^+]$ from 3.18 ± 0.15 SEM mEq/l to 2.25 ± 0.22 SEM mEq/l ($P < 0.01$). The above results suggest that secretion by the CP can be blocked completely and that the CP is responsible for 50-60% of total CSF fluid production. (This work was supported by U.S.P.H.S. Grants NS 12869 and GM 00153).

767 A MODEL FOR THE LOCATION OF BASIC MYELIN PROTEIN (MBP) AND PROTEOLIPID (PL) ON CAMELLAR MYELIN. Rodman G. Miller The Salk Institute for Biological Studies, P.O. Box 1809, San Diego, Ca. 92114. London and coworkers have shown that the MBP can be protected from proteolytic attack by partial insertion into a lipid monolayer at the air water interface (e.g., BBA 311, 420). Plateau and coworkers (J. M. B. 75, 6976) have shown that introduction of MBP into a lipid bilayer system induces a double bilayer repeat pattern as measured by X-ray diffraction studies. The degree of hydrophobicity of the PL and freeze fracture studies of the incorporation of the PB into artificial vesicles (e.g., Vail, et al. BBA 345, 463) indicate that the PL is located in the hydrophobic matrix of the myelin membranes. Freeze fracture of myelin which has been fixed and which has been freshly frozen indicates a pleomorphism of the membrane particles, suggesting that the PL alone cannot be responsible for the particles. Freeze fracture also indicates a structural link between particles (neurokeritin formation) which must extend across both the main dense line and the interperiod line (Pinto da Silva and Miller, PNAS 72, 4046).

These lines of evidence suggest a model for the location of the two major proteins in myelin membranes in which the MBP extends through at least one membrane and interacts with the PL in anotner membrane. Arguments of economy and arguments arising from the swelling properites of meylin (e.g., Finean, and Millington, J.BC. 3, 89 and Bornstein and Raine, Lab. Invest. 35, 391) suggest that the MBP may traverse a second membrane and interact with the PL in a third.

Below is a sketch of this model.



This work was done during the tenure of an MDA Fellowship.

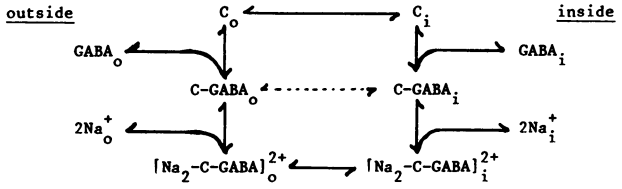
768 ANALYSIS OF CEREBELLAR NEURONAL CELL SURFACE PROTEINS DURING GROWTH AND DIFFERENTIATION IN VITRO. Lee N. Minier*, Robert S. Lasher, and Paul F. Erickson*. Anat. Dept., Univ. Colo. Med. Center, Denver, Colorado 80262.

A number of morphogenetic processes may be regulated through the interaction of specific proteins located at the cell surface. Since proper function of the central nervous system is highly dependent upon faithful recognition and matching of given sets of neuronal cells, it is important to have a knowledge of the organization of the neuronal cell surface. We have used dispersed cell cultures of 2-day old rat cerebellum as a developmental model to determine the surface composition of neuronal and non-neuronal cells. Enzymatic radioiodination coupled with discontinuous SDS-polyacrylamide gel electrophoresis methods were used to detect and resolve the cell surface protein complement. To ensure the purity of neuronal samples for analysis after labeling, small aggregates of neurons together with fasciculi were gently loosened from the surface of the culture dish with a sharpened tungsten needle, and then collected using a micropipette attached to a micromanipulator. The preliminary results may be summarized as follows: a) the classes of neuronal cell surface proteins were generally constant during development; radioautographs indicated between nine and fourteen discernible peptides falling in the range of 3.0×10^4d and 1.5×10^5d. b) considerable radioactivity traveled with the electrophoretic front, indicating labeling of low molecular weight macromolecules c) radioautographs of gels derived from cultures containing a greater relative proportion of mitotic neuroblasts and undifferentiated neurons contained 2-3 prominent bands which decreased markedly in those gels obtained from cultures having differentiating or more mature neurons. While the qualitative aspects of the surface profiles remained fairly constant during neuronal differentiation, quantitative differences occasionally appeared which may be due to variability between individual cell cultures. d) some dissimilarities existed between the labeling patterns of neuronal and nonneuronal cells.

The cell surface protein complement of nonneuronal cerebellar cells did not appear to change significantly during growth in culture. One of the major cell surface components of the non-neuronal cell population, the LETS or CSP protein, was shown by indirect immunofluorescence microscopy to appear as an increasingly complex reticular network on the surface of some nonneuronal cells. As reported previously, the LETS protein was never detected on the surfaces of neuronal cells. Research Support: NIH Training Grant GM01981 (LNM), NIH Grant NS13133 (RSL,PFE).

769 KINETICS OF CARRIER-MEDIATED γ-AMINOBUTYRIC ACID (GABA) TRANSPORT IN PINCHED-OFF PRESYNAPTIC TERMINALS (SYNAPTOSOMES). M.T. Nelson* and M.P. Blaustein, (SPON: C.M. Rovainen), Dept. Physiol. and Biophys., Washington U. Med. Sch., St. Louis, MO 63110.

GABA uptake by synaptosomes from rat brain occurs via the voltage-sensitive, carrier-mediated co-transport of two Na^+ ions with one GABA (J. Membrane Biol. 30: 153, 1976). Martin's (J. Neurochem. 21: 345, 1973) observations on high-affinity GABA uptake kinetics, which we have verified, fit the carrier model shown below. According to this model, the binding of the transported substances is ordered, and GABA binds before the Na^+ ions; only free carriers (C) and fully-loaded carriers ($[Na_2\text{-C-GABA}]^{2+}$) can cycle. The derived kinetic model has been tested by measuring GABA efflux from rat brain synaptosomes preloaded with ^{3}H-GABA; Ca-free, EGTA-containing (0.5 mM) media were employed to minimize the extrusion of GABA by exocytosis (cf. Cotman et al., J. Physiol. 245: 475, 1976). Some GABA efflux appears to be carrier-mediated since it is stimulated by external GABA in the presence of Na (Simon et al., J. Neurochem. 23: 981, 1974). We confirmed this observation, but found that ^{3}H-GABA efflux is inhibited by external GABA when Li is the predominant external cation. This implies that external GABA can bind to the carriers in the absence of external Na, thereby limiting the number of carriers available at the inside - presumably because the GABA-loaded carriers (C-GABA) cycle slowly, if at all, in the absence of external Na. We also found that depolarizing agents (veratridine or elevated external potassium concentrations) enhance ^{3}H-GABA efflux into Ca-free, EGTA-containing media; this suggests that carrier-mediated GABA efflux involves the net exit of positive charge (e.g., Na^+ moving out with the GABA, which is a zwitterion at neutral pH).



[Supported by USPHS grant NS-08442.]

770 EARLY STAGES OF EXOCYTOSIS CAPTURED BY RAPID-FREEZING. R. L. Ornberg* and T. S. Reese. LNNS, NINCDS, NIH, Bethesda, MD 20014 and Marine Biological Laboratory, Woods Hole, MA 02543.

The interactions between secretory granules and the plasmalemma which lead to exocytosis in various secretory cells have been studied in considerable detail with morphological techniques. Since the effects of chemical fixatives used in these studies to stop the secretory process undoubtedly progress from the outside in and are rather slow, there is reason to question whether current views of the earliest stages of exocytosis are representative of their actual configurations and frequencies. In order to minimize distortions, we rapidly froze the secretory blood cells (amebocytes) from the horseshoe crab, Limulus polyphemus and used freeze-fracture and freeze-substitution techniques to prepare them for electronmicroscopy. Limulus amebocytes are particularly advantageous for studying secretion in that they: 1) are essentially the only blood cell in the crab and are highly concentrated ($2x10^5$ cells/ml) making centrifugation and purification unnecessary; 2) are large (10-20 μm diameter) with many large secretory granules; 3) secrete clotting proteins very rapidly upon exposure to endotoxin; 4) can be reversibly inhibited by propanolol (1 mM). Freshly drawn amebocytes were stimulated by application to an endotoxin-soaked filter paper on a freezing stage and frozen after various times. There was a progressive loss of granules up to two minutes when all but a few amebocytes were devoid of granules. Also, there were progressively more images in thin sections of granules fused with the plasmalemma, and the freezing even preserved the concentration gradient of granule contents as it was released from the cell. Amebocytes frozen 3-15 seconds after endotoxin showed the earliest precursors of exocytosis. Unlike the smooth plasmalemma of inhibited cells, these stimulated cells have variable numbers of outward bulges and flat-bottomed inward depressions as well as small exocytotic granule openings. Comparison of freeze-fracture and thin section micrographs showed that the outward bulges are where small cisterns of endoplasmic reticulum expanded against the plasmalemma. It was at the inward depressions where secretory granules usually contacted the plasmalemma. Fractures through these regions of contact occasionally revealed single, minute (500 A) perforations where a granule membrane had fused with the plasmalemma. We could find no particle specializations or rearrangement associated with these sites, nor any changes in the contents of granules in this position. We conclude that granules are readied for exocytosis when the plasmalemma puckers in to contact them. We infer from their frequency that this contact stage is relatively long-lived but ends suddenly when a minute perforation occurs between the closely opposed membranes, and rapidly widens to release the granule contents.

771 POLYPEPTIDE COMPOSITIONS AND Ca^{2+}- AND cAMP-PROMOTED PHOSPHORYLATION OF MEMBRANE PROTEINS OF SYNAPTIC SUBFRACTIONS. Elena H. Petrali*, Brenda J. Thiessen*, Peter N. Dessens* and Prakash V. Sulakhe. Dept. Physiol., Univ. Sask., Saskatoon, Canada.

Polypeptide (PP) profiles of membrane fractions from rat cerebral cortex (CC) were analyzed by SDS (1%)-PAGE on slab gel (10%, Laemmli). Synaptosomes (SN) contained 46 PP bands; the molecular mass (M) in K daltons of major PPs was - 230, 165, 130, 107, 105, 58, 55, 54, 48, 40 and 36. Synaptic vesicles (SV) contained 30 PP bands; major PPs were similar to those of SN and in addition contained 280, 260, 250 and 200. Synaptic plasma membranes (SPM) contained 50 PP bands and major PPs were similar to those of SN. Synaptic junctions (SJ) contained about 24 PP bands and M of major PPs was 270, 130, 55, 54, 34 and 30. Post-synaptic density (PSD) contained about 4-6 PP bands and M of major PP was 58 and 55. SN, SPM and SJ isolated from 8-day old rat CC contained fewer PPs - SN contained 4 major PPs of 58, 55, 54 and 47; SPM about 30 PPs and protein profile relatively similar to adult SN; SJ, about 15 PPs and M of major PPs were 55, 32 and 30. All fractions, except PSD, from adult rat CC contained endogenous protein kinase (ePK); ePK was progressively enriched in SJ. In the absence and presence of Triton (Tx) about 14 PP bands of SN were phosphorylated (autoradiography); cAMP (μM) promoted (↑) phosphorylation (PR) of 280 (doublet), 80, 76 and 59 regions, the extent of stimulation being greater with Tx. Ca^{2+} (μM) ↑ PR of 175, 80, 76, 64, 55 and 51; SPM exhibited similar pattern of PR (± Tx). SJ showed PR of 280, 83 and 79 regions and which was ↑ with cAMP but not Ca^{2+} (± Tx). Purified myelin contained ePK that phosphorylated 18K and 16K PPs (identified as myelin basic proteins), PR of which was profoundly ↑ with Ca^{2+} (but not cAMP). SV showed PPs of multiple bands (± Tx); in the absence of Tx, cAMP ↑ PR of 250 while Ca^{2+} ↑ PP of 250, 65, 60 and 55; with Tx present similar results were obtained except that Ca^{2+} ↑ PP of 55 was considerably reduced. PSD even in the presence of exogenous PK showed little PP. Stimulatory effects of cAMP (-Tx) on PP of 83 and 79 regions could not be observed in either SPM or SJ prepared from 8-day old rat CC, although some effect was detectable with SN. Ca^{2+}, but not cAMP (-Tx), ↑ turnover of phosphate in 83, 79, 65, 60 and 55 regions of SN, SV and SPM. From these findings we suggest that Ca^{2+} acts pre-synaptically and ↑ PP of 65 and 55 regions of SV and 83 and 79 regions of SN; this may be involved in Ca^{2+}-induced release of transmitter from SV. cAMP action at the post-synaptic membrane is localized to 83 and especially 79 regions; cAMP ↑ PP of 79 occurs following synaptogenesis and may be of significance in neurotransmitter-induced changes in excitability of the post-synaptic cell.

772 CORRELATION OF INTRAMEMBRANOUS PARTICLES WITH α-BUNGAROTOXIN BINDING IN CULTURED CHICK MYOTUBES: QUANTIZATION OF PARTICLE CLUSTERS. D.W. Pumplin, Dept. Anat., Univ. Md. at Baltimore and LNNS, NINCDS, NIH and S.A. Cohen, LDN,NICHD,NIH. Bethesda, Md. 20014.

Acetylcholine receptor (AChR) distribution on chick skeletal muscle fibers developing in tissue culture was mapped using a specific ligand, α-bungarotoxin, covalently bound to the fluorescent dye, tetramethylrhodamine (TMR-α-BGT). The myotubes were subsequently freeze-fractured, and complementary replicas were obtained of cytoplasmic and external leaflets of the sarcolemma. There was excellent correlation between patches of intense fluorescence$_o$specific to TMR-α-BGT binding and clusters of large (~100 A) angularly shaped particles and the suggestion that the particles represent the ultrastructural correlate of the AChR has been previously reported (Cohen and Pumplin, Soc. for Neuroscience Abstracts, Vol. III, 1977). This correspondence was found on noninnervated muscle fibers grown in the routine manner as well as in the presence of tetrodotoxin (TTX). In the latter, the size of both the fluorescent and the particle patches was greater. These particles were distributed in about a 2:1 ratio between the cytoplasmic and external leaflets, but otherwise resembled closely particles seen at postsynaptic membranes of neuromuscular junctions. Innervated myotubes in cultures to which small fragments (explants) of spinal cord had been added were also fractured as were lengths of the neurites (previously visualized with Nomarski optics) overlying them. We have seen organizations of particles in neuronal plasmalemmas apposing the muscle which may represent precursors to active-zone specializations. When present these are found along some but not all short segments (several microns) of nerve-muscle contact which have intense fluorescence and clusters of large angular particles in the sarcolemma immediately adjacent to the nerve. Fluorescent patches and particle clusters were absent along the lengths of many neurites contacting the muscle but often were present away from sites of contact. The cluster size (number of particles/cluster) was determined for a large number of clusters on muscle fibers in each of the three categories above. The distribution of cluster sizes was not smooth, but contained peaks which were multiples of a "unit size" of 10-12 particles. Many examples of membrane depressions containing about this number of particles were also seen. Such "quantization" puts contraints on the possible mechanisms by which particle clusters are formed and degraded.

773 CROSS TALK BETWEEN BARE AND MYELINATED AXONS IN SPINAL ROOTS OF DYSTROPHIC MICE. Michael Rasminsky. Division of Neurology, Montreal General Hospital and Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada.

In spinal roots of dystrophic mice some axons are bare and others are ensheathed with inappropriately thin myelin. Impulses are generated ectopically in these spinal root axons; some of the ectopic activity is due to ephaptic conduction or cross talk between adjacent single fibers (Rasminsky, Ann. Neurol. 3:351, 1978). The present experiments were designed to examine conduction in both the exciting and excited fibers in the immediate vicinity of sites of cross talk.

Spontaneous activity on lumbosacral spinal roots of 129B6F1/J dy/dy dystrophic mice was recorded differentially between pairs of Ag or Pt-Ir electrodes separated by 200-300 μm. This recording technique permits identification of the direction of propagation of each impulse as centrifugal or centripetal. The site of origin of an ectopically arising impulse can be identified as the site of change in polarity of the action potential recorded from the fiber in question. Simultaneous recordings were made from a pair of reference electrodes held immobile at a site on the root a few mm remote from an ephapse and a pair of mobile electrodes which was moved in steps of 100 μm between successive recording sites over the 4-6 mm straddling the ephapse. With this recording configuration the impulse in the excited fiber will traverse the reference electrodes after the events of interest near the ephapse have occurred. Transmission of the impulse in the excited fiber past the reference electrodes was used to initiate pre-triggered averaging of events occurring at the mobile electrodes.

In the pairs of fibers studied so far, there was a striking difference in the conduction velocity (CV) in the exciting and excited fibers. In the exciting fiber impulses were conducted past the ephapse relatively slowly (CV ≈1 m/sec) and CV was uniform for more than 1 mm on either side of the ephapse. In the excited fiber the local CV in the immediate vicinity (within 1 mm) of the ephapse was >4 m/sec for propagation of the impulse both centrifugally and centripetally. Propagation over the next 2 or 3 mm away from the ephapse usually proceeded at a non-uniform CV in both directions in the excited fiber.

These results suggest (but do not definitively prove) that the exciting impulse is conducted in a bare axon in which conduction is continuous (Rasminsky et al, Brain Res. 143:71,1978) and that the excited impulse arises in a myelinated fiber in which conduction is saltatory but relatively slow due to thin myelin. Myelinated fibers may be more susceptible to cross excitation than bare axons because the current required to stimulate a myelinated fiber to activity is theoretically much less than that required to excite a highly capacitive bare axon.

774 ATPASE ACTIVITIES IN RETINAL PIGMENT EPITHELIUM AND CHOROID. Michael V. Riley*, Barry S. Winkler, Jennifer Benner* and Ellen M. Yates*. Institute of Biological Sciences, Oakland University, Rochester, Michigan 48063.

The Mg^{2+}-, the Na^+-K^+- and the HCO_3^--dependent ATPase activities of retinal pigment epithelium-choroid tissue from grassfrog, bullfrog and rabbit have been measured. In the frogs, Mg^{2+} ATPase activity was between 1 and 2 μmols P_i liberated $hr^{-1}mg^{-1}$protein, while Na^+-K^+ ATPase and HCO_3^- ATPase were about 20% and 25% of this activity respectively. Corresponding values for the rabbit were 8.5 μmol P_i $hr^{-1}mg^{-1}$protein, 8% and 9%. Na^+-K^+ ATPase activity in each species was completely inhibited by 2 mM calcium, while HCO_3^- ATPase was unaffected by calcium or ouabain.

The pigment epithelium-choroid preparations of bullfrog and rabbit were scraped to yield suspensions of isolated epithelial cells, relatively free of other tissue components, and a residual fraction which contained some epithelial cells, choroidal elements and red blood cells. Na^+- K^+ ATPase activity was present in the epithelial cells of both species and in the choroid of only the frog. HCO_3^- ATPase and Mg^{2+} ATPase activities were roughly equally distributed in each of the bullfrog fractions, but in the rabbit were concentrated in the choroid.

The magnitude of the ion-dependent ATPase activities in the bullfrog are comparable to the magnitude of the ion fluxes measured across similar preparations in other laboratories. These results suggest that the ATPases of the pigment epithelium are capable of mediating the active transport of sodium ions, and possibly of bicarbonate ions, that is postulated to account for the observed short circuit current across this cell layer.

775 PHOTOLABELING OF SURFACE PROTEINS OF RAT BRAIN SYNAPTOSOMES. James C. Schaeffer, Michael Hurst*, Mark Ediger*, and Michael Peck*. Dept. of Chemistry, University of Missouri, Kansas City, Missouri, 64110.

A number of functionally important presynaptic receptors and transport systems are associated with synaptosomal membranes. Since at least components of these receptor and transport systems are integral proteins partially exposed to the extracellular medium, non-specific photolabeling experiments have been performed in an attempt to determine the apparent molecular weights of the surface proteins of synaptosomes and thus shed light on the size distribution of these functional presynaptic proteins.

Whole rat brain synaptosomes were prepared by the procedure of Gurd, et al. (J. Neurochem. 22:281, 1974) and photolyzed in the presence of 100μM of tritium labeled N-4-azido-2-nitrophenylglycine at 4°C for one hour. All experiments were run at pH 7.4 to insure that the photolabel was completely ionized and therefore non-penetrable by diffusion. Furthermore, low temperatures were employed to prevent label uptake by any non-specific synaptosomal transport system. After photolysis, the labeled synaptosomes were separated by centrifugation at 10,000 x g, washed twice with isotonic saline, freeze-thawed, solubilized in sodium dodecylsulfate (SDS), heated to 100°C and dialyzed. The resulting solubilized synaptosomal protein mixture was resolved into eighteen bands by SDS-gel electrophoresis. One millimeter gel slices were solubilized and counted. Radioactivity was detected in slices corresponding to molecular weights of 115kD, 91kD, 72kD, 62kD, 54kD, and 41kD. These results indicate that synaptosomal surface proteins fall within a broad molecular weight range, but do not include polypeptides whose molecular weights are less than 40kD.

Supported in part by the UMKC Research Council.

776 CELL JUNCTIONS BETWEEN THE PHOTORECEPTOR CELLS OF DROSOPHILA. Rudolf H. Schinz*(SPON: J.E. Mittenthal). Purdue University, West Lafayette, IN 47907

It has been known for some time that there are desmosomes between the membranes of two neighboring dipteran photoreceptor cells. We found still another type of photoreceptor cell contact in freeze-fracture studies of the Drosophila retina. Within this cell contact, the protoplasmic membrane leaflet showed a rather loose and irregular pattern of ridges, which under high magnification displayed a particulate substructure. The exoplasmic leaflet of the adjacent cell showed furrows corresponding to the ridges. These cell contacts were found throughout the retina, but favorable preparations yielded pictures showing that the structures occur also between two neighboring photoreceptor cells. These structures satisfy the criteria for cell junctions published by Satir and Gilula (1973). Moreover, because there were no conspicuous structures at the corresponding sites in thin sections, other than a gap of about 150Å between the neighboring membranes, the observed cell junction corresponds most closely to a "continuous junction", found in various other invertebrate tissues. The physiological significance of this type of junction is still poorly understood. (Supported by NSF BNS77-18647 and NIH EY00033 granted to W.L. Pak)

777 THE USE OF ANTIBODY + COMPLEMENT TO GAIN ACCESS TO THE INTERIOR OF PRESYNAPTIC TERMINALS. Erik S. Schweitzer and Mordecai P. Blaustein, Department of Physiology and Biophysics, Washington University Medical School, St. Louis, MO 63110.

Treatment of synaptosomes with antiserum directed against synaptosomal membranes (Ab) and complement (C') results in a rapid release of intracellular potassium. This release does not occur after treatment with antiserum alone, or with normal serum + C'. Ab + C' treatment releases 40% of the total K content of intact synaptosomes, while osmotic lysis or saponin treatment (procedures that appear to disrupt completely the permeability barrier at the plasma membrane) release 50% of the total K.

The Ab + C' reaction can be carried out in separate steps. Binding, but not pore formation, occurs at 0°. When the incubation medium is warmed to 30° after a 20 minute incubation at 0°, K release is complete in less than 5 minutes. This latter step can be carried out in a medium containing sub-micromolar Ca^{2+}.

The perm-selectivity of the complement-induced pores is consistent with a pore size of 25 Å, as has been suggested by Michaels and Mayer (Biophys. J. 21: 125a, 1978). The pores are large enough to allow the rapid release of intra-terminal K and, presumably, to cause the collapse of the membrane potential; however, they are too small to permit the soluble cytoplasmic enzyme, lactate dehydrogenase, to escape. In addition, preliminary EM studies indicate that Ab + C' treatment does not lead to gross morphological disruption of the synaptosomes.

This treatment may allow the manipulation of the intra-terminal ion and metabolite composition without the loss of macromolecules or subcellular organelles, such as synaptic vesicles, microtubules, endoplasmic reticulum, and mitochondria. [Supported by USPHS grant NS-04882.]

778 GENESIS AND DEVELOPMENT OF SERTONERGIC VESICLES IN THE IDENTIFIED GIANT CEREBRAL NEURON (GCN) OF *APLYSIA*. Ludmiela J. Shkolnik* and James H. Schwartz. Dept. Physiol., Div. of Neurobiol. & Behav., Columbia Coll. of Phys. & Surg., New York, N.Y. 10032.

In order to trace the genesis of the serotonergic vesicle in the cell body and its subsequent development in axons and nerve terminals, we have injected ^{3}H-N-acetylgalactosamine directly into the cell body of GCN in the isolated *Aplysia* central nervous system. Membrane glycoproteins, labeled by incorporation of the ^{3}H-sugar precursor, are rapidly transported along the axon in the cerebrobuccal connective to the buccal ganglion where they can serve to identify terminal varicosities of the injected neuron by electron microscopic radioautography.

Labeled synaptic varicosities, examined after conventional glutaraldehyde-osmium fixation, revealed a heterogeneous population of vesicles. Two regions can be defined within varicosities by the types of vesicles they contain. One region contains an equal number of dense-cored (DCV) and empty vesicles, 80-90 nm in diameter. Filling the other region are small (50 nm) lucent vesicles, many of which cluster along active zones; occasional DCVs, some of which abut the membrane, are also present in this region. Similar serotonergic terminals have now been described in other invertebrates and in the vertebrate brain indicating that serotonergic terminals have common morphological features in phylogenetically distant animals. On the other hand, only one characteristic and different type of vesicle occurs along the entire length of GCN's axon. This is a 70-80 nm *lucent* vesicle containing a lucent 50 nm vesicle, one inside the other. Similar compound vesicles are also seen in the cell body and axon hillock, where they are occasionally attached to short tubules, 30-35 nm wide. These compound vesicles have previously been shown to be the only significantly labeled organelle in the axon of GCN after intrasomatic injection of ^{3}H-serotonin. When we treated the tissue with $KMnO_4$ or chromium salts, the lucent compound vesicles became intensely dense-cored, indicating that they contain endogenous serotonin.

We suggest that the lucent compound vesicle observed in the cell body and axon is formed by budding of smooth endoplasmic reticulum and is the precursor of the synaptic DCV, which reaches maturity only within the varicosity. By an exocytotic process, which occurs only once for each DCV, the DCV contributes its inner membranous component to the presynaptic membrane and releases both transmitter and core proteins into the synaptic cleft. Thus, although the DCV functions primarily to store transmitter, its inner membrane component is also the source of the small vesicles observed clustered along active zones, which mediate the routine and *repeated* release of neurotransmitter.

779 LOCALIZATION OF RAT BLOOD-CSF BARRIER Na-K ATPase Quentin R. Smith* and Conrad E. Johanson* (Spon:W. Stevens Dept. of Pharmacology, Univ. of Utah Col. Med., Salt Lake City, Utah 84132

Two properties of ouabain, the biphasic effect on Na-K ATPase and its low permeativity, were utilized to investigate the localization of the Na-K pump to the apical or basolateral membrane of the blood-CSF barrier. Adult rats were injected i.p. with ouabain at a dose of either 0.01 or 10 mg/kg (or vehicle) and sacrificed 1 hr later for analysis of Na and K. The extracellular fluid space and residual erythrocyte volume were measured with tritiated inulin and ^{51}Cr-tagged-RBC, respectively. With the lower dose, no effect on electrolyte concentration was detected in any fluids or tissues examined. At the 10 mg/kg dose, analysis of choroid plexus (CP) epithelial cell concentration of electrolytes by compartmentation analysis revealed that cell [K] rose by 13% to 171 mmole/kg H_2O and cell [Na] fell by 45% to 25 mmole/kg. The cerebral cortical cell [K]/[Na] increased, but not significantly. Although plasma [K] increased by 101% to 9.41 mmole/L and plasma [Na] decreased by 7% to 140 mmole/L, no significant alteration was seen in CSF electrolytes. Erythrocyte and skeletal muscle cell electrolytes changed in the directions expected for an inhibited Na-K pump.

The electrolyte data for the CP strongly suggest a stimulation of the Na-K pump in this tissue subsequent to the intraperitoneal administration of 10 mg/kg ouabain. This, along with the lack of effect of the 0.01 mg/kg dose, is consistent with an apical membrane model for the Na-K pump of the rat blood-CSF barrier. (Supported by NIH Grant Nos. NS-04553 and GM 00153)

780 CALCIUM ION-STIMULATED ENDOGENOUS PROTEIN KINASE CATALYZED PHOSPHORYLATION OF MYELIN AND ITS BASIC PROTEINS. Prakash V. Sulakhe, Elena H. Petrali* and Brenda J. Thiessen* (SPON: J.W. Phillis). Dept. Physiol., Univ. Sask., Saskatoon, Canada.

Myelin-enriched particulate fraction (R) and purified myelin (M) isolated from the white matter of rat brain were phosphorylated by the endogenous (Mg^{2+}-supported) protein kinase (ePK). With either R and M, myelin basic proteins of molecular mass 18,000 (MPII) and 16,000 (MPI) were the major substrates for phosphorylation (PP) and their PP accounted for greater than 85% of the phosphate incorporated into R or M. Low concentrations of Ca^{2+} markedly stimulated PP of R, M as well as MPI and MPII. Triton X-100 (Tx) at 0.05% increased both Ca^{2+}-independent (basal) and Ca^{2+}-stimulable PP. The maximal stimulation (3- to 5-fold) of PP by Ca^{2+} was observed at 10 μM (free) Ca^{2+} with the half-maximal at about 0.5 μM; Ca^{2+} greater than 1.0 mM caused significant inhibition of PP of R, M, MPI and MPII. cAMP (0.01-100 μM), showed no stimulation of PP in the absence of Tx; with Tx, only modest (30%) but consistent stimulation of PP of M, R, MPI and MPII was noted. Calcium increased the apparent affinity of ePK-catalyzed PP of M, R, MPI and MPII for Mg^{2+} (0.2 mM with Ca^{2+} vs. 2.0 mM without Ca^{2+}) but not for ATP; Tx increased the V_{max} without changing the apparent affinity for Mg^{2+}, ATP, or Ca^{2+}. In the presence of Ca^{2+} and Tx the apparent affinity towards cAMP of ePK-catalyzed PP was increased nearly 100-fold. Tx even at 3 μmole/mg protein failed to solubilize Ca^{2+}-dependent ePK while cAMP-dependent ePK was solubilized only poorly (up to 20%). Tx effects on basal and Ca^{2+}-dependent ePK-catalyzed PP are hence unrelated to solubilization of ePK by Tx. The PP of R, M, MPI and MPII was rapid, reaching maximum between 1-2 min at 30°C; this was true for ± Tx and/or Ca^{2+}. Inhibition of phosphatase (PPase) by NaF failed to increase the initial rate of PP under a variety of conditions. PPase was present and was capable of dephosphorylation (dPP) of MPI and MPII and appear to be stimulated by Ca^{2+} but not cAMP. Amongst the various divalent cations tested, Mg^{2+} was most effective in supporting basal ePK and Ca^{2+} was by far the most effective stimulator of Mg^{2+}-dependent basal ePK. Ca^{2+} stimulated PP of polypeptides similar to MPI and MPII was seen with the particulate and not the soluble fractions of white matter; similar PP was absent from (a) particulate and soluble fractions of the gray matter (b) axolemmal and oligodendroglial cell membrane fragments (c) synaptic plasma membranes, synaptic junctions and post-synaptic density. These results show the presence of a highly Ca^{2+}-sensitive ePK in myelin that catalyzes PP of myelin basic proteins. ePK is tightly bound to the myelin and appears buried within the membrane matrix.

781 TWO Na,K-ATPases OF BRAIN: BIOCHEMICAL, KINETIC, AND DISTRIBUTIONAL DIFFERENCES. Kathleen J. Sweadner Dept. Neurobiology, Harvard Med. Sch., Boston, Mass. 02115.

The large subunits of two different Na,K-ATPases from mammalian brain can be separated by polyacrylamide gel electrophoresis in SDS. The two bands differ by ~500 in apparent molecular weight. Both bands are phosphorylated by γ-[^{32}P]ATP in the presence of Na^+ and are dephosphorylated in the presence of K^+. The bands cannot be interconverted by incubation of crude homogenates of brain at 37°C, or by mild tryptic or chymotryptic proteolysis. The two bands differ in their sensitivity to digestion with trypsin, to crosslinking with Cu^{++}-o-phenanthroline, and in their reactivity to N-ethyl maleimide. In the rat, there are two Na,K-ATPase activities in brain with dramatically different affinities for cardiac glycosides; the high affinity species of Na,K-ATPase can be assigned to the band with the higher apparent molecular weight, and the low affinity species to the other band. The two ATPases may differ functionally, in that the activity of the high affinity species is stimulated 15-20 fold by K^+, while the low affinity species has a high basal activity in the absence of K^+, and is stimulated only 2-5 fold.

Attempts to localize each of the two species have given the following results: the low affinity, lower apparent molecular weight species is indistinguishable from the Na,K-ATPases found in kidney, cardiac muscle, skeletal muscle, and cultured sympathetic neurons. Both species are found together in white and in gray matter, in retina, and in synaptosomal plasma membrane. A heterogeneous mixture of non-neuronal cells cultured from whole brain shows only the species which is found in the peripheral tissues. The higher affinity, higher apparent molecular weight form unique to brain and retina has not yet been isolated free of the other species, but it is most likely that it is associated with some, but not all, classes of neurons. The possibility that the two ATPases modulate synaptic activity in different ways deserves further investigation.

782 PLASTICITY OF DENDRITIC SPINE SYNAPSES OF THE DENTATE GYRUS: ASSOCIATION OF SPINE APPARATUS WITH SYNAPTIC SPINULES. Sally Tarrant* and Aryeh Routtenberg (SPON: W. Bondareff). Cresap Neuroscience Laboratory, Northwestern University, Evanston, ILL 60201.

Ultrastructural analysis of serial sections reveals two distinct types of dendritic spines present in the molecular layer of the dentate gyrus of the adult rat hippocampal formation. One is small and slender, spine head circumference $< 1.5\ \mu$ in single sections, with a straight synaptic cleft, no spine apparatus and no synaptic spinules. The other is large, with an irregular shaped spine head, circumference $> 1.5\ \mu$ in single sections and is observed to contain spine apparatus and synaptic spinules. As we have recently described (Tissue and Cell, 9 (3), 1977, 461-473), the synaptic spinule is a protrusion of dendritic spine cytoplasm and membrane, which is devoid of postsynaptic density, into the presynaptic terminal region within the active zone of the synapse. Coated vesicles are observed on the extremities of the presynaptic membrane portion of the spinule; ribosomes and filamentous material are present in the spinule cytoplasm.

We have previously reported observations of an association between spine apparatus and spinule-containing synapses, i.e., all spines which terminate in spinules, contain a spine apparatus in the cytoplasm. We have now observed membranous structures which extend from spine apparatus to the postsynaptic membrane region in these synapses. It is speculated that these elements, spine apparatus and synaptic spinules, are labile membranous structures within certain dendritic spines. Preliminary evidence regarding the lability of synaptic spinules is suggested by our observations of increased number of spinules counted following knife cuts which axotomized the parent granule cells. To determine whether this increase is due to an increase in the number of spinules or to an elaboration of already existing ones will require study of serial sections.

An examination of serial sections has revealed that the presence of synaptic spinules and spine apparatus in all dendritic spines is about 10%. When only the large spines are considered, at least 40% of these dendritic spines contain synaptic spinules. If a considerable number of certain dendritic spines are in fact labile, then perhaps electrophysiological events related to potentiation (Lomo, Exp. Br. Res., 12, 1976, 36-63) or memory formation (Collier and Routtenberg, Brain Research, in press) may be related to this lability. (Supported by MH25281 and NSF 19388 to A. R.)

783 PERMEABILITY OF THE FROG PERINEURIUM UNDER CONDITIONS OF STRETCH AND HYPERTONICITY. Ananda Weerasuriya*, Robert E. Taylor and Stanley I. Rapoport. NINCDS, NIH, Bethesda, Md. 20014 and NIA, NIH, Baltimore, Md. 21224.

The permeability to ^{14}C-sucrose of the isolated perineurium of the sciatic nerve of female R. pipiens was measured at room temperature at rest length, when the perineurium was stretched and after the perineurium had been subjected to hypertonic treatment. The perineurium was removed as a cylindrical tissue from the sciatic nerve. The ends of the perineurial cylinder were mounted and sealed on cannulae. Ringer fluid was infused at a constant rate into the inlet cannula and was collected in scintillation vials from the outlet cannula. The preparation was placed in a stirred isotonic Ringer bath containing ^{14}C-sucrose. The permeability coefficient is defined as the tracer flux per sec, from bath to interior, per unit concentration gradient across the perineurium divided by the perineurial surface area. Mean permeability was calculated to be 5.6 ± 0.27 (S.E.M., n=45)$\times 10^{-7}$ cm sec^{-1}. Stretch of the perineurium by 10% from rest length reversibly increased perineurial permeability to ^{14}C-sucrose by a factor of 2. A 20% stretch increased permeability by a factor of 5, but the increase was not fully reversed when the preparation was returned to rest length. The perineurial permeability increased in a tonicity-dependent fashion in isotonic Ringer fluid after it had been immersed for 25-50 minutes in a Ringer bath made hypertonic by the addition of either NaCl or sucrose. NaCl addition produced a greater increase in permeability than did a sucrose solution of equal osmolality; the increase in both cases was irreversible. The threshold for a significant increase in the perineurial permeability was a 25 minute immersion in a Ringer bath containing an additional 1.0 M of NaCl. If the perineurial cylinder was inverted and the inverted preparation was immersed in a Ringer bath made hypertonic with NaCl, an increase in permeability was noticed, but in a Ringer bath made hypertonic with sucrose no increase in permeability was detected. The experiments quantify, for the first time, the diffusional restriction that the perineurium places on exchange between the endoneurial space and general extracellular fluid space, and indicate that stretch or hypertonicity can reduce this restriction.

784 IDENTIFICATION OF LECTIN BINDING POLYPEPTIDES IN MICROSOMAL FRACTIONS OF PIG CEREBELLAR CORTEX. John G. Wood and Francis I. Byrd. Department of Anatomy, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163.

We have identified smooth membrane cisternae in cerebellar Purkinje cell dendrites and axons which appear to be specialized in their capacity to bind certain lectins (J. Cell Biol., 63:541-549, 1974; Brain Res., 118:15-26, 1976) and which may serve as part of a transport system in these processes. In order to identify and characterize the macromolecules on smooth membrane systems which bind different lectins, we first isolated microsomal fractions from the molecular layer (hand dissected) of pig brain cerebellar cortex, an area rich in Purkinje cell dendrites containing the cisternae. The microsomal fractions were solubilized in sodium lauryl sulfate (SDS) and dithiothreitol (DTT) and subjected to electrophoresis in a 12.5% SDS slab gel. The slab gel was cut into strips and one strip was stained for protein using Coomassie Brilliant Blue dye. The other strips were treated to remove SDS exactly as described by Fairbanks et al., (Biochem., 10:2606-17,1971) using isopropyl alcohol and acetic acid. The strips were then "stained" for lectin binding sites using Concanavalin A (Con A), wheat germ agglutinin (WGA) and castor bean (CB), three lectins which we have extensively used in cytochemical experiments. In all cases peroxidase was used to mark the sites of lectin staining of the gel pattern; Con A and peroxidase were applied sequentially (Exp. Cell Res., 64:232-236, 1971) and WGA and CB were conjugated to peroxidase before being used to stain the gel. Several Coomassie Blue positive bands were also stained by each of the lectins. The staining pattern with WGA and CB were very similar, a result in accord with our cytochemical results (J. Cell Biol., 75:117a, 1977). The staining with Con A differed, however, with several new lectin positive bands apparent, especially in the high molecular weight region of the gel. Since Con A stains the smooth membrane cisternae in Purkinje dendrites while WGA and CB do not (J. Cell Biol., 63:541-549, 1974; 75:117a, 1977), the additional Con A positive bands may represent a family of carbohydrates lining the specialized cisternae. Further study of this problem will be possible by employing a wider range of lectins in comparative cytochemical and gel labeling studies. Supported by NS-12590 and the Sloan Foundation (JGW).

785 CLOSE ASSOCIATION OF SELECTIVE ACID HYDROLASES WITH RAT CNS MYELIN. Shuichi Yamaguchi*, Eisuke Hanada*, and Kunihiko Suzuki, Departments of Neurology & Neuroscience, The R. F. Kennedy Center, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Constituents of normal CNS myelin are known to turn over metabolically during the life of the animal. Turnover of such a highly extended plasma membrane may require an unusual metabolic machinery. Contrary to the earlier concept, an increasing number of enzymes are being found associated with purified myelin preparations. The results of the present study suggest that even some of the acid hydrolases, generally considered to be of lysosomal localization, may also be closely associated with myelin.

Myelin was isolated from adult rat brain according to Norton & Poduslo. Judged by the electron microscopic appearance and the essentially undetectable activities of UDP-glc:ceramide glucosyltransferase and the magnesium-dependent neutral sphingomyelinase, our myelin preparations were of the generally accepted purity with no more than 5% impurities. Acid hydrolase activities in purified myelin could be divided into two distinct groups. The specific activities of the first group were 3.3 ± 0.7(SEM)% of those in the starting homogenate. This group included β-hexosaminidase, β-glucosidase, acid phosphatase, and arylsulfatase B. On the other hand, the specific activities of the second group of hydrolases were 40.2 ± 2.1(SEM)% of those in whole homogenate. This group included arylsulfatase A, 4-MU β-galactosidase, galactosylceramidase, G_{M1}-ganglioside β-galactosidase, sphingomyelinase, α-fucosidase, and α-mannosidase. These activities could not be removed by washing the purified myelin with various aqueous salt solutions. The activities of the hydrolases in myelin showed pH optima similar to those in whole homogenate. When the purified myelin was arbitrarily fractionated into light, medium and heavy subfractions, the heavy myelin had the highest specific activities of the acid hydrolases. The medium myelin showed specific activities approximately half of those in the heavy myelin. The light myelin was least active with acid hydrolases. These findings suggest that a group of selective acid hydrolases may be either intrinsic to myelin or present in structures closely associated with myelin, such as axolemma, the lateral loops, or the junctional area between myelin and the oligodendroglial cell body. It is noteworthy that the hydrolases found in purified myelin have their natural substrates in myelin --- sulfatide, galactosylceramide, sphingomyelin, G_{M1}-ganglioside, and myelin glycoproteins, while no myelin constituents appear to be the natural substrates for those hydrolases that are not found in myelin. The possible degradation of myelin constituents in situ by the associated acid hydrolases is under investigation. (Supported by NS-10885, NS-03356, and HD-01799 from the U.S.P.H.S.)

786 MODIFICATION OF SH GROUPS OF THE MUSCLE MEMBRANES OF A MARINE CRUSTACEAN RESULTS IN OSCILLATORY SPIKE ACTIVITY.* C. Zuazaga de Ortiz and J. del Castillo. Lab. of Neurobiol., Med. Sci. Campus, U.P.R., San Juan, P.R. 00936.

Reagents that modify SH groups usually block excitability in a variety of excitable membranes. However, we have found that several sulfhydryl reagents induce oscillatory spike activity in the electrically inexcitable muscles of the fresh water crustaceans Xiphocaris elongatus and Atyas occidentalis (C. Zuazaga de Ortiz and J. del Castillo, to be published). The purpose of the present work is to determine if this effect is specific for fresh water crustaceans or if it is more general, and it is found also working on muscles from marine species. The ventroabdominal flexor muscle of the marine shrimp Stenopus hispidus was used for this purpose. The reagents used were N-ethylmaleimide (NEM), maleimide and 4-cyclopentene-1,3-dione (4-CPD). Exposure of Stenopus muscle to these reagents (2mM, 5-10 min) induces action potentials with low membrane accomodation. Depolarization by injected current causes repetitive spike activity. Free SH groups are required for this effect to appear. If before applying the sulfhydryl reagents, the muscle is exposed to an organic mercurial which blocks the SH groups, the effect is prevented. Furthermore, 1,3-cyclopentanedione, the saturated analog of 4-CPD, has no effect. The spikes are not affected by tetrodotoxin (50μM) but are reversibly blocked by Mn^{2+} (50mM), suggesting thay are partially due to Ca^{2+} ions. It is proposed that the induction of excitability in crustacean muscles by the above reagents depends upon the modification of the cysteinyl residues to carbonyl containing thioethers which allows the occurrence of new interactions between the proteins that control the ionic conductances of the membrane.

*Supported by grants Nos. NS-07464, NS-14938 and GM-05784 from the USPHS. (Contribution No.86 of the Laboratory of Neurobiology).

MEMORY AND LEARNING

787 THE BLOCKADE OF CONDITIONED REINFORCEMENT BY PIMOZIDE. Richard J. Beninger* and Anthony G. Phillips. Dept. Psychol., Univ. British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Psychomotor stimulant drugs which serve as dopamine agonists have been shown to potentiate conditioned reinforcement (Hill, 1970; Robbins, 1975). However, the involvement of dopaminergic neurons in the establishment of conditioned reinforcement has not been investigated. As a direct test of this possibility, the present experiment examined the effect of the dopamine receptor blocker pimozide on the establishment of conditioned reinforcement in which a tone was paired with food reward. The experimental design consisted of five groups (N=6) of food-deprived rats each tested in three distinct phases. Phase one measured preference for each of two levers one of which produced a tone. In phase two, with the levers absent, the tone was paired with food over four sessions. Phase three again measured preference for each of the two levers. Control rats injected with saline during phase two showed a significant increase in preference for the tone lever in phase three. An additional control group which received pellets explicitly unpaired with the tone in phase two showed no change in lever preference in phase three. A third group was tested in phase two under the influence of 1.0 mg/Kg pimozide; they were observed to eat all the pellets readily during phase two but showed no change in preference for the tone lever in phase three. The similar performance of a group which was under the influence of pimozide during phases two and three ruled out the possibility of state dependent learning. A final group was injected with pimozide 60-70 mins after each session of phase two and during phase three. This group showed a shift in preference for the tone lever thus ruling out the possibility that the state dependent learning group failed to show conditioning to the tone because of a drug-induced motor impairment. These results implicate dopaminergic neurons in the learning process which underlies the establishment of conditioned reinforcement.

Hill, R.T. In Amphetamine and Related Compounds (eds.) E. Costa and S. Garrattini, New York: Raven Press, 1970, pp.781-795.
Robbins, T.W. Psychopharmacology, 45, 103-114, 1975.

788 SEPARATION OF ASSOCIATIVE FROM NON-ASSOCIATIVE SHORT LATENCY CHANGES IN MEDIAL GENICULATE AND INFERIOR COLLICULUS DURING DIFFERENTIAL CONDITIONING AND REVERSAL IN RATS. Dorwin Birt, Robert Nienhuis*, and Marianne Olds*. Div. Biol., 216-76 Calif. Inst. of Tech., Pasadena, CA 91125.

Results obtained in a recent experiment in this laboratory drew attention to the possible contribution of subtle non-associative processes to changes in the early latency unit responses obtained in MGB and IC of the rat during the learning of a differential appetitive conditioning task. An important possible source of such non-associative effects was shown to arise from differences in the interval between a previous food pellet presentation and the following CS+ or CS-. To determine whether previously reported changes in MGB and IC were due to associative processes or to non-associative ones, the present experiments were carried out using a counterbalanced stimulus presentation schedule which placed the CS+ and CS- at the same average time following a previous food pellet. Criteria adopted for characterizing a change as associative required that the response to the CS+ be enhanced relative to that to the CS- throughout repeated reversal sessions.

The results obtained support previous findings of early latency associative changes in MGB, and are consistent with the idea that such changes are most likely to occur in the medial division of this nucleus. The findings in the IC thus far do not support the notion that this nucleus participates in this process. However, in view of the complex structure of the IC and the fact that not all of its subdivisions were sampled, these negative findings must be viewed with caution. The results also support the view that the anatomical distribution of neurons participating in the elaboration of associative changes may be more restricted than that of neurons participating in non-associative ones.

789 BIOCHEMICAL AND BEHAVIORAL EFFECTS OF STREPTOVITACIN A IN MICE. C.A. Boast and B.W. Agranoff. Neuroscience Laboratory Building, University of Michigan, Ann Arbor, MI 48109.

Disruption of long-term memory formation by protein synthesis inhibitors has been documented in several species. In goldfish these agents are injected into the cranial cavity, while in mice a parenteral route has been most commonly employed. Experiments to establish a neuroanatomical site of action of the inhibitors have been limited by technical factors. For example, small brain size has precluded localization studies in the goldfish. In experiments with mice (Eichenbaum et al, Brain Res. 101:171, 1976) we found memory loss following bilateral intracerebral injections of cycloheximide (CXM, 30 µg) into striatum, hippocampus (Hpc), amygdala (AM) or posterior lateral thalamus, but not in other brain regions examined. Partlow et al, (Trans. Am. Soc. Neurochem. 9:212, 1978) recently reported that injections of CXM directly into rat AM can result in amnesia. Similar injections into the internal capsule do not impair memory. In the present experiments we have employed the antibiotic streptovitacin A (SVA), a potent protein synthesis inhibitor. SVA in saline was injected into ether-anesthetized mice through 26 g cannulae during 35 sec. One µg of SVA in 0.5 µl was injected bilaterally into ventral Hpc at various times following one-trial inhibitory avoidance training. Retention was tested 24 h after training. Amnesia resulted from injections immediately after or 1 h following training, while a 6 h delay was less effective, a result consistent with the well-established time-dependence of memory susceptibility. We found that 0.1 µg of SVA in 0.05 µl injected into the ventral Hpc immediately following training was ineffective, while 1 µg of SVA in 0.05 µl resulted in amnesia. An intermediate dose and vol (0.6 µg SVA in 0.03 µl) yielded variable results. These results indicate a dose-dependent, volume-independent, amnestic effect of SVA. SVA (1.0 µg in 0.05 µl) injected bilaterally into dorsal Hpc resulted in amnesia, but was ineffective when injected into cortex overlying ventral Hpc or corpus callosum overlying dorsal Hpc. The behavioral results are compared with studies on the extent of regional inhibition of protein synthesis assessed by radioautography and scintillation counting of brain samples following unilateral intracerebral injection of SVA combined with a systemic ^{14}C-methionine pulse. (Supported by Grants MH-12506 and NIMH 013831.)

790 DECREASES IN EXCITABILITY OF CORTICAL NEURONS TO EXTRACELLULARLY DELIVERED CURRENT AFTER EYEBLINK CONDITIONING, EXTINCTION, AND PRESENTATION OF US ALONE. J. Brons* and C.D. Woody, UCLA Med. Center, Los Angeles, CA. 90024

The excitability of 304 neurons in pericruciate cortex to extracellularly delivered nA currents was studied in awake cats after conditioning (click CS-glabella tap US); during extinction (US-CS); and during presentation of US or CS alone in untrained animals. The CR and UR each consisted of a short latency concurrent eyeblink and nose twitch. Extracellular unit activity was recorded with 15-50 MΩ, 1.4M K+ citrate filled glass microelectrodes. Up to 6 nA positive current pulses (10 msec width, 10 Hz) were passed extracellularly through the recording electrode to determine the threshold level of current required to elicit spikes on 50% of the stimulus pulses. Cortical neurons were classified as ultimately projective to eye (orbicularis oculi), nose (levator oris), both eye and nose, or neither eye nor nose musculature on the basis of averaged EMG responses to the cortical stimulation used to measure cell excitability.

Neurons which individually projected their activity to eye, nose, and both eye and nose muscles concurrently required significantly more extracellular current for spike initiation in all cats given the glabella tap US (i.e. conditioning, extinction, and US only groups) as compared with the cats given the CS only, ($F(3, 13) = 6.11$, $p < .01$). Neurons that did not project to either eye or nose muscles did not show any consistent differences in excitability among the four behavioral groups.

The results indicate that a glabella tap, which elicits a reflex eyeblink mediated by brainstem pathways, also produces decreases in excitability to weak (up to 6nA) extracellularly applied currents in neurons of sensorimotor cortex. Thus, serial presentation of a US during Pavlovian conditioning or extinction can produce non-associative alterations in cortical neural excitability as well as other associative effects. Studies of the latter have been reported elsewhere (Woody and Black-Cleworth, J. Neurophysiol., 1973; Brons et al., Fed. Proc., 1978). Different cellular mechanisms appear to support alterations in excitability to weak nA extracellular current than support altered excitability to intracellularly injected current (Woody et al., J. Neurophysiol. 1976; Woody and Gruen, Soc. Neurosci. Abstr., 1977). Supp. by BNS76-06886.

791 ENHANCEMENT OF THE LOCAL INHIBITORY EFFECT OF DOPAMINE ON A MOTOR CONDITIONED RESPONSE IN CATS TREATED WITH MICROINJECTION OF 6-HYDROXYDOPAMINE IN THE CAUDATE NUCLEUS. H. Brust-Carmona and C. Reyes Vázquez*. Depto. de Fisiología, Div. de Investigación, Fac. de Medicina, UNAM. México 20, D.F., México.

Different observations have shown the involvement of catecholamines as inhibitory neurotransmitters in the caudate nucleus (CN). Dopamine (DA) microinjections enhance the suppression of a lever pressing motor conditioned response (SMCR). Therefore, we proposed that lesions of the dopaminergic terminals of CN would diminish the SMCR.

Cats were conditioned to press a lever (MCR) to obtain 0.5 ml of milk while a small light was on; no reinforcement was given when it was off, and the animals learned to suppress (SMCR) the MCR. After three training sessions, the cats, under pentobarbital anesthesia, received bilateral injections of 6-hydroxydopamine (6-OHDA) into the head of the CN, in doses of 5, 10, 20, 40, 80 and 160 μg. After two or three days the conditioning was started anew. MCR was not affected by any of the injections in any session. In contrast, the lower doses (5, 10, 20 μg) significatively increased ($P < 0.05$) SMCR while the 80 and 160 μg doses decreased it ($P < 0.05$). The 40 μg dose did not produce any differences from those injected with NaCl. To further evaluate the differences the lever pressing for all sessions of the animals in each group was pooled. The U-test comparing the totals showed that the differences are statistically significant at the level of $P < 0.05$. The results support the postulation that DA acts as inhibitory neurotransmitter, but they also led us to conclude that low doses produce sensitization by denervation as postulated by other authors. To test this possibility, another group of cats was conditioned similarly and afterwards bilateral cannulae were chronically implanted in the head of CN. After a recovery period the effect of 4 microinjections of NaCl or DA, applied one every other day, was tested and, afterwards, one 6-OHDA dose (20 μg) or NaCl (5 μl) was injected. Two days later a new series of microinjections of NaCl or DA was repeated. The cats injected with NaCl showed a slight increase of lever pressing in the suppression condition, while DA produce a decrement of the lever pressing. This depressor effect was more intense after the 6-OHDA application and was not observed after NaCl injection. It is important to mention that the lever pressing in the reward situation did not change with any of the treatments.

792 COMPARING NEURAL PLASTICITY IN THE HIPPOCAMPUS DURING CLASSICAL CONDITIONING OF THE RABBIT NICTITATING MEMBRANE RESPONSE TO LIGHT AND TONE. Steven R. Coates and Richard F. Thompson. Dept. Psychobiology, UCI, Irvine, CA 92717.

Unit activity was recorded from the CA1 area of dorsal hippocampus in New Zealand White rabbits (Oryctolagus cuniculus) during conditioning. The conditioning paradigm involved using a corneal air puff unconditioned stimulus (UCS) to classically condition nictitating membrane movement to the presentation of either a tone or a light conditioned stimulus (CS). Twelve rabbits were divided equally into 3 stimulus presentation groups. In the first group the stimulus presentations consisted of pairing the light with the air puff. In the second group, initially, the tone was paired with the air puff. Once conditioning had been established, the light was then paired with the air puff. The third group received random, unpaired presentations of either the light or air puff. For all 3 stimulus presentation groups, the CS was presented for 350 msec and the UCS was presented for 100 msec. For the paired presentations, the CS and UCS were overlapping and coterminating. Systematic increases in patterns of unit activity occurred during conditioning to both the light and tone CS. The plasticity associated with both types of CS was found to be similar in terms of pattern, magnitude and time course. When animals were shifted from one CS to the other, conditioned behavioral responding ceased and hippocampal activity was markedly reduced. Relearning, both in terms of behavioral responses and hippocampal unit activity, was slower than original learning.

These data demonstrate that the large increase in hippocampal unit activity in this learning paradigm is not dependent on the specific properties of the CS.

793 IMPAIRED MOTOR MEMORY AND INTACT MOTOR SKILLS ACQUISITION IN ANTEROGRADE AMNESIA. Neal Cohen and Larry R. Squire. Departments of Neurosciences and Psychiatry, UCSD, School of Medicine, La Jolla, CA 92093.

Studies of human amnesia have reported that acquisition of perceptual-motor skills is spared in patients who otherwise are deficient in committing new information to long-term memory. In normal subjects, kinesthetic (motor) memory seems to exhibit certain unique characteristics. These observations have sometimes been interpreted to mean that the organization of motor information and its neurological substrate are special in some way. The present study explored further the status of motor skill acquisition and motor memory in the amnesic syndrome.

Patients receiving bilateral electroconvulsive therapy (ECT) and the chronic amnesic patient N.A. were given the rotary pursuit task to test motor skill acquisition, and a lever-positioning task to test motor memory. Rotary pursuit required patients to maintain contact with a small target on a rotating disc. Lever-positioning required patients, in the absence of visual cues, to reproduce horizontal criterion movements (ranging from 28.5 to 71.5 cm) after a variable interval (0, 12, 60 sec).

In the rotary pursuit task, these patients exhibited a normal rate of acquisition over three weekly sessions. In the lever positioning task, the patients were able to reproduce criterion movements as well as control subjects in the 0-sec delay condition. However, patients were slightly impaired in the 12-sec delay condition and markedly impaired in the 60-sec delay condition.

These results clearly demonstrate that, in the amnesic syndrome, motor memory can be impaired despite a preserved capacity for motor skill acquisition. The finding that motor memory is impaired raises difficulty with the notion that motor information in general enjoys a special neurological status. Possible differences between motor skills acquisition and motor memory will be discussed.

794 ATROPINE INJECTIONS IN THE ANTERIOR AND POSTERIOR CAUDATE NUCLEUS: EFFECTS ON PASSIVE AVOIDANCE. Sara E. Cruz-Morales*, Flavio A. López-Miro* and Roberto A. Prado Alcalá. Physiol. Dept. Sch. Med. Ntnt. Univ. of México, México City, México.

Cholinergic blockade of the caudate nucleus (CN) produces significant deficits in learned behaviors. Further, differential impairments are found, in active avoidance, after dorsal or ventral blockade of the head of the CN.

The present results deal with the effects of atropine injections in different areas of the CN.

Rats were implanted with cannulae in the anterior or posterior CN, and later trained in a two - compartment box. During the first session, the latency to cross from one compartment to the second compartment, in which a footshock was delivered, was noted. Twenty-four hr later this procedure was repeated, except for the footshock (retention session). An unimplanted group of rats was also trained. Half of the implanted rats were injected through each cannula with 60 μg of atropine, and the other half with saline, 1 min after the first training session.

It was found that the saline groups and the group injected with atropine in the posterior CN had retention scores that were not statistically different from the unimplanted group. In contrast, the group injected with the anticholinergic drug in the anterior CN displayed a significative impairment in retention ability.

These results further support the hypotheses that: a) a cholinergic mechanism is involved, within the CN, in learned performance, and b) the caudate nucleus is not functionally homogeneous.

795 AMNESIA IN CHICKS AND MICE INDUCED BY 3,4 DEHYDRO-DL-PROLINE. Joel L. Davis and Arthur Cherkin. Psychobiology Research Laboratory, VA Hospital, Sepulveda, CA 91343.

Intraventricular injection of L-proline (L-PRO; 6.0 µmols/chick) or its analog baikiain (4,5 dehydro-L-pipecolic acid; 1.5 µmol/chick) induces retroactive amnesia without brain seizure or isoelectric activity. D-PRO is non-amnestic. We now report that 3,4 dehydro-DL-proline (DHP), an analog of PRO and baikiain, has amnestic properties. We injected chicks intracerebrally with 10 µl/hemisphere of 150 mM L-PRO or DHP, or 75 mM DHP, 1 min after one-trial training to suppress the peck response to a stainless steel bead. Avoidance was conditioned by coating the bead with an aversive liquid (methyl anthranilate) immediately prior to training. Retention of avoidance was tested 24 hr later using the uncoated bead; reduced avoidance scores and increased peck scores indicate impaired memory.

Compound	Dose/Chick (µmols)	N	Avoidance Score (%)	Peck Score ($\sqrt{p}$±S.D.)
DHP	1.5	57	47.3	1.29±1.54
DHP	3.0	49	12.2	2.48±1.35
L-PRO	3.0	39	51.3	0.93±1.15
L-PRO	6.0	304	34.5	1.59±1.49
D-PRO	6.0	296	56.1	0.77±1.15

The results demonstrate that DHP is an effective amnestic agent at a dose (3 µmols) below the amnestic dose of L-PRO (6 µmols); for DHP and L-PRO at 3.0 µmols, the avoidance scores ($p<0.001$; χ^2 test) and peck scores ($p<0.0001$; t-test) differ significantly. The amnestic effect of 1.5 µmols of DHP is significantly less than that of 3.0 µmols of DHP (peck score, $p<0.0001$) but not significantly greater than that of 3.0 µmols of L-PRO ($p>0.23$). Previous scores for 6.0 µmols of L- and D-PRO are tabulated for reference. No seizure spiking or isoelectric activity from chick ectostriata were found in EEG recordings under DHP or PRO conditions resembling the behavioral paradigm.

We injected DHP intracranially into mice 1 or 60 min after one-trial passive avoidance training. Mice (Swiss HLA-sw/ICR males, 50-65 days old) were injected through implanted cannulae with 5 µl/hemisphere of 150 mM DHP (1.5 µmols) or saline, then tested 24 hr later. Results indicate a significant amnestic effect for DHP compared to saline controls at the 1-min interval. Delaying the injection for 60 min abolished the amnestic effect, suggesting a retrograde effect.

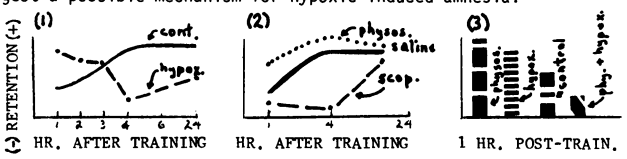
Compound	Injection Interval (min)	N	Median Latency (sec)	U	p
3,4 DHP	1	13	20.0	26.5	<0.01
Saline	1	26	158.0		
3,4 DHP	60	9	184.0	45.0	>0.05
Saline	60	10	151.0		

796 HYPOXIC-INDUCED AMNESIA IN PASSIVE AVOIDANCE LEARNING: EVIDENCE FOR A DYNAMIC CHOLINERGIC INVOLVEMENT. Reginald L. Dean* and Raymond T. Bartus, Warner-Lambert/Parke-Davis Res., Ann Arbor, MI

Memory for a brief, one-trial learning experience improves during the first few hours after training. Thus, if retention is tested too soon after training, memory is poor, but rapidly improves with time (McGaugh, Sci., 153, 1966). An amnestic treatment given soon after training also requires time before the amnesia is manifested. Thus, memory immediately after recovery from the systemic trauma shows little amnesia, but gradually worsens with time (Sara, Phys. & Behav., 13, 1974). Using a single trial, passive avoidance task, we investigated possible mechanisms for these phenomena.

The first study confirmed that mice tested within 1 hr after training showed poor retention, but improved over the next 3 hrs, reaching a peak 4 to 6 hrs after training. Exposure to hypoxia (3.5% O^2) immediately after training produced no amnesia during the first 3 hours after training. In fact, retention was paradoxically improved 1 to 2 hrs after the training/hypoxic treatment. Significant amnesia was not manifested until 4 hrs (Fig.1)

Next, we evaluated whether changes in cholinergic processes may be involved in both the gradual memory improvement and delayed amnesia. Scopolamine (1.0 m/k) given immediately after training produced temporary amnesia, mimicking the anoxic treatment 4 hrs post-training. Physostigmine (.1 m/k) given immediately after training improved retention, mimicking the paradoxical effects of the anoxic treatment 1 hr after training (Fig. 2). Finally, combined anoxia and physostigmine immediately after training (which both improved retention 1 hr after training), produced amnesia on the 1 hr retention test, implying that their combined effects, i.e.; increasing post-synaptic cholinergic levels, produced synaptic blockade (Fig. 3). These results, when considered with recent hypoxia/biochemical data (Gibson & Blass, J. Neuroch., 27, 1976; Sterling, et al., Neurobiol. of Aging, 1976), imply that a primary effect of hypoxia may involve an initial dumping of cholinergic pools (temporarily facilitating retention), followed by inadequate cholinergic synthesis (leading to amnesia within a few hours). These data therefore support the notion of an important, dynamic role of cholinergic mechanisms in new learning and suggest a possible mechanism for hypoxic-induced amnesia.



797 NOREPINEPHRINE INJECTIONS INTO AMYGDALA IMPAIR PASSIVE-AVOIDANCE LEARNING. Maureen E. Ellis 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, 3mA footshock and then tested for retention of the footshock experience 30 min or 24 hr later. Retention was evaluated as an increase in latency to approach the lick tube relative to pretreatment latency. Immediately or 12 hr after the footshock, rats were injected bilaterally via stainless steel cannulas chronically implanted into the amygdala with norepinephrine (NE, dose range: .02ßug, .05ug, .1ug, 1ug) dissolved in 1ul special buffered saline (SBS). Rats injected with 1ug NE immediately after footshock did not show retention (amnesia) when tested 24 hr later. In contrast, rats injected immediately after footshock with doses less than 1ug NE or injected 12 hr post-footshock with 1ug NE did show retention, i.e. they showed an increase in approach latency, which was similar to unoperated and SBS controls. Additionally, rats injected with 1ug NE + 1.5ug propranolol (a B-adrenergic blocking agent) or 1.5ug propranolol alone also showed retention. To test for possible proactive and toxicity effects of 1ug NE injection immediately following a footshock, rats were given a 30 min retention test and monitored for food consumption, water intake, and body weight over the subsequent 24 hr period. Rats showed retention at the 30 min test and no changes occured in food and water consumption or body weight over the 24 hr period. These results suggest that the noradrenergic system within the amygdala and possibly interconnected neural systems is involved in storage and retrieval of aversive experiences.

798 CHANGES IN DENDRITIC SPINES OF THE DENTATE MOLECULAR LAYER DURING CONDITIONING. Eva Fifková and A. Van Harreveld. Dept. Psych., Univ. Colorado, Boulder, CO 80309 and Calif. Inst. Technol., Pasadena, CA 91125.

Following a classical conditioning paradigm we have observed an enlargement of dendritic spines at the projection site of the perforant path in the dentate molecular layer (Neurosci. Abstr. 1977). Present experiments were aimed at a comparison of the effect of conditioning and pseudoconditioning on spine dimensions in the dentate molecular layer. Forty-seven mice (18 g) had twice a day 50 pellets (45 mg) available for 2 consecutive days in experimental cages. On the third day animals were at random assigned either to the conditioning, pseudoconditioning or control procedures. In a single 2-hr session, 50 trials of tone followed by a pellet were presented during conditioning and 50 trials of a random presentation of tone and pellet were given during pseudoconditioning. Controls spent 2 hrs in the training cage with 50 pellets available. Since these control mice were on a food restricting regimen similar to the experimental ones they were referred to as "starved controls." As another control served 22 mice which had food ad libitum and which were never placed into the training cage. In conditioned mice the area of dendritic spines was significantly larger in the middle (12%; $p<0.01$) and distal third (20%; $p<0.01$) of the dentate molecular layer as compared to "starved controls." Comparing the conditioned animals with controls the difference was 10% ($p<0.02$) and 17% ($p<0.01$), respectively. In pseudoconditioned mice spines in the middle and distal third were also larger but the difference relative to either of the controls did not attain the level of significance. Both controls had spines of comparable dimensions in the middle and distal third. However, spines in the proximal third of "starved controls" were significantly smaller (6%; $p<0.05$) than of the controls. Thus, it seems that conditioning induces a significant spine enlargement in the dentate molecular layer which is restricted to the projection site of the perforant path. Supported by NIMH grant MH 27240.

	n	Dentate Molecular Layer Proximal 1/3	Medial 1/3	Distal 1/3
Conditioning	12	1.249±0.055	1.372±0.031	1.643±0.048
Pseudocondi.	23	1.233±0.033	1.348±0.061	1.456±0.033
"Starved control"	12	1.110±0.042	1.224±0.024	1.360±0.046
Control	22	1.181±0.028	1.251±0.027	1.402±0.041

Mean values ± standard errors of the average spine area in arbitrary units in different parts of the dentate molecular layer.

799 INSTRUMENTAL RESPONDING BY SINGLE NEURONS UNDER DISCRIMINATIVE STIMULUS CONTROL IN CERVEAU ISOLE CATS. Ana L. Figueroa* and James J. Keene. Dept. Physiol., Sch. Med., Univ. Puerto Rico, San Juan, PR 00936.

A recent paper (Physiol. Psych., 5: 181, 1977) presented evidence of intracranial self-stimulation and escape in unanesthetized cerveau isolé rats, where the operant response was derived from the cortical EEG. We now report that similarly prepared unanesthetized, post-collicular, pretrigeminal cerveau isolé cats (N=7) are able to manipulate single cell activity as an instrumental response (IR) in an operant learning paradigm.

Trials consisted of (1) a discriminative stimulus (SD+)--a small light near the eye, and (2) a reinforcing stimulus (SR)--a 0.5 sec train of 40 Hz, 0.5 msec cathodal pulses delivered by an isolated constant current device to rewarding medial forebrain bundle sites, or in other runs, to aversive midbrain reticular sites. Trials were presented with a random variable ITI of 20 sec in blocks of 50. An IR was defined as an increased unit firing rate comparing pre-SD+ and SD+ periods in a given trial; thus, random responding produced IRs on 50% of the trials. A PDP 11/20 computer determined IR occurrence over real time.

For each unit recorded, habituation blocks (HAB) presenting the SD+ alone were run until performance stabilized around 50% for 3 consecutive blocks. Next, 4 or more acquisition blocks (ACQ), in which the SR was presented at the end of the SD+ if an IR was made, were run. A unit "acquired" an IR if there was (1) a sig. difference ($p < .05$) in % performance between HAB and ACQ blocks, and (2) a sig. change ($p < .05$) in % performance from random responding (50%). Increased and decreased % performances were considered approach and avoidance learning respectively.

Of 109 well-isolated units studied, 34 (31%) acquired IRs. The table below shows the mean (± SEM) changes in % performance from 50% for units showing approach, avoidance, and no learning.

	APPROACH (N=26)	AVOIDANCE (N=8)	NON-IR (N=75)
HAB & EXT blocks...	6.41 ± 1.80	-6.08 ± 4.50	1.67 ± 1.46
ACQ blocks.....	19.90 ± 2.13	-24.97 ± 4.19	1.01 ± 1.08

The interpretation that these 34 cells located in cortex, diencephalon, and basal ganglia showed instrumental responding is supported by the following: (1) The animal could limit IRs to the SD+ period. (2) IRs by a given cell could be acquired and extinguished (EXT) repeatedly. (3) Many cells also discriminated between the SD+ and a second light. (4) Comparing among the 3 groups above or between HAB & EXT blocks and ACQ blocks in each of the groups, there were not sig. differences in spontaneous firing rate or in post-SR unit responses. (5) The cerveau isolé prevents somatosensory feedback. We propose that the present method be used to map units involved in volitional or intentional control of learned behavior. (Supported by NIH Grant RR-8102.)

800 MEDIAL FOREBRAIN BUNDLE LESIONS AND PAVLOVIAN CONDITIONING OF CORNEORETINAL POTENTIAL AND HEART RATE CHANGES. James S. Francis, Dept. of Pharmacology, University of Houston, Houston, Texas; Linda L. Hernandez*, Donovan D. Clark*, & D. A. Powell, Neuroscience Laboratory, VA Hospital and University of S.C., Columbia, S.C.

Ten rabbits received bilateral radio frequency lesions in the far lateral hypothalamus interrupting the fibers of the medial forebrain bundle (MFB). A second group of eight rabbits served as operated sham controls with the electrode lowered into the hypothalamus but no current passed through its tip. Corneoretinal potential (CRP) and heart rate (HR) CRs were assessed in a simple Pavlovian conditioning experiment in which adaptation, acquisition, and extinction sessions were administered. A 1216 Hz, 80 db, 500 msec tone was the CS, and a 3 ma, 300 msec paraorbital electric shock was the US. At the end of conditioning training, (a) free field activity, (b) paraorbital shock thresholds and (c) HR URs were determined. The Ss were then sacrificed by decapitation and regional CNS concentrations of serotonin and norepinephrine determined in the hippocampus and cerebral cortex.

It was found that MFB lesions had no effect on the number of trials to reach a CRP acquisition criterion of 10 consecutive CRs or on an extinction criterion of 10 consecutive trials without the occurrence of a CR. Total number of CRP CRs also did not differ significantly for the two groups. However, the HR CR was accelerative in the lesioned group during both adaptation and acquisition, whereas it was decelerative in the sham-operated group. Since bradycardiac HR CRs are typically obtained in the normal rabbit, MFB lesions reversed the topography of this response, but had no effect upon the CRP response. Similarly, free field activity, shock thresholds and the HR UR were unaffected by MFB lesions. Hippocampal and cortical 5-HT and NE were significantly depleted by the lesions, but these monoamine levels were uncorrelated with the behavioral effects. This latter finding suggests that the reversal of the HR CR was due to disruption of descending MFB fibers to brain stem cardiac control centers rather than ascending cortical and/or hippocampal modulatory mechanisms.

801 DEVELOPMENT OF DISCRIMINATIVE UNIT ACTIVITY IN CINGULATE CORTEX AND ANTEROVENTRAL THALAMUS DURING CONDITIONING AND OVERTRAINING IN RABBITS. Michael Gabriel, Kent Foster*. and Edward Orona*. Dept. Psychol., Univ. Texas, Austin, TX 78712.

Multiple unit activity was recorded in the cingulate cortex (CC) and in the anteroventral nucleus of the thalamus (AV) during conditioning of a discriminative avoidance response (locomotion) in rabbits. A previous study had shown a greater unit response in CC to the positive conditioned stimulus (a tone paired with footshock) relative to the negative conditioned stimulus (a tone randomly interspersed with the positive stimulus, but never paired with footshock). The discriminative neuronal effect in CC developed at an early stage of conditioning, but discrimination in AV did not develop until the behavioral discrimination was well-learned. Here we extend these findings by examining new locations (30 in CC and eight in AV), by analyzing neuronal activity in relation to behaviorally defined stages of conditioning, and by testing the persistence of neuronal discrimination during overtraining in the conditioning task. The results replicated our previous findings in showing the first neuronal discrimination in CC during the session in which the rabbits first achieved significant behavioral discrimination. The first neuronal discrimination in AV occurred at a later stage of conditioning, during the session in which the strict criterion of behavioral discrimination was met. The early developing discrimination in CC was restricted to the deep layers (V-VI) of CC. Certain locations within the superficial layers (I-IV) of CC showed late developing neuronal discrimination that was coincident with the late developing discrimination in AV. This outcome suggests that the late developing discrimination in AV is fed back to the superficial layers of CC via the thalamocortical projection known to interconnect these structures. Finally the present study showed that once established the neuronal discriminations in CC and in AV persisted throughout six sessions (720 trials) of overtraining which occurred after the rabbits attained the strict criterion of behavioral discrimination. Thus thalamic discrimination does not replace cortical discrimination. Rather once acquired both effects persist in parallel for a considerable period of training.

802 OPIATE ADMINISTRATION INTO THE AMYGDALA: EFFECTS ON MEMORY PROCESSES. Michela Gallagher and Bruce S. Kapp, Dept. Psychol., Univ. of Vermont, Burlington, VT 05401.

The function of opioid peptides in brain regions not generally associated with pain processing remains largely undetermined. For example, opiate administration into the amygdala complex, an area possessing high concentrations of opiate receptors and opioid peptides, does not reliably alter pain sensitivity. This finding is in basic agreement with other research indicating that amygdala lesions do not alter pain sensitivity. However, opioid peptides within this region may participate in other functions for which the amygdala has been implicated. Since numerous studies have reported that manipulating amygdala activity alters memory processes, particularly for aversive experiences, this experiment examined the effects of post-training administration of opiate agents into the amygdala of rats on retention of passive avoidance conditioning.

Male Sprague-Dawley rats were surgically prepared with cannulae positioned at the dorsal surface of the amygdala complex. Intracerebral injections were administered bilaterally in a 0.5 µl volume of a Krebs Ringer phosphate solution. The opiate agents used were the agonist levorphanol, its inactive enantiomer dextrorphan, and the antagonist naloxone. Administration of levorphanol immediately after training produced dose-dependent decreases in retention measured 24 hrs. later compared to vehicle injected and unoperated control groups. The effects obtained with levorphanol were observed to be stereospecific and time-dependent; neither dextrorphan nor delayed administration of levorphanol for six hours after training exerted a significant effect on retention. Administration of the antagonist naloxone into the amygdala immediately after training significantly increased retention. This effect was likewise dose-dependent and time-dependent. In addition, combined naloxone and levorphanol administration into the amygdala immediately after training blocked the retention deficit produced by opiate agonist administration. Finally, injections of opiate agents comparable to those which significantly altered retention when injected into the amygdala did not significantly alter retention when administered into the basal ganglia 1.0 mm dorsal to the amygdala complex. These data suggest a role for amygdala opioid peptides in time-dependent memory processes.

Supported by NIH grant RO3 01577-01, and a research scientist development award KO2 MH00118 (NIMH) to B.S.K.

803 INTEROCULAR TRANSFER OF PATTERN DISCRIMINATION LEARNING AS A FUNCTION OF AGE IN THE DEVELOPING CHICK. Karen E. Gaston* (SPON: R. W. Sperry). Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Complete optic decussation in birds restricts direct visual input from each eye to the contralateral hemisphere, making interocular transfer of visual learning dependent on interhemispheric connections. An intact supraoptic decussation (DSO) is apparently necessary for transfer of pattern discrimination learning in adult birds. The extent of interocular transfer of this type of learning in chicks might be expected to vary directly with age and the functional maturity of the participating interhemispheric connections. The present experiment is the first stage of a developmental study designed to establish the degree of interocular transfer of pattern discrimination learning in newly-hatched chicks and to determine whether the extent of transfer varies with age, possibly as a result of progressive myelination of a forebrain fiber system such as the DSO.

In the first phase of the investigation, 24 3-6 day-old domestic chicks were trained monocularly to perform a simultaneous two-choice visual pattern discrimination (+,Δ) for heat reward and then were tested for interocular transfer of the discrimination learning. After each animal was shaped to peck in an operant chamber, one of its eyes was covered with an occluder and the discrimination was trained to a criterion of 36/40 correct responses. The discrimination was readily learned, with nearly all animals reaching criterion in less than 300 trials. Immediately following the acquisition session the chicks were retrained to criterion on the discrimination problem with either the same eye (monocular retention, n=12) or the other eye (interocular transfer, n=12). The chicks retested with the trained eye demonstrated excellent retention of the discrimination task. In marked contrast, animals tested for interocular transfer showed no significant savings. Initial performance (errors in the first 40 trials) and total trials and errors to criterion with the second eye were not appreciably different from those of the earlier acquisition session with the first eye. These results indicate that, under the present experimental conditions, pattern discrimination learning fails to transfer from the trained to the untrained eye in very young chicks, in contrast to the findings in adult birds and to the good transfer reported for one-trial passive avoidance and for an object discrimination in newly-hatched chicks. Progressively older chicks are presently being tested on the pattern discrimination in order to determine whether there is a positive correlation between behavioral transfer and the maturation of interhemispheric connections.

Supported by USPHS Grant # MH-03372.

804 EEG DEPRESSION IS NOT ESSENTIAL FOR L-PROLINE INDUCED RETROGRADE AMNESIA. L. K. Gerbrandt* and J. L. Davis. Dept. Psychol., CSUN, Northridge, CA 91324 and Psychobiology Research Laboratory, VA Hospital, Sepulveda, CA 91343.

Previous experiments (Gerbrandt et al., 1977) have shown EEG and multiple unit depression within 20 sec (rapid onset and lasting 20 sec) under amnestic levels of L-proline (L-PRO). Since activity like spreading depression (S.D.) has been shown to cause amnesia (Buresova and Bures, 1969), the question is raised whether amnesia produced by L-PRO includes a S.D. induced component. The objective of these experiments is to compare electrophysiological and amnestic effects of KCl and L-PRO at doses of KCl sufficient to produce EEG depression similar to those observed after L-PRO injections. Chicks (N=6), 44±12 hrs old, were implanted with bilateral electrodes in the ectostriata. The following day, after 15-min adaptation and baseline period, chicks were trained with methyl anthranilate (MeA) to mimic interactions of training and injection in the behavioral paradigm and then injected intracerebrally with 10 µl/hemisphere of 300 mM L-PRO (N=6), or 250 mM KCl (N=6). EEG was recorded for 11 min after injection. The 250 mM concentration of KCl was chosen because preliminary experiments indicated it would yield a reliable EEG depression comparable to that by 300 mM L-PRO. None of the injections produced isoelectricity or seizure spiking. Comparable durations and amounts of depression were found for KCl and L-PRO.

To test whether 250 mM KCl induces a S.D. related amnesia, we injected chicks intracerebrally with 10 µl/hemisphere of 300 mM L-PRO (N=90), 300 mM D-PRO (N=85), or 250 mM KCl (N=87) 1 min after a 10 sec one-trial training of the chicks to suppress their "innate" peck response to a small, shiny bead. Peck suppression, with avoidance of the normally attractive bead, was conditioned by coating the bead with an aversant (MeA). Retention of the avoidance response was measured 24 hr later using the dry, uncoated bead; increasing pecking indicated impaired memory retention. Results confirmed the amnestic effects of L-PRO relative to D-PRO ($p<.0001$), and to KCl ($p<.005$). KCl did not differ significantly in its amnestic effects from D-PRO ($p>.10$).

These results indicate L-PRO-induced amnesia cannot be duplicated by another agent which produces a comparable EEG depression. Therefore, these data support the Van Harreveld - Fifkova theory that L-PRO-induced amnesia is dependent upon blocking the effects of glutamate release and is not merely the result of EEG depression independent of a glutamate mechanism.

805 RIGHT HEMISPHERE SUPERIORITY IN THE PERCEPTION OF FACIAL EMOTION. Edward C. Hansch*, Francis J. Pirozzolo, Jackson Beatty*. Department of Psychology, University of California, Los Angeles, California 90024.

A special role for the right hemisphere in the processing of affective stimuli has been suggested recently in the ability to respond to emotionally-laden questions (Schwartz, 1975) and in the perception of nonverbal human emotional voices (Carmen & Nachison, 1973). This work corresponds with clinical neurological evidence for a diminished capacity to respond to emotionally-charged stimuli in patients with right hemisphere brain damage (Wechsler, 1972). While facial recognition has been reliably demonstrated as a right hemisphere specialized skill (Pirozzolo & Rayner, 1977), the laterality of facial emotion perception has not been studied.

The present experiment examined hemispheric differences in the perception of both emotional and non-emotional stimuli, utilizing a forced-choice reaction time procedure. Four types of trials were employed: facial emotion, emotional word, facial recognition, and neutral word recognition. Word and facial stimuli occurred interspersed so that laterality effects could not be attributed to an "attentional bias".

Subjects were required to decide whether an orally-presented cue matched a fifty msec exposure of a lateralized stimulus. Left visual field presentation of facial emotion trials yielded significantly shorter latencies than did those for the right visual field. Latencies for response to word stimuli were shorter in the right visual field than in the left.

These results strongly support the notion of right hemisphere specialization for the perception of facial emotion, and cast doubt on the alternative hypothesis that a constant attentional bias to a given visual field mediates laterality effects. This evidence would also seem to support the clinical observations that patients with right hemisphere disease have difficulty discriminating and comprehending affectively-charged stimuli (Heilman, Scholes & Watson, 1975; Tucker, Watson & Heilman, 1977).

806 NEURAL ACTIVITY RECORDED IN THE ABDUCENS AND OCULOMOTOR NUCLEI DURING NICTITATING MEMBRANE CONDITIONING IN THE RABBIT. T.A. Harrison*, C.F. Cegavske (Dept. Psychology, SUNY, Binghamton, NY 13901) and R.F. Thompson (Dept. Psychobiology, UCI, Irvine, CA 92717).

This experiment was designed to characterize the neural activity in the motor output systems controlling movement of the rabbit nictitating membrane (NM) during acquisition and extinction of the classically conditioned NM response to tone CS and airpuff UCS. Multiple unit activity (MUA) was recorded in each animal during conditioning from electrodes chronically implanted in 1) the ipsilateral abducens (VI) nucleus, and 2) the dorsocaudal portion of either the ipsilateral or the contralateral oculomotor (III) nucleus. These areas have been determined to control NM extension (indirectly, via activation of the retractor bulbi muscle of the orbit), and, potentially, NM retraction (via a slip of the levator palpebrae superioris muscle attached to the NM), respectively (Cegavske, Thompson, Patterson & Gormezano, JCPP, 1976, 90, 411-423; Harrison, Torigoe & Cegavske, in preparation).

The conditioning procedure consisted of 13 blocks of trials (one block contained one CS-alone trial followed by 8 CS-UCS paired trials) per day for two days or until the rabbit reached criterion of 100% responding in one block of trials. Extinction consisted of 104 CS-alone presentations per day for two days. Data were recorded on audio tape for later analysis by computer. Histograms of the number of cells firing per 3 msec time bin were constructed for each trial for comparison with NM magnitude and latency data.

The patterns of increases and decreases of MUA in the oculomotor and abducens nuclei in relation to the NM response were examined. Comparisons of the abducens MUA and the NM response confirmed previous work (Cegavske, Patterson & Thompson, submitted). Abducens MUA was highly correlated in time and magnitude with the NM response over the course of extension and retraction of the NM. Increases in MUA consistently began before the onset of NM extension, and decreased prior to full retraction of the NM. Increases in oculomotor MUA began after abducens MUA had already increased. The relationship between the NM response onset and oculomotor activity depended upon the topography of the response. The temporal relationships of the behavioral response and the MUA recorded from both areas were maintained over the course of acquisition and exinction, whether the response was a UCR or a CR. It is concluded that changes in patterns of MUA recorded in these motor nuclei during conditioning reflect performance of the NM response, and not other aspects of conditioning.

807 FOUR MEMORY CHANNELS FOR SHUTTLE-BOX LEARNING IN THE RAT. Ivan Izquierdo, Deusa A. Vendite*, and Elaine Elisabetsky*. Dept. Biochem., Inst. Biosciences, UFRGS, 90000-Porto Alegre, Brazil.

Rats were trained to make shuttle responses to a 5-sec buzzer using 1.5 mA footshocks as a reinforcement in two different modes: a) classical conditioning (DP), in which the two stimuli were paired (i.e., given contiguously) on every trial, regardless of whether the animals made shuttle responses to the buzzer or not: b) avoidance without stimulus pairing (DC), in which the buzzer-shock interval was varied at random between 5 and 35 sec on every trial and the shocks were contingent upon omission of a shuttle response to the preceding buzzer. With both procedures, training sessions consisted of 50 trials at 10-40 sec intertrial intervals. One or seven days after training the animals were submitted to a retest session using either the same paradigm in which they had been trained, or the other one. Thus, it was investigated whether information acquired through each mode was available for retrieval only in that mode or in the other one as well, and existence of the following four possible memory channels could be tested: DP-DP, DC-DC, DP-DC, and DC-DP. In untreated animals, only the first three channels were manifest. The DP-DP channel was already in operation on day 1, and suffered no build-up or decay from then to day 7. The DC-DC channel declined between day 1 and day 7. The DP-DC channel became manifest only on day 7. Finally, animals trained in DC made no more responses in DP retests than naive animals submitted to DP for the first time. The effect of an ip. injection of d-amphetamine sulfate (1 mg/Kg), metrazol(10 mg/Kg), or nicotine (0.2 mg/Kg), given immediately after the end of the training session, was investigated. The DP-DP channel was insensitive to any of the treatments. Operation of the DC-DC channel was enhanced by amphetamine and by metrazol, but not by nicotine. Operation of the DP-DC channel was enhanced by metrazol, but not by any of the other two drugs. Finally, nicotine "unclogged" the normally "dormant" DC-DP channel. The effect on the four channels of immediate post-training hippocampal spreading depression was also investigated. This was produced by applying KCl crystals onto this structure through implanted cannulae, and was monitored electrographically. Control rats had the cannulae but received no KCl. Hippocampal spreading depression in the treated group lasted for 1-3 hs. It had no effect upon operation of the DP-DP or DC-DC channels, but it enhanced that of the other two channels(DC-DP and DP-DC). These data do not support any of the hypotheses that integrity of hippocampal function is in any way essential for memory consolidation, and suggest instead a role for this structure in the processing and sampling out of recently acquired information.

808 MORPHINE AND NALOXONE ALTER MEMORY IN THE RAT. Robert A. Jensen, Joe L. Martinez, Jr., Rita B. Messing, Vina Spiehler*, Beatriz J. Vasquez, Bernard Soumireu-Mourat*, K.C. Liang*, and James L. McGaugh. Department of Psychobiology, School of Biological Sciences, University of California, Irvine, CA., 92717, U.S.A.

The effects on memory storage of peripherally administered naloxone (Nx) and morphine (Mor) were studied. Male F-344 rats (90 days old) were trained in a one-trial inhibitory avoidance step-through task with either a 500 or 750 μA, 0.5 sec footshock. Immediately after training, the rats received an i.p. injection of either Mor or Nx. Nx was administered in divided doses, 30 min apart, of Sal, 0.1, 1.0, or 10.0 mg/kg, while Mor was given in a single dose of Sal, 1.0, 3.0, 10.0, or 30.0 mg/kg. Nx facilitated retention measured 24 hrs later. Rats that received 1.0 mg/kg Nx showed significant facilitation of retention compared to controls with either a 500 μA (χ^2=10.3, df=1, p<.01) or a 750 μA footshock (χ^2=7.02, df=1, p<.01). Rats that received Mor, trained using a 750 μA shock, showed significant amnesia with both the 1.0 mg/kg dose (χ^2=6.01, df=1, p<.02) and the 3.0 mg/kg dose (χ^2=6.72, df=1, p<.01).

To test the receptor specificity of the effects, the capacity of Mor to attenuate the facilitatory effect of Nx was examined. Rats were trained as before with a 500 μA footshock, and were given divided doses of either Sal, Nx (1.0 mg/kg), or a mixture containing both Mor (30.0 mg/kg) and Nx (1.0 mg/kg). All rats received 2 injections 30 min apart. Rats that received Nx showed significant facilitation of retention (χ^2=4.2, df=1, p<.05). However, Mor blocked the facilitatory effect of Nx since animals that reveived the mixture differed significantly from the Nx alone group (χ^2=6.04, df=1, p<.02), but not from the controls (χ^2=2.27, df=1, p>.10).

The effect of intraventricular administration of Mor was then studied. Indwelling cannulas were stereotaxically implanted above the lateral ventricle and, after a 1-week recovery period, rats were trained and given an intraventricular injection (2.0 μl) of either Ringer's solution or Mor (0.3, 3.0, 20.0, or 40.0 μg). The 40 μg dose of Mor produced significant facilitation of retention (U=166.5, z=2.14, p<.05), while the 3.0 μg treatment caused significant amnesia (U=116, z=2.38, p<.02).

These data indicate that posttraining administration of Nx facilitates memory, while Mor has a biphasic effect. The findings that Mor attenuates the Nx-induced facilitation of memory, and that intraventricular administration of Mor also affects memory suggest that the effects are mediated by central opiate receptors having a memory modulatory function. (Supported by USPHS grants MH 12526 and AG 00469 (J.L.McG.), Postdoctoral Fellowship MH 05358 (R.A.J.), and a grant from the McKnight Foundation (J.L.McG.).

809 THE CENTRAL NUCLEUS OF THE AMYGDALA: BULBAR PROJECTION AND INVOLVEMENT IN HEART RATE CONDITIONING. Bruce S. Kapp, Robert C. Frysinger*, and Michela Gallagher, Dept. of Psychology, and James S. Schwaber, Dept. of Anatomy, University of Vermont, Burlington, Vermont 05401.

As a part of an effort to assess the involvement of specific amygdala systems in the development of heart rate conditioning, bilateral lesions were made in the dorsomedial amygdala of eight New Zealand rabbits producing damage confined to the central nucleus and proximal fibers of passage. Two weeks following surgery the subjects received 15 presentations of the CS alone (1000Hz, 92dB, 5 sec tone) followed by 45 paired CS-US conditioning trials. The US was a 0.5 sec 2.0 mA eyelid shock coincident with the offset of the CS. The lesioned animals were significantly impaired in the development of conditioned bradycardia compared to unoperated and operated control animals (p's < .02).

Since the conditioned bradycardia response is mediated by the vagus nerves in the rabbit (Fredericks et al., 1974), the possibility was examined that a direct projection exists from the amygdala to the dorsomedial medulla, a region subserving cardiovascular reflexes and containing cells of origin of vagal cardioinhibitory fibers in this species. Bilateral and unilateral injections of horseradish peroxidase (HRP; 0.05-0.2μl, 30%) were placed in nine rabbits at the border of the medial nucleus of the nucleus of the solitary tract and the dorsal motor nucleus, both at the level of the obex and 750 micra rostral to the obex. After 24-48h the medulla and the amygdala were sectioned at 40 micra and processed using benzidine as a chromogen. HRP-labelled amygdala neurons were found to be restricted to the central nucleus, largely within the dorsomedial quadrant and in the rostral half of the central nucleus. We are now using autoradiographic and anterograde HRP techniques to determine more precisely the projections of these neurons.

Although the functional significance of this amygdala-bulbar projection is at present a matter for speculation, it is noteworthy that (1) the origin of this projection is in an area which when lesioned results in deficits in the acquisition of conditioned bradycardia, and (2) a terminal zone of this projection appears to be within a region which subserves cardiovascular reflexes and contains cells of origin of cardioinhibitory fibers.

Supported by NIH Grant KO2 MH00118 and by a UVM Research Development Award.

810 ETHANOL-INDUCED FACILITATION OF INHIBITORY AVOIDANCE PERFORMANCE IN MICE. R. D. Malcolm, K. E. Lovett* and R. L. Alkana, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

It has been suggested that consolidation decrements may underly ethanol-induced memory impairment. However, recent findings in mice using post-training ethanol injections do not support this hypothesis. To further explore the effects of ethanol on memory storage, ethanol was administered to C57 Bl/6j male mice immediately after training on a "step-through", one-trial inhibitory avoidance task at a 150 μA shock level. The ethanol was given i.p. at doses of 0.75, 3.0 and 4.5 g/kg (15% w/v). A 0 g/kg control group received the 4.5 g/kg volume of normal saline. Retention was tested in the non-drug state one week after training. Latencies to step-through were recorded to a maximum of 360 sec. In this experiment, immediate post-training injection of ethanol significantly improved retention latencies at 3.0 g/kg (median - 287 sec) and 4.5 g/kg (median - 204 sec) when compared to controls (median - 77 sec) (p<0.05; 2-tailed Mann Whitney U-test). One control and 10 experimental animals reached the 360 sec ceiling. There was a non-significant trend toward increased retention at the 0.75 g/kg dose (median - 141 sec)(p<0.1). Immediate post-training injections of 4.5 g/kg ethanol in the absence of foot shock did not significantly change latencies when compared to saline controls, suggesting that the ethanol injection was not aversive. In contrast to post-training treatment, injection of ethanol 10 minutes before training significantly impaired retention. The present findings do not support the consolidation hypothesis of ethanol's acute amnestic effects. These findings agree with earlier studies suggesting that post-training administration of ethanol enhances memory storage. Further studies are necessary to replicate and extend these preliminary findings.

811 INTRAVASCULAR ERYTHROCYTE AGGREGATION AND SHORT-TERM MEMORY IN RATS AND RHESUS MONKEYS: AN EVALUATION OF THE ROLE OF BLOOD SLUDGING IN AGE-RELATED, COGNITIVE DECLINES. John G. Marriott, Raymond T. Bartus and Ronald E. Voigtman*. Pharm. Dept., Warner Lambert/Parke-Davis Research Labs, Ann Arbor, MI 48105.

Several studies tested the hypothesis that an increase in erythrocyte aggregation or blood sludging, concomitant with the aging process, is responsible for the impairment of short-term memory (STM) commonly found in aged humans. Clinical reports of the therapeutic benefits of long-term anticoagulant treatment in senile patients prompted the current investigations into the role of blood sludging in memory impairments. First, blood sludging was induced in male, hooded rats by intravenous injection of high molecular weight dextran (mean mol. wt.=370,000). Analysis of blood sludging produced by dextran as measured by erythrocyte sedimentation rate (ESR) showed profound sludging after doses of 300 m/k, which persisted for several days. In contrast to the effects on ESR, a second study showed that Dextran 500 did not affect the accuracy of STM in rats working on a delayed alternation task. Discrete trial, operant procedures were used in this study which required the animal to remember from trial to trial the position chosen on the previous trial and to respond to the opposite (alternate) side. Accuracy of delayed alternation performance, used as a measure of STM, was not affected by dextran administration. The behavioral effects of dextran under these procedures were limited to a general, dose-dependent suppression of responding. Animals completed 52% fewer trials during the session following administration of 300 m/k of dextran as compared to control performance.

To test further the hypothesis of sludging-induced STM impairments in aging, the degree of blood sludging in behaviorally impaired, aged rhesus monkeys was compared with that of normal, young monkeys. In previous studies using automated, delayed response procedures, this colony of aged monkeys was shown to exhibit significant impairment of STM similar to that reported in human, geriatric patients. Complete separation in delayed-response performance between groups of young and old monkeys was found in these earlier studies. In contrast, the comparison of young and old monkey blood showed almost total overlap in degree of blood sludging, and therefore, no significant difference between the two age groups.

Thus, blood sludging was not associated with STM impairments in rats administered IV dextran or in aged monkeys. These results do not support the notion that cognitive declines in aging, specifically in STM, are due to the effects of gross changes in blood sludging. The clinical reports of salutary effects resulting from long-term, anticoagulant therapy are likely due to actions of such drugs other than their anti-sludging properties.

812 PERIPHERAL 6-HYDROXYDOPAMINE (6-OHDA) ALTERS THE FACILITATORY EFFECT OF AMPHETAMINE ON MEMORY. Joe L. Martinez, Jr., Robert A. Jensen, Rita B. Messing, Beatriz J. Vasquez, Bernard Soumireu-Mourat*, Debora Geddes*, K. C. Liang*, and James L. McGaugh. Department of Psychobiology, School of Biological Sciences, University of California, Irvine, CA 92717, U.S.A.

We investigated, (1) the effects of peripherally administered 6-OHDA on acquisition of an inhibitory avoidance task, and (2) the effects of d-amphetamine sulphate (AMPH) on learning and retention in rats sympathectomized with 6-OHDA.

Male ARS Sprague-Dawley rats (90 days old) were trained in a one-trial inhibitory avoidance task. Rats in several groups received either SAL, 5.0, 25.0, or 100.0 mg/kg 6-OHDA, intravenously, and were then trained 24 hours later with either a 500 or 750 μA, 1 sec footshock. A retention test was given 72 hours later using a 600 sec cutoff latency. Post-mortem assay of norepinephrine (NE) concentrations in heart tissue showed that 5.0 mg/kg 6-OHDA reduced NE concentrations to about 25% of control; 25.0 mg/kg to 13%; and 100 mg/kg to 7% of control. There was no significant correlations between NE depletion of individual animals and their retention scores. Only the 5.0 mg/kg dose of 6-OHDA at 500 μA produced a significant retention deficit (U= 155.5, p<.05). Performance on the training trial was not altered by 6-OHDA since entrance latencies of rats given either SAL, 5.0, or 100.0 mg/kg 6-OHDA did not differ [$F(2,47)$= 2.38, p>.05].

In the next experiment, either SAL, 5.0, or 100.0 mg/kg 6-OHDA was administered to animals as before, and they were trained using a 500 μA footshock. Immediately following training rats received either no injection, SAL, 0.25, 1.0, or 4.0 mg/kg AMPH, i.p. A 4.0 mg/kg dose of AMPH given to rats not receiving 6-OHDA significantly facilitated retention performance [$t(28)$= 2.42, p<.05]. For rats that received 6-OHDA before training, the facilitatory dose of AMPH was decreased to 0.25 mg/kg [5.0 mg/kg 6-OHDA, $t(36)$= 2.36, p<.05; 100.0 mg/kg 6-OHDA, $t(23)$= 2.13, p<.05].

These findings are evidence that denervation of peripheral presynaptic terminals alters the facilitatory effect of amphetamine. Moreover, the results point to the importance of peripheral systems in learning and memory and suggest that part of the action of amphetamine may be peripherally mediated.

[Supported by UPHS grants MH 12526, AG 00469 (JLMcG); Postdoctoral Fellowships MH 05429 (JLM Jr), MH 05358 (RAJ); BNS 76-17370 (JLMcG); and the McKnight Foundation (JLMcG)].

813 ELECTRICAL STIMULATION OF THE ABDUCENS NUCLEUS IN CLASSICAL CONDITIONING OF THE RABBIT'S NICTITATING MEMBRANE RESPONSE. Frederick W. Mis, f. Gormezano*, David Rosewall*, and John A. Harvey, Dept. of Psych., Univ. of Iowa, Iowa City, Iowa, 52242.

Extension of the rabbit's nictitating membrane (NM) is reliably elicited by applying electric shock to the paraorbital region (unconditioned stimulus, UCS). Movement of the NM is a passive response accompanying retraction of the eyeball into the socket. Neural control of this unconditioned response (UCR) originates within the motor neurons of the abducens nucleus. The present studies examined the ability of direct electrical stimulation of the abducens nucleus to serve as either a CS or UCS in classical conditioning of the NM response. The first experiment examined for 7 rabbits, in a within subjects design, the relationship between a variety of stimulation parameters and UCR amplitude. Our findings indicate that UCR amplitude was a function of the total energy applied via the electrode and an inverse function of the linear distance from the abducens nucleus to the electrode tip.

In the second experiment, we assessed the efficacy of abducens nucleus stimulation as the UCS. The conditioning paradigm consisted of a 200 msec tone CS followed by a 40 msec train of brain stimulation. Of the 26 rabbits which continued to manifest a UCR throughout the 20 training sessions, 16 developed substantial levels of conditioned responding (at least 50% CRs during a session). Analysis of UCR amplitude further suggested that there was an inverse linear relationship between UCR amplitude and distance from the abducens nucleus.

In the third experiment we sought to determine if stimulation applied to the abducens nucleus could effectively serve as a CS. The conditioning paradigm consisted of an 80 msec train of brain stimulation (CS) followed 120 msec later by a peripheral 50-msec paraorbital shock (UCS). Five of the six rabbits in this experiment reached asymptotic levels of conditioned responding (90% CRs) with 240 conditioning trials. Histological examination revealed that the tips of the electrodes were within 1.2 mm of the abducens nucleus.

Our findings suggest that the neural tissue necessary for integration of the sensory information related to the manifestation of a CR is located in the vicinity of the abducens nucleus.

Supported by Grants NSF BNS 76-84561 and NIMH 2 RO1 MH16841-10.

814 ROLE OF SUPRAOPTIC DECUSSATION IN INTEROCULAR TRANSFER IN CHICKS: AGE DEPENDENCE? Nancy A. O'Connell* (SPON: R.W. Doty). Center for Brain Research, University of Rochester, Rochester, N.Y.14642

The supraoptic decussation (DSO) has been shown to be responsible for interocular transfer in birds. Meier (Psychol. Forsch. 34: 220, 1971) found that section of at least 75% of the DSO prior to training severely impaired interocular transfer of both color and pattern discrimination in pigeons. However, it was not known whether DSO actually produces a memory trace in the non-exposed hemisphere or merely provides it with access to an engram which remains in the exposed hemisphere. To test this, chicks were raised for 2 weeks monocularly in a cage where red and green colored feed was continuously available. For each chick one or the other color of feed was made aversive by adding methyl anthranilate. The DSO was then cut and eye cover reversed. Interocular transfer was considered to be present if the chick, when hungry, pecked at the previously preferred color of feed at least twice as often (and > 100 pecks) as it pecked at the other when tested for a minimum of 5 min in the presence of unadulterated feed of both colors. Of 15 chicks with > 90% of DSO sectioned, 13 showed interocular transfer (p < .01), and 5 others with >75% of DSO sectioned also showed interocular transfer. This suggests that memory was stored bilaterally, since the supraoptic decussation was unavailable for readout during testing. The possibility remained that the DSO might be unnecessary for transfer of this preference. Thus, the DSO was transected 1-2 days after hatching. These chicks were trained monocularly for 2 weeks, then tested. In this case 12 of 14 chicks with section of > 75% DSO still showed transfer. This seems to conflict with the results of Meier, but it is conceivable that newly hatched chicks can compensate for lack of an intact DSO while adult pigeons cannot. To see if newly hatched chicks differ from slightly older ones, and to have a better age-matched control for the original operated group, a third experiment was done. DSO was cut in 16-day old chicks which were then trained monocularly for 2 weeks and tested for retention and transfer. All 5 unoperated control chicks showed transfer and retention, as did all 5 chicks with anterior and pallial commissures cut but with DSO intact. All 5 chicks with > 75% of DSO cut learned color preference with the trained eye, but this preference did not transfer to the untrained eye. Thus, although the experiments are continuing, it is tentatively concluded that a bilateral engram is produced when DSO is intact, and that DSO is necessary for interocular transfer of color discrimination if the chick is raised with it intact, but that the critical role in interocular transfer is assumed by some other system if the DSO is absent during early visual experience. (Supported by USPHS Grant NS 03606 from NINCDS and NIMH fellowship MH 05257).

815 PRENATAL Co^{60} IRRADIATION EFFECTS ON GROWTH, BEHAVIOR, AND PERSISTENCE OF IMPAIRMENT IN SQUIRREL MONKEY OFFSPRING. J.M. Ordy, K.R. Brizzee, T. Beavers, P. Medart*, Tulane Univ., Delta Primate Center, Covington, LA 70433

Abnormalities of the brain are some of the more frequently cited postnatal manifestations in offspring exposed to irradiation during pregnancy. Until recently, most research on prenatal hazards, including radiation effects on the brain, has been done with rodents. High-dose effects that produce gross brain malformations are readily demonstrable in most animal models. Consequently, increased interest has been directed toward the squirrel monkey as a "diurnal primate model" for studying more subtle low-dose effects on impaired growth rates or developmental delay, sensory, learning, motor capacity, and the persistence of early damage into maturity. The specific aims of this study were to examine the effects of prenatal 0, 10, 100 and 200 rads of Co^{60} radiation on: 1) differences in postnatal growth rates between control and irradiation offspring to differentiate transient developmental delay from permanent abnormal development, 2) development of reflexes and neuromuscular coordination from birth to maturity, 3) sensory, learning, motor capacity, eye-hand lateralization, light-dark activity, emotionality, exploratory behavior and social development. Depending upon dose, significant decreases in body weight, head-circumference and crown-rump length were observed between control and irradiated offspring at birth. Comparisons of postnatal growth rates indicated developmental delay during the first six months after birth in the 100R offspring. According to the neurological evaluations, the postnatal development of reflexes and the maturation of neuromuscular coordination was significantly slower and less coordinated in the irradiated offspring from birth to two months of age. Low dose main effects on sensory-learning-motor capacity, eye-hand lateralization, emotionality, exploration, light-dark activity and social development were confounded with age, sex, and phenotype. Also, no significant differences have been observed between control and 100R irradiated offspring in sensory-learning-motor capacity from 1 to 2 years after birth. However, significant differences have been observed in visual discrimination reversal learning between control and 100R but not 10R irradiated offspring 2 years after birth. Followup studies are in progress to establish low dose 10R threshold effects and to determine if the prominent early effects of 100R and 200R persist into maturity. (Supported by grants NIH HD09942 and NIH RR00164-16).

816 HIPPOCAMPAL AND CORTICAL CONTROL OF PAVLOVIAN CONDITIONED AUTONOMIC AND SOMATIC RESPONSES. D. A. Powell, Shirley Buchanan,* Linda Hernandez,* Neuroscience Laboratory, VA Hospital and University of S. C., Columbia, SC 29201 and James Francis, Department of Pharmacology, University of Houston, Texas 77004.

Four experiments were conducted in which the effects of hippocampal, cortical control, and sham operate lesions were studied upon differential classical conditioning of corneoretinal potential (CRP) and heart rate (HR) changes in rabbits. Tones of different frequencies were CSs and paraorbital electric shock was the US. In the first experiment the CRP discrimination was slightly impaired during acquisition but severely impaired during reversal training. HR CRs were more accelerative in both hippocampectomized and cortical lesioned animals compared to sham lesioned Ss during both acquisition and reversal training. In addition hippocampal lesioned Ss revealed accelerative HR changes during both adaptation and extinction sessions relative to cortical and sham control Ss. However, an earlier experiment, which studied simple CRP and HR classical conditioning in hippocampal lesioned Ss, revealed enhanced bradycardiac HR CRs in the hippocampectomized Ss relative to control Ss. Subsequently experiments in the present series of investigations revealed that damage to the posteroventral hippocampus and/or damage to the cingulate cortex produced accelerative HR CRs, whereas damage restricted to the dorsal hippocampus produced enhanced decelerative HR CRs relative to control Ss. These results suggest a possible differential role of the dorsal and ventral hippocampus in modulating cardiac changes, possibly related to attentional dysfunctions. These data also implicate the cingulate cortex in mediating the cardiac inhibition accompanying Pavlovian conditioning.

817 ASYMMETRICAL STATE DEPENDENT LEARNING PRODUCED BY BENACTYZINE AND PHYSOSTIGMINE IN RATS. D. L. RICKETT* (SPON: T.-M. Shih). U. S. Army Biomedical Laboratory, CSL, APG, MD 21010.

In two separate studies using rats, 6.0 mg/kg of benactyzine or 0.6 mg/kg of physostigmine were tested for state dependent properties in a one-trial, step-through passive avoidance task. Both of the studies used the 2 x 2 factorial design with isotonic saline serving as the control condition (N state) for both drugs (D state). Twenty minutes prior to training or testing, each rat received an i.p. injection of the appropriate drug or an equivalent volume of saline. Neither benactyzine nor physostigmine had any effect on performance per se, as evidenced by comparisons of the groups trained and tested in the D state with groups trained and tested in the N state. Both benactyzine and physostigmine produced asymmetrical dissociation from the D to the N states; rats trained in the N state and tested in the D state were no different from those trained and tested in the same state, either N or D. These results are taken to support the notion that pertubation of the absolute level of cholinergic activity, regardless of direction, is more critical to retrieval of information during training than during testing. Furthermore, the pertubation in itself does not disrupt information storage and retrieval mechanisms when those changes which existed during training are redintegrated during testing.

818 ASSEMBLIES OF NEURONS IN BRAIN FUNCTION AND MEMORY--THEORY AND EXPERIMENT. Kathleen J. Roney and Gordon L. Shaw. Physics Department, University of California, Irvine, California 92717.

We discuss the possible identification of the often hypothesized assembly with the clusters of neurons that have been firmly identified neuroanatomically. Assemblies of neurons are important to brain function in that they could: maintain reverberatory firing activity for periods of the order of a second, provide stability against local brain damage, provide statistical reliability to insure a reproducible response to one stimulus presentation, and insure a reasonable memory storage capacity. This last property issues from a theory of memory developed by Shaw and Little (Shaw, Brain Res. Bull. 3:107, 1978). We note that we cannot determine the size of an assembly from the properties above; the actual dimensions must be determined electrophysiologically. From the theory, however, we envision a network of 10^3 to 10^4 highly interconnected neurons, corresponding to a cortical column (present throughout the cortex; Goldman and Nauta, Brain Res. 122:393, 1977), divided into assemblies consisting of roughly 20 neurons (the number of stimulus presentations needed to determine a reproducible post stimulus histogram (PSH)). In response to a stimulus, the network (column) will be excited into one of many different sequences of (averaged) firing patterns of the assemblies. Columns then interact, either directly or through thalamocortical pathways. The theoretical assemblies may be the "clusters" or "bundles" of apical dendrites of pyramidal cells found in the somatosensory cortex of the cat, rat, and rabbit, originally referred to by Peters and Walsh (J. Comp. Neurol. 144:253, 1972) and Fleischhauer, et al. (Anat. Entwicklungsgesch. 136:213, 1972). These clusters appear to be a fairly general feature of the mammalian brain, having been found in spinal cord, thalamus, formatio reticularis and cerebral cortex (Scheibel, et al., Exp. Neurol. 42:307, 1974). The clusters are smaller than columns and consistent with the size of our assemblies. Peters and Walsh consider it likely that the clustered dendrites have a common afferent input, consistent with the averaging of action potentials done within the theoretical assembly. It is also possible that dendrodendritic gap junctions between neurons in a cluster could serve as a basis for averaging within an assembly. Whether the clusters are electrically functional or merely developmental units needs to be determined. We propose and discuss the details of several possible experiments involving both electrophysiological and neuroanatomical measurements to determine whether these clusters are our assemblies. Though difficult, the experiments are doable; the large importance of identifying clusters as electrophysiologically functional assemblies makes the effort worthwhile.

819 BRAIN METABOLISM AND THE ACQUISITION OF NEW BEHAVIORS: EVIDENCE FOR SECRETION OF TWO PROTEINS INTO THE BRAIN EXTRACELLULAR FLUID AFTER TRAINING. Victor E. Shashoua. Mailman Research Center, McLean Hospital and Harvard Medical School, Belmont, MA 02178.

Immunochemical and double labeling experiments were used to demonstrate that at least two out of three brain cytoplasmic proteins, whose metabolism is markedly influenced by behavior, are products which are secreted into the extracellular fluid (ECF) of goldfish brain. Even after one hour of labeling, the extracts of goldfish brains with 0.32 M sucrose were found to contain highly labeled proteins. Electrophoretic analyses of the proteins, on SDS-polyacrylamide gels, indicated that an increased incorporation of [^{3}H]valine occurs for trained animals as compared to untrained controls ([^{14}C]valine) at specific protein bands migrating at 32,000 and 26,000 daltons. The proteins in the ECF gave identical precipitin bands against rabbit antisera to goldfish brain proteins whose metabolism was responsive to the acquisition of new patterns of behavior. Preliminary characterization studies of the proteins indicate that they are glycoproteins with an amino acid composition containing acidic to basic amino acid ratios of about 2/1. These results are consistent with the hypothesis that the cells which contain the proteins and whose location in the ependymal zone was determined by immunohistochemistry (L. Benowitz and V.E. Shashoua, Brain Research 136:227-242, 1977) can secrete the products into ECF. These findings also provide an explanation for our previous observations that antisera to β and γ can inhibit the formation of a long-term memory (V.E. Shashoua, Proc. Natl. Acad. Sci. USA 74:1743-1747, 1977). It is not necessary for the IgG molecules to get into cells to find their targets since the antigens are being secreted and are present in the extracellular space. It is, therefore, quite possible that the functional sites of the proteins are away from the locus of their synthesis. (This work was supported by grants from The McKnight Foundation and the National Institute of Neurological and Communicative Disorders and Stroke.)

820 **DEVELOPMENT OF LEARNING AND MEMORY IN MICE AFTER BRIEF PARADOXICAL SLEEP DEPRIVATION.** **P. Shiromani*, Baruch M. Gutwein* and William Fishbein. Psychobiology Laboratory, CCNY, New York, N.Y. 10031.**

Recently Bloch and his colleagues (Brain Res. 49: 367-369, 1973) have demonstrated that a brief augmentation of paradoxical sleep (PS) occurs immediately following two-way active avoidance learning. They have also shown that the retention of the learned response is impaired if the PS augmentation is prevented. In addition, Pearlman and Greenberg (Anim. Learn. Behav. 1: 49-51, 1973) have shown that selective PS deprivation (PSD), either via drugs or the 'water tank' procedure, for 3 hours immediately following two-way active avoidance or discrimination learning in the rat produces marked retention deficits; no amnesia is observed if PSD is delayed until 3 hours after training. Collectively these experiments seem to suggest a critical time period for memory consolidation. This period extends up to 3 hours after training and is characterized by PS or conditions compatible with the occurrence of PS.

The present series of experiments attempt to further elucidate the above findings. Several experiments are performed to assess, in mice, the effects of 3 hours PSD, via the 'water tank' procedure, on active and inhibitory avoidance learning. Results indicate no amnesia in experimentally treated mice. An attempt is then made to induce amnesia by administering ECS immediately after 3 hours PSD; this procedure also fails to produce amnesia. We conclude that in mice PS immediately after aversively motivated training is not essential for memory consolidation. Our results, however, do not subtract from the long-term effects of PS on memory that we have previously reported (Behav. Biol. 19:425-464, 1977).

821 EVIDENCE FOR THE SEQUENTIAL PARTICIPATION OF INFERIOR TEMPORAL CORTEX AND AMYGDALA IN STIMULUS-REWARD LEARNING. Brenda J. Spiegler* and Mortimer Mishkin, NIMH, Bethesda, MD 20014

On a test of one-trial learning of stimulus-reward associations, monkeys showed marked impairment after lesions of either the anterior part of inferior temporal cortex (area TE) or the amygdala (A). By contrast, they showed only mild loss after lesions of either the posterior part of inferior temporal cortex (area TEO) or the fusiform-hippocampal gyrus and hippocampus (FHH). Preoperatively, the monkeys had been trained to remember the reward value of an object on the basis of a single acquisition trial in which the object covered either a peanut reward (positive object) or an empty well (negative object). On the test trial, an average of 20 seconds later, the object was paired with a grey card (cf. Gaffan, JCPP 86: 1100, 1974). On this test trial the object was positive if it had been positive on the acquisition trial; otherwise the grey card was positive. A new object was used on every trial, and the trials were presented in sets of two: that is, separate acquisition trials for each of two objects preceded separate, randomly ordered test trials with each of those objects. Also, most sets consisted of one positive and one negative object. These procedures ensured that the animals could perform correctly only by remembering the objects' reward values and not by other strategies. On reaching the preoperative criterion, the monkeys received one of the four bilateral temporal lobe lesions (TEO, TE, A, or FHH) with the outcome as described above.

The finding of impairment after both the TE and the amygdala lesions fits the view (Jones & Mishkin, Exp. Neurol. 36: 362, 1972) that stimulus-reward learning in vision is mediated by a functional chain linking the visual system to the limbic system through relays in the inferior temporal area. Area TE is considered to be the last purely visual link in this pathway. A previous study (Mishkin & Oubre, Neurosci. Abs. 2: 1127, 1976) showed that damage to TE, but not to other temporal lobe structures (TEO, A, or FHH), impairs performance on a one-trial learning test of object recognition as distinguished from object-reward association. Presumably, the impairment after TE lesions in the present study was due to this same basic recognition disorder. The impairment after amygdala lesions, however, not being attributable to a recognition disorder, appears to reflect instead a disorder in object-reward association. The results thus point to object recognition and object-reward association as consecutive processes that depend in part on the sequential participation of area TE and the amygdala.

822 LOCALIZATION OF LESION IN A NOTED CASE OF CHRONIC ANTEROGRADE AMNESIA. Larry R. Squire and Robert Y. Moore. Departments of Psychiatry and Neuroscience, UCSD, La Jolla, CA 92039.

In man, chronic anterograde amnesia can result from dysfunction in the medial temporal region of the brain (case H.M.) or from neuropathological changes in the diencephalic region (Korsakoff disease). In the case of the diencephalic region the dorsal thalamus, the mammillary bodies, and the terminal aspects of the fornix have been associated with amnesia but there is disagreement as to what constitutes the minimal lesion in these regions.

We have recently been able to obtain information about the site of injury in the chronic amnesia patient N.A. In 1960, this individual sustained a stab wound to the brain as the result of a fencing accident. Since 1960, N.A. has had great difficulty in committing day-to-day events to long-term memory. His amnesia is remarkably pure, occurring in the absence of any known cognitive defect other than memory loss. The defect is more severe for verbal than for nonverbal material.

During the past year N.A. consented to three C.A.T. scans. The results consistently demonstrated a small lucency to the left of midline, at the level of the pineal calcification, corresponding to the position of the left dorsal medial thalamic nucleus.

We cannot rule out the possibility that damage has also occurred to the adjacent anterior nucleus or to fibers of the mammillary thalamic tract. The lesion did not involve the fornix or the mammillary bodies. These findings do not exclude the possibility that lesions of the mammillary bodies may result in amnesia in some cases. The results from this patient emphasize the importance of the dorsal thalamus in memory functions.

823 A PROPOSED EXPERIMENT FOR THE INVESTIGATION OF THE "MEMORY OF SPEED". George Vroulis. Dept. Psychophysiology., TRIMS, Med. Center, Houston, TX 77030.

An experimental task is proposed for the measurement of the memory of speed of an event by human subjects. The experimental task in question involves the measurement of visual temporal behavior by having a subject to adjust a rotating disk, with a point light source attached to its periphery, ("subject's disk"), to the angular velocity of another identical reference disk (experimenter's disk") which lies right above the "subject's disk".

Small variations of the above mentioned experimental arrangement involve the use of one disk only with the subject having to memorize a reference angular velocity and be able to reproduce it 5-10 sec later.

It is speculated that saccadic and pursuit movements must mediate those tasks together with some form of short term memory which must encode and store enough angular velocity attributes during the receptive phase, which, after a rehearsive phase, must be reproduced during the executive phase.

This particular short term memory is named "Short Term Memory of Speed" (STMS) and pertains to the memory of the speed of an event.

It is assumed that this type of memory has not been coined as such by the literature and may not involve the traditional coding of information into auditory or articulatory or visual representations, but may encode and store the very eye-movement information that was necessary to perceive the angular velocity movements in the first phase.

By examining concomitantly the EOG and EEG activities, during the three separate phases of the experiment, and correlating them with the accuracy of "speed-reproduction", one may theorize about the roles of both eye movements and specific brain area activities in the STMS.

Preliminary results using the above mentioned task, (matching angular velocities) indicated that normal subjects, can replicate 3.16 Revolutions Per Minute (12 inches diameter disk) to within 9% accuracy, when they observe both the reference (top) and the subject's (bottom) disks, and to within 13% when they are exposed 20 sec to the reference angular velocity (i.e. 3.16 RPM) and they have to reproduce it 5 sec later starting at 0 RPM.

824 ENCODING DEFICITS IN ANTEROGRADE AMNESIA. C. Douglas Wetzel* and Larry R. Squire (SPON: W.T. Schlapfer). Departments of Neurosciences and Psychiatry, UCSD, School of Medicine, La Jolla, CA 92093.

Studies of the alcoholic Korsakoff syndrome have suggested the hypothesis that the amnesic syndrome may in part depend on a failure to encode information with the elaborateness characteristic of normals. Since Korsakoff disease involves some cognitive defects other than amnesia, it has not been clear whether this explanation of amnesia can be usefully applied to other examples of the amnesic syndrome. Patients receiving bilateral electroconvulsive therapy (ECT), the chronic patient N.A., and a Korsakoff patient were given verbal memory tests designed to assess their ability to encode information along graphic, phonemic and semantic dimensions. In order to induce these different levels of encoding, the subjects were queried as to whether or not the word was in upper or lower case letters, whether it rhymed with another word, or whether it was an instance of a semantic category. Results with the Korsakoff patient agreed with findings in a population of Korsakoff patients reported by other investigators. Korsakoff patients tend to fail to encode semantic dimensions of words relative to their controls except in the very simplest version of this task. In contrast, patients receiving ECT and the chronic amnesic N.A. did not exhibit a selective defect in semantic encoding. First, these patients were deficient on all dimensions of encoding. Second, they showed a pattern similar to controls that included the superiority of the semantic encoding condition. Third, the retention performance of these amnesic patients could be duplicated by normal subjects who were tested at long intervals after learning. The results lead to the following conclusions: 1) Korsakoff patients appear to have more severe semantic information processing deficits than that observed in other more pure kinds of amnesia; 2) Normal forgetting shares some similarities with the amnesias found with ECT and the patient N.A.

825 VARIATIONS ON A THEME BY LASHLEY: LESION EXPERIMENTS ON THE NEURAL MODEL OF ANDERSON ET AL. Charles C. Wood* (SPON: T. Allison). Neuropsychology Lab., VA Hospital, West Haven, CT 06516

J.A. Anderson and his colleagues (Psychol. Rev., 1977) recently presented a neural model of memory in which every neural element participates in every memory. Here I report simulated lesion experiments on neural elements in the Anderson et al. model that are similar in spirit to Lashley's classic experiments from which the concepts of "mass action" and "equipotentiality" were derived. The purpose of these experiments was twofold. The first was to examine the effects of systematic variations in lesion size and location on the performance of the model, and to compare them to lesion effects in real nervous systems. The second was to use the model to assess the degree to which principles of neural organization such as localization of function and equipotentiality can be inferred from lesion experiments. A neural model is particularly useful for this purpose because: a) the mechanisms of information processing and storage can be completely and quantitatively specified; and b) lesion size and location can be precisely controlled and systematically manipulated.

The model is represented mathematically in matrix algebra form. Each of N input neurons is assumed to be connected synaptically to each of N output neurons, and the patterns of activity in the input neurons and output neurons are represented as N-element vectors. A given pattern of input activity becomes associated with a given pattern of output activity by making the synaptic strengths between input and output neurons equal the vector product of the activity in the input and output neurons. This mechanism of association is closely related to that originally postulated by Hebb, and similar mechanisms have been proposed by Kohonen (I.E.E.E. Trans. Comput., 1972; Neurosci., 1977).

The effects of systematic variations in lesion size and location were assessed by removing specific combinations of input and output neurons and testing the model's association performance. In all, 65,024 lesions were made for each of 100 different sets of randomly selected input and output vectors. When expressed as average results over large numbers of lesions, the deficits in the model's performance provide axiomatic examples of Lashley's concepts of mass action and equipotentiality. That is, increasing lesion size produced increasing performance deficits regardless of the particular neurons removed. However, certain individual lesions produced highly selective deficits that have been widely interpreted as strong evidence for localization of function. As in the mammalian visual system, lesions including virtually the entire population of neurons were necessary to abolish performance. (Supported by the Veterans Administration and NIMH Grant MH-05286).

MONOAMINERGIC SYSTEMS

826 BEHAVIORAL EFFECTS OF LESIONS OF THE MEDIAN NUCLEUS OF THE RAPHE. Karen E. Asin, David Wirtshafter, Ernest W. Kent. Dept. of Psych. Univ. Il.-Chicago Circle, Chicago, Il. 60680.

In the past, we have demonstrated that damage to the median nucleus of the raphe (MR) results in a number of behavioral deficits which are similar to those seen following hippocampal damage. For example, we have reported that MR lesions retard extinction of a straight alley task (Neurosci. Abst.2,1976) decrease distractability and impair the acquisition of a single alternation response (Neurosci. Abst.3,1977). In the current study we further investigate the consequences of MR lesions on tasks which have been found to be sensitive to damage to limbic structures.

MR lesioned rats have been found to perseverate non-reinforced responding in a T-maze and this behavior cannot be characterized as either response or stimulus perseveration (Asin et al. Neurosci Abst. 3, 1977). To further investigate the nature of this behavior, control and MR lesioned rats were given two non-reinforced trials in a T-maze. During the first trial, one arm of the maze was blocked so as to prevent entrance into that side. During the second trial, one minute later, the block was removed and it was found that both control and MR lesioned rats responded by entering the previously blocked arm. This suggests that MR lesioned animals, similar to hippocampals, fail to perseverate forced choices.

To determine whether MR lesioned rats perseverate learned as well as spontaneous responses, the acquisition and reversal of a T-maze position habit was studied. MR animals were found to acquire the initial response as readily as controls, but were clearly impaired on its reversal. This result was obtained in animals trained to a criterion and in subjects given extensive over-training. Acquisition and reversal of a brightness discrimination task will also be discussed.

In another test, the effects of median raphe damage on a conditioned emotional response (CER) were assessed. Control and MR rats were trained for 3 days to drink water in an operant box. On the fourth day, the tube was removed and rats were shocked in the box for 30 seconds. On the following days, controls showed a significantly larger suppression of drinking than did MR animals. Impaired acquisition of a CER has also been reported following limbic lesions.

Finally, PCPA has recently been reported to eliminate latent inhibition in a shuttle box (Solomon et.al.,P&B,1978). We now report the similar observation that pre-exposure to the conditioned stimulus impairs shuttle box acquisition in control but not MR subjects. Since hippocampal lesions have been found to produce similar results, it is possible that the serotonergic projection to the hippocampus is involved in latent inhibition.

827 ALPHA-ADRENOCEPTOR MEDIATION OF ADRENERGIC-SEROTONERGIC INTERACTION IN DORSAL RAPHE. Jay M. Baraban* and George K. Aghajanian, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Several lines of evidence indicate that the serotonergic (5-HT) cells of the dorsal raphe are subject to adrenergic influence. Drug treatments which impair adrenergic tone reduce 5-HT cell activity. For example, administration of either 1) clonidine (10 μg/kg, i.v.), which acts as a pre-synaptic α-agonist to decrease impulse flow, (Svensson et al., Brain Res., 92:291, 1975) or 2) reserpine (5 mg/kg, i.v.), which impairs noradrenergic transmission, suppresses the firing rate of 5-HT cells in the dorsal raphe.

An α-adrenoceptor is implicated in the suppression produced since the centrally active α-blocker piperoxan, but not the β-blockers sotalol and propranolol, slows 5-HT cell activity (Gallager and Aghajanian, Eur. J. Pharmacol., 39:341, 1976). Furthermore, the inhibition produced by both reserpine and low doses of clonidine can be abruptly reversed by a higher dose of clonidine (200 μg/kg, i.v.), which acts as a central post-synaptic α-agonist. In addition, we have recently found that WB-4104 (60 μg/kg, i.v., Boehringer Ingelheim), a potent α-antagonist, totally suppresses 5-HT cell firing in the dorsal raphe.

The dorsal raphe, as well as the adjacent central grey, receives a dense adrenergic input as demonstrated by biochemical, histofluorescence and immunocytochemical techniques. Thus, the α-adrenoceptors mediating the adrenergic-serotonergic interaction could be located in or near the dorsal raphe itself. This possibility is supported by the following observations from single cell recordings in chloral hydrate anesthetized rats: 1) both α-blockers, piperoxan and WB-4104 when applied iontophoretically gradually slow 5-HT cell firing; and 2) locally iontophoresed norepinephrine (NE) reverses the cessation of activity produced by either reserpine or a low dose of clonidine.

The post-synaptic α-adrenergic agonist phenylephrine is only weakly active at central pre-synaptic α-receptors located in the locus coeruleus (Cedarbaum and Aghajanian, Eur. J. Pharmacol., 44:375, 1977). In contrast, we have observed that it is equipotent to NE in its ability to restore 5-HT cell firing following the administration of either reserpine or a low dose of clonidine. Therefore, we suggest that the receptors mediating the adrenergic influence on dorsal raphe activity are of the post-synaptic α-adrenergic type.

Supported by USPHS Grants MH-17871; MH-14459; MSTP GM-02044 (J.M.B.) and the State of Connecticut.

828 **EFFECT OF SYSTEMIC APOMORPHINE ON FIRING RATES OF DOPAMINE CELLS IN RATS WITH STRIATAL KAINIC ACID LESIONS.** **M.D. Baring, J.R. Walters, E.K.Silbergeld, N. Eng*, S. DeSantis*, and J.M. Lakoski. Experimental Therapeutics Branch, NINCDS, Bethesda Md. 20014.**

Systemic apomorphine (APO) causes a rapid decrease in firing rate of nigral dopamine (DA) cells projecting to the striatum. APO, a DA agonist, may be acting both directly at DA receptors on DA cell bodies and indirectly through a striato-nigral feedback loop. To examine the effect of systemic APO in the absence of feedback from the striatum, we recorded firing rates of DA cells in rats with striatal kainic acid(KA) lesions, since KA is thought to kill cell bodies, leaving incoming axons intact. Rats were unilaterally injected with 2 μg KA in 1 μl .1 M NaH2PO4 buffer at stereotaxic coordinates (Koenig & Klippel) A 7.9, L 2.6, and 5.7 mm below skull surface. At 7 and 14 days after injection, striatal DA content and DOPA accumulation after DOPA decarboxylase inhibition had not changed significantly in the KA-injected striatum as compared to the contralateral side. Glutamic acid decarboxylase activity at 7 days had fallen to 22% of control in the striatum, and to 38% of control in the substantia nigra (SN). In the caudate-putamen, nearly complete loss of neurons was seen for 2-3 mm between A 9.4 and A 6.0. Cell loss also occurred in the globus pallidus; other areas were variously affected.

Extracellular single unit recordings were made from DA cells (one cell per rat) in 11 control and 14 KA-lesioned rats anesthetized with chloral hydrate; recordings from KA-lesioned rats were made in the SN ipsilateral to the lesion, 9-17 days after injection. APO was given at 2 min intervals in increasing amounts which doubled the previous cumulative dose; cumulative doses ranged from .32 to 320 μg/kg (.001 to 1 μM/kg). Despite major loss of striatal neurons, we saw no discernible change in the response of nigral DA cells to systemic APO at these doses. The firing of all cells was inhibited by APO; the cumulative dose at which at least half the cells showed 50% or greater inhibition of firing was 20 μg/kg for both control and KA-lesioned rats. At none of the 11 cumulative APO doses was there a significant difference in average % inhibition between control and KA-lesioned rats, nor was there a significant difference in the average dose which produced 50% inhibition. These results agree with the observation of DiChiara et al (Brain Res., 1977) that KA lesion of the striatum does not block APO's effect on DA synthesis, and our finding that APO's ability to reverse the increase in DOPA accumulation induced by γ-butyrolactone is not reduced in KA-lesioned rats.

While this study does not rule out the possibility that loss of striatal input causes subtle changes, or that changes might be seen during recovery of firing or after chronic APO, it supports the idea that the acute effect of APO on DA cell firing is predominately a direct effect on the DA cell.

829 DYNAMICS OF ENDOGENOUS RELEASE OF CATECHOLAMINES FROM RAT MEDIAL BASAL HYPOTHALAMIC (MBH) FRAGMENTS IN PERIFUSION. J. Becker* and V. D. Ramirez* (SPON: F. Delcomyn). Dept. of Physiology and Biophysics and Neural and Behavioral Biology Program, Univ. of Illinois, Urbana IL 61801.

The release of catecholamines from brain tissue in vitro has been demonstrated, but most systems use radioactively labeled monoamines or precursors as their indicator of release. We report a perifusion method in which it is possible to measure in the perifusate the endogenous concentration of DA and NE, without disturbing the initial pool of these putative neurotransmitters or the initial metabolic conditions of the neural tissue.

Adult male and female Holtzman rats were killed by decapitation, brains removed, MBH dissected out and placed in Krebs phosphate buffer glucose-albumin medium with ascorbic acid (0.1%) and pargyline (80 mM), pH 7.4, on ice. Twelve MBH's (~140 mg wet weight) are halved at the 3rd ventricle with one half going to the experimental the other to the control chamber, placed in a water bath at 37°C and medium described above is flowed in at a rate of 400 μl/4 min; air is bubbled in continuously. Perifusate is collected on ice in perchloric acid (final concentration 0.1N $HClO_4$). The tissue is allowed to equilibrate for 16 minutes. Duplicate 20 μl samples are assayed the same day using a radioenzymatic assay with a sensitivity of 32-16 pg for both NE and DA.

The spontaneous release rate (pg/mg/min) over the initial 50 min of perifusion for males is, NE: 2.67±0.28; DA: 1.79±0.13. In females, NE: 7.19±0.32; DA: 4.69±0.28. The release of both amines is irregular with occasional large NE pulses and more regular episodic DA discharges. The concentration in tissue (ng/mg) for males is, NE: 1.3±0.08(10); DA: 0.43±0.03(10). In females, NE: 1.4±0.07(2); DA: 0.35±0.02(2). In males, 60 mM K^+ triggers an immediate pulse of DA (299% of control) followed by a larger more sustained pulse of NE release (569% of control). In the absence of Ca^{++} the pulsatile secretion of NE is inhibited but not that of DA; the K^+ induced stimulation of NE is reduced and the DA surge abolished. At 0°C the mean secretion of DA and NE (control, NE: 2.83±0.31; DA: 1.58±0.08; 0°C, NE: 1.89±0.19; DA: 1.37±0.07) was reduced but pulses were still observed. The response to K^+ of the MBH's at 0°C was abolished.

In conclusion, we see sex differences in the spontaneous release of NE and DA, the latter of a magnitude consistent with values determined in vivo in the portal vessel. The endogenous secretion can be manipulated by changes in ions and T^o. Therefore, we propose the perifusion system of brain fragments as a viable in vitro technique for studying endogenous catecholamine release. Supported in part by RIAS Grant (NSF SER 76-18255).

830 SPATIAL PREFERENCE AND DOPAMINERGIC ASYMMETRIES IN THE RAT. Gregory Lucas Belenky, Claire Mays*, Richard Samaha*, G. Jean Kant, and James L. Meyerhoff. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20012.

Spatial preference, operationally defined as turning preference in a T-maze shock-escape apparatus, was determined in twenty-four female rats. The T-maze measured 62 cm by 44 cm. The grid floor of the stem delivered 0.05 watts of scrambled foot shock. The arms were shock free. Rats were given ten trials a day for ten days. Preferences emerged in the first ten trials and were highly correlated with preferences in the remaining 130 trials ($r = 0.61$, Student's $t = 3.61$, $df = 22$, $p < .001$). One month after the completion of the ten days of behavioral testing, the rats were decapitated and their brains removed. The left and right striatum, left and right nucleus accumbens/olfactory tubercle, and left and right hippocampus were dissected and separately assayed for dopamine in the case of the striatum and the accumbens/tubercle, and for norepinephrine in the case of the hippocampus. The average concentrations of dopamine (μg/gm ± SEM) were: left accumbens/tubercle 4.51 ± 0.21, right accumbens/tubercle 4.12 ± 0.14; left striatum 8.35 ± 0.40, right striatum 7.95 ± 0.49. The average concentrations of norepinephrine (μg/gm ± SEM) were: left hippocampus 0.36 ± 0.02, right hippocampus 0.38 ± 0.02. There were no statistically significant side to side differences. The ratios of assayed neurotransmitter left to right were calculated for the three paired regions and these ratios correlated with the previously determined spatial preference expressed as percent turns to the left after either ten trials or 140 trials. The ratio of dopamine left to right in the nucleus accumbens/olfactory tubercle showed a significant, positive correlation with the degree of leftward turning preference after ten trials ($r = 0.50$, Student's $t = 2.70$, $df = 22$, $p < .02$) and after 140 trials ($r = 0.436$, Student's $t = 2.27$, $df = 22$, $p < .05$). No significant correlations were found between either the ratio of left to right striatal dopamine or left to right hippocampal norepinephrine and leftward turning preference after either ten trials or 140 trials. Taken together these findings support a role for dopaminergic asymmetries in spatial preference and focus attention on mesolimbic dopaminergic asymmetries as a possible substratum of spatial preference in intact animals.

831 INCREASE IN SEROTONIN:N-ACETYLTRANSFERASE ACTIVITY FOLLOWING NEURAL STIMULATION OF THE RAT PINEAL. C.W. Bowers* and R.E. Zigmond (SPON: P.B. Dews). Dept. of Pharmacol., Harvard Med. Sch., Boston, MA 02115

The activity of serotonin:N-acetyltransferase (NAT) has a diurnal rhythm in the rat pineal gland, increasing 40 to 100-fold during periods of darkness. Pharmacological and anatomical evidence suggests that this nocturnal increase in NAT activity is related to an increase in the activity of the sympathetic nerves innervating the pineal from the superior cervical ganglion (SCG). However, attempts to measure an increase in electrical activity of these nerves in response to darkness have produced contradictory results. The magnitude of the increase in NAT activity makes the pineal gland an appropriate system for studying the neuronal control of the biochemistry of target cells. Therefore, we sought to determine whether electrical stimulation of the preganglionic trunk of the SCG would increase NAT activity in the pineal.

Albino rats were anesthetized and the cervical sympathetic trunks were stimulated bilaterally at 10 Hz, using current intensities twice that required to produce maximal exophthalamus of the ipsilateral eye. Animals were taken during the dark part of their cycle and exposed to light for 45 minutes before the beginning of the stimulation. Immediately following the period of stimulation the pineals were removed and frozen. The NAT activity increased linearly with the duration of stimulation up to 60 minutes, and by 2 hours the NAT activity had reached levels equivalent to the peak night levels seen in intact animals. Stimulation of animals during the light part of their cycle revealed that the pineal is less responsive to one hour of nerve stimulation during the day than during the night. The NAT activity (expressed in picomoles of acetylated product formed per 20 min per pineal ± the standard error of the mean) increased from an unstimulated value of 37 ± 9 to a value of 159 ± 22 following stimulation during the day, and from 278 ± 44 to 3580 ± 693 following stimulation at night. These experiments demonstrate that increases in pineal NAT activity similar to those seen in intact animals can be induced by electrical stimulation of the preganglionic trunk of the SCG. The responsiveness of the pineal to this stimulation varies during the normal light/dark cycle. (Supported by NIH grant NS 12561 and Amer. Heart Assoc. grant #76723.)

832 TOLERANCE TO THE BEHAVIORAL EFFECTS OF METHYLPHENIDATE AFTER DAILY AND INTERMITTENT ADMINISTRATION. Anne M. Brewin* and M.W. Emmett-Oglesby. Dept. Pharmacol. N.Tex. St. U./Tex. Col. Osteo. Med., Denton, TX. 76203.

This experiment tested the assumption that daily drug administration is a necessary condition for the development of tolerance to the behavioral effects of methylphenidate (MP). Thirty-two rats were trained to press a lever with water as a reinforcer and then switched to a DRL 18 second schedule, 45 minutes per session, which generated low rates of responding. After 74 training sessions, responding had stabilized, and subjects were injected with MP, twice weekly, 20 minutes pre-session. Dose-effect data were obtained for seven doses of MP ranging from 1.25 to 33.5 mg/kg. MP produced dose-dependent increases in response rate and decreases in reinforcements earned. A second series of dose-response measurements showed a reduced drug effect, and a third administration of the 10 mg/kg dose further reduced the drug effect. These data suggested that intermittent administration of MP resulted in a reduced drug effect. Therefore, the effect of daily and intermittent administration of MP were further determined for the 10 mg/kg dose. In this phase, eight rats received MP daily 20 minutes pre-session, eight rats received MP daily immediately after the session, and sixteen rats received MP twice weekly 20 minutes pre-session. The animals receiving daily MP pre-session developed tolerance to the rate-increasing and reinforcer-decreasing effects after 40 administrations; the animals receiving MP twice weekly developed partial tolerance to the rate-increasing effects after 20 administrations. Animals receiving MP post-session did not develop tolerance. After 82 sessions of chronic administration, test doses of MP, d-amphetamine, l-amphetamine, methamphetamine, and saline were given to all three groups 20 minutes pre-session. The results demonstrated tolerance to the effects of MP and cross-tolerance to the behavioral effects of the amphetamines after daily or intermittent administration of MP before the testing session. Thus, prior exposure to the behaviorally disruptive effects of a drug of the amphetamine class might subsequently decrease the effects of these drugs. (Supported by Faculty Research Grant #34150.)

833 CHARACTERISTICS OF COCAINE RESISTANT ACCUMULATION IN RAT CEREBRAL CORTEX. G.H. Burrows, M.M. Myers*, and E.D. Hendley. Dept. Physiology and Biophysics, Col. Med., Univ. Vermont, Burlington, Vermont 05401.

A major mechanism for the inactivation of extracellular norepinephrine in rat cerebral cortex is thought to be its accumulation in adrenergic nerve terminals. A sodium dependent, high affinity, transport system ($uptake_1$) has been described which saturates at approximately 0.4uM l-norepinephrine (l-NE). In view of the large (>1.0uM) concentrations of l-NE one would expect to find in central nervous system adrenergic synapses, we have directed our studies toward the characterization of a second accumulation system that is known to exist in rat cerebral cortex which saturates at concentrations much higher than 1.0uM. We have reported (Fed. Proc. 36:381, 1977) that this second system contrasts with $uptake_1$ in that it is not blocked by 10uM cocaine; it shows extremely rapid initial rates of accumulation; and it is markedly inhibited by the removal of calcium ions from the incubation medium. We have named this second system "cocaine resistant accumulation"(CRA).

We report here that when rat cortical tissue is incubated for one minute at 31°C in the presence of 1.0uM ^{3}H-l-NE, if, instead of 10uM cocaine, either 100nM desmethylimipramine or a sodium-free choline chloride medium, or both, are used to block $uptake_1$, CRA does not appear to be altered in either amount or calcium sensitivity. We have also examined this system in brain slices, crude synaptosomal homogenates, and in purified synaptosomes. The purified synaptosomes showed a 500% increase in the amount of calcium-sensitive ^{3}H-l-NE accumulation compared with brain slices, and a 60% increase when compared with crude homogenates, suggesting that CRA may, like $uptake_1$, be occurring in nerve endings. As with $uptake_1$, CRA was blocked by millimolar dinitrophenol and the regional distribution appears to follow the distribution of NE nerve terminals in the brain. In contrast with $uptake_1$, CRA did not appear to be stereo-selective with regard to l-NE accumulation. Because of the high capacity of CRA and its apparent association with NE nerve terminals, CRA may serve a major role in clearing the high concentrations of NE presumed to occur during the release of the transmitter from the nerve terminal.

Supported by USPHS grant #01-25811.

834 EFFECTS OF CHRONIC MONOAMINE OXIDASE INHIBITORS ON CENTRAL NORADRENERGIC SYSTEMS. Iain C. Campbell*, Dorothy W. Gallager, Dennis L. Murphy and John F. Tallman. National Institute of Mental Health, Bethesda, MD 20014.

The effects of the monoamine oxidase inhibitors (MAOI) were examined in animals chronically treated with low doses of clorgyline (MAO Type A inhibitor) and pargyline (MAO Type B inhibitor) in a regimen which results in the inhibition of specific MAO forms. Animals treated with clorgyline showed a 25% decrease in the number of β-adrenergic receptors as measured by the binding of 1-[^{3}H]-dihydroalprenolol with no change in the affinity of the receptors. This effect persisted for at least one week following the final dose of clorgyline. By 3 weeks the number of receptors had returned to control levels. Acute clorgyline did not lead to desensitization of β-adrenergic binding. In contrast, chronic pargyline leads to a very small decrease in β-adrenergic binding (approximately 10%) and acute pargyline was without effect. These receptor effects may be corrolated with the differential effects of the inhibitors on norepinephrine (NE) levels. Consistent with desensitization observed in β-adrenergic receptor binding studies, electrophysiological data suggests that α-adrenergic function is also disrupted by chronic pretreatment with MAOIs. Twenty-four hours following the last daily dose of the inhibitor, animals were anesthesized with chloral hydrate and prepared for extracellular recording of the NE-containing cell bodies in the nucleus locus coeruleus (LC). Minimal to nonexistent spontaneous activity of the LC neurons was observed in rats chronically pretreated with MAOI in contrast to control rats (given daily saline injections) whose LC cells displayed the spontaneous firing patterns and rates typical for this region. However following the administration of the α-adrenergic antagonist, piperoxane, in chronically treated rats, a return of spontaneous activity in LC cells was observed. Electrophysiological effects of chronic MAOI treatment are also currently being investigated in a predominantly β-adrenergic postsynaptic region. This data suggests that continuous occupancy of NE receptors by NE in chronic MAOI-treated animals results in the inhibition of NE cell firing and desensitization of NE receptors.

835 COMPARATIVE EFFECTS OF SYNAPTIC STIMULATION ON TYROSINE HYDROXYLASE, DOPA DECARBOXYLASE, AND DOPAMINE BETA HYDROXYLASE ACTIVITIES IN THE SUPERIOR CERVICAL GANGLION OF THE RAT; PHARMACOLOGICAL ANALYSIS OF THE REGULATION OF TYROSINE HYDROXYLASE ACTIVITY. A. Chalazonitis*, P.J. Rice* and R.E. Zigmond (SPON: U.C. Drager). Dept. of Pharmacol., Harvard Med. Sch., Boston, MA 02115

The activities of tyrosine hydroxylase (TH), dopa decarboxylase (DDC) and dopamine beta hydroxylase (DBH) were measured in the superior cervical ganglion 72 h after electrical stimulation of the cervical sympathetic trunk in vivo. Continuous stimulation at 10 Hz for 60 min produced a significant increase in enzyme activity per ganglion (expressed as a percentage of the contralateral unstimulated ganglion ± S.E.M.) of both TH (166 ± 13%) and DBH (156 ± 14%) but not of DDC (105 ± 3%) (n = 12). Stimulation of the sympathetic trunk with trains of stimuli (40 Hz for 250 msec every sec for 60 min)--thus applying the same total number of stimuli as above--produced similar results. TH and DBH activities were increased dramatically (173 ± 9%, 153 ± 16% respectively) and there was a small (110 ± 3%) increase in DDC. These results demonstrate that direct stimulation of the preganglionic nerves innervating the superior cervical ganglion produces a significant increase in TH and DBH activities with little change in DDC activity and suggest that the increase in TH and DBH activities are more responsive to the number of stimuli applied than to the pattern of stimulation.

Nicotinic, muscarinic and α-adrenergic receptors have been implicated in ganglionic transmission. Previous experiments demonstrated that nicotinic blocking drugs, such as chlorisondamine, largely block the increase in TH activity produced by preganglionic nerve stimulation (Chalazonitis and Zigmond, Proc. Soc. Neurosci., 1977). To determine whether muscarinic and/or α-adrenergic receptors are also involved in the regulation of TH activity, animals were stimulated in the presence of appropriate antagonists at doses which block these receptors but do not interfere with nicotinic transmission. Preganglionic stimulation with 40 Hz trains (250 out of every 750 msec) for 90 min produced a similar increase in TH measured 72 h later whether the stimulation was performed in the presence (215 ± 18%; n = 4) or absence (188 ± 21%; n = 5) of atropine sulfate (125 μg/kg/min infused i.v.). In the same manner, stimulation with 40 Hz trains for 30 min produced a similar increase in the presence (156 ± 13%; n = 9) or absence (153 ± 10%) of dihydroergotamine mesylate (3.3 mg/kg injected i.v. prior to the start of stimulation). Therefore, muscarinic or α-adrenergic receptors do not play a major role in the increase of TH activity produced by synaptic stimulation. (Supported by NIH grant NS 12651 and NIH training grant NS 07009).

836 MONOAMINE-CONTAINING STRUCTURES IN THE POSTERIOR SALIVARY GLAND OF THE *OCTOPUS VULGARIS*: FLUORESCENCE HISTOCHEMICAL AND ULTRASTRUCTURAL STUDY. Tanemichi Chiba, Yuji Yaku*, and Makoto Kato*, Dept. Anat., Sch. Med., Chiba University, Chiba 280, Japan.

The posterior salivary gland(PSG) of the *Octopus vulgaris* has been paid attention by some workers due to its high content of monoamines including octopamine and the existence of chromaffin cells. Matus(1971) classified two types of epithelia(named type A and B) and identified a distinct proportion of epithelial cells of type A as chromaffin cells which also exhibited positive formaldehyde induced fluorescence.

The purpose of the present study was to clarify the localization of monoamines in the PSG by a combined histochemical and ultrastructural studies.

Two monoamine-containing structures were identified: one is a network of green-yellowish fluorescent nerve terminals surrounding tubular gland and the other is the glandular epithelial cell with brilliant granular yellow fluorescence. Spectrofluorimetric analyses revealed the existence of dopamine-like spectral pattern (excitation max. 410 μm, emission max. 480 μm and after exposure to HCl vapour for ten second, excitation max. shifted to 380 μm which persisted even after additional ten minutes exposure to HCl vapour) in the former and serotonine-like spectral pattern (excitation max. 410 μm, emission max. 510 μm) in the latter structures.

Monoamine-containing(MA) cell granules in the epithelial layer were found to be argentaffin by Bodian's stain and by Tramezzani-'s silver stain for Epon embedded sections. MA cell granules were PAS negative in contrast to surrounding most exocrine cells which stain in variable degrees for PAS.

After the observation of glyoxylic acid induced fluorescence preparation, the same specimens were processed for electron microscopy. It was confirmed that monoamine containing dense granules(0.5 to 3 μm in diameter) were embedded in the amorphous peripheral zones which were divided into many compartments by the membrane. MA cells extend their processes in both apical and basal portions and the nucleus were located in the basal portion surrounded by well developed rough endoplasmic reticulum and Golgi apparatus. Potassium permanganate seemed to preserve the cell better than glutaraldehyde or formaldehyde.

Most parts of the MA cell surface are covered by the sheath of supporting cells and occasionally axon varicosities were seen to synapse on MA cells.

The present results revealed that MA cells compose neurosecretory complex in the epithelial layer of the PSG suggesting paraneuronic nature of the cell. It is not clear whether MA cells work through endocrine, exocrine or paracrine mechanism.

837 DOPAMINE AGONIST PRETREATMENT ALTERS LSD'S ELECTROPHYSIOLOGICAL ACTION FROM DOPAMINE AGONIST TO ANTAGONIST. Greg R. Christoph* and Barry L. Jacobs (SPON: T.J. Sejnowski). Prog. in Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08540.

We previously reported that low doses of LSD (25-50 μg/kg i.v.) significantly decreased the firing rate of dopamine-containing neurons in the substantia nigra of chloral hydrate anesthetized rats (Life Sciences, 21, 1585-1596, 1977). These effects were blocked, or reversed, by administration of haloperidol (100 μg/kg i.v.), and were not mimicked by brom-LSD, a peripherally active non-hallucinogenic congener of LSD. These and other electrophysiological data were supportive of the hypothesis, derived from behavioral and neurochemical experiments, that LSD can act as a dopamine agonist in the CNS. In addition, neurochemical experiments measuring dopamine specific receptor binding or synthesis of dopamine sensitive c-AMP indicate that LSD can also act as a dopamine antagonist. The present electrophysiological experiments support such an hypothesis. Dopamine-containing neurons were identified on-line by their firing rate (2-7 spikes/sec), and spike duration (> 2 msec). Histological analysis confirmed that the cells were in the substantia nigra. In contrast to the generally depressant effects of LSD alone, when LSD (50 μg/kg i.v.) was administered to rats whose nigral cell discharge had been reduced by 45% below baseline by d-amphetamine (mean dose = 1.45 mg/kg i.v.), the firing rate increased to within 7% of the original baseline. A second injection of this dose of LSD further increased the discharge rate, to a point 4% above the original baseline. A similar pattern was observed when this dose of LSD was administered to rats whose nigral unit activity had been depressed by apomorphine pretreatment (50 or 100 μg/kg i.v.). Brom-LSD, which shares LSD's dopamine antagonist, but not agonist, properties also produced these reversal effects. Finally, pretreatment with LSD (200 μg/kg i.v.) produced a 250% increase in the dose of d-amphetamine required to produce a 50% decrease in nigral discharge rate. All of these effects of LSD resemble those of classical central dopamine antagonists such as haloperidol. We hypothesize that the shift in LSD's action from that of dopamine agonist to antagonist by prior dopamine agonist treatment may be mediated by a conformational shift in the state of the dopamine receptor. (Supprted by NIMH grant MH-23433).

838 NEURAL INPUTS TO THE SELF-STIMULATION REGION IN THE SULCAL PREFRONTAL CORTEX OF THE RAT: HORSERADISH PEROXIDASE STUDY. Ronald M. Clavier and Charles R. Gerfen*. Dept. Anat., Northwestern Univ. Med. Sch., Chicago, IL 60611.

The prefrontal cortex, dorsal to the rhinal sulcus of the rat has been related to intracranial self-stimulation (ICSS). However, the anatomical substrates specific to this behavior have not been determined. While there is evidence of a causal role in ICSS for efferents from the sulcal cortex (Clavier and Corcoran, 1976), no data exist as to the possible contribution of afferents to this region.

As a preliminary approach to defining the role of sulcal afferents in ICSS, we have examined each of these systematically with the use of the retrograde transport of horseradish peroxidase (HRP). Specifically, 0.08-0.10 µl of 30% HRP (Boehringer) was injected unilaterally via a 30 g cannula over 15 min., using the same stereotaxic coordinates as those used for chronic ICSS electrode implantation. Histochemical reaction was with 3,3' diamonobenzidene or benzidene dihydrochloride (Sigma).

Labelled perikarya were seen consistently within the region of the ipsilateral medial forebrain bundle, rostral to the mesencephalic border. In view of the finding (Lindvall et al., 1978) of an A10 dopaminergic projection to the sulcal cortex, the presently labelled neurons may constitute the rostral aspect of that cell group. Lindvall's group reported A10 cells from the level of the posterior mammilar nucleus to the rostral interpeduncular nucleus after sulcal HRP injections; however, their injections showed possible spread to the caudate nucleus as evidenced by labelling in the substantia nigra (SN). A similar SN and A10 pattern was seen after HRP injections confined to the caudate nucleus (Carter and Fibiger, 1977). In the present study, there was no evidence of SN or caudal A10 labelling. Thus the presumed A10 cells referred to in our study may be distinct from those reported previously.

Labelled neurons were also seen in the dorsal raphe nucleus. These may be responsible for the sulcal serotonergic innervation described by Tassin et al. (1978).

Thalamic inputs to the sulcal cortex included a projection from the mediodorsal nucleus, as well as the ventromedial and parafascicular nuclei. Projections from the locus coeruleus and the amygdala were also seen. Each of these results confirms previous demonstrations that employed orthograde transport or neurophysiological techniques.

Ongoing studies into the possible role of each of the sulcal afferents described herein are discussed.

(Supported by NIMH Grant 1 R01 MH 30296-01).

839 SIMULTANEOUS LOCALIZATION OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH) AND CATECHOLAMINES (CA) IN RAT MEDIAN EMINENCE. C.J. Clayton*, T.H. McNeill and J.R. Sladek, Jr. (SPON. K. Bignall). Dept. Anat., Univ. Rochester, Rochester, NY 14642.

Evidence indicates that CA play a role in the regulation of LHRH. Both substances are found abundantly in the median eminence (ME). In this study a technique for the simultaneous visualization of CA and LHRH in rat ME was used. Also, the distribution of LHRH reaction product in freeze-dried tissue was compared to that found in tissue fixed in Bouin's solution.

Brains from adult male Sprague-Dawley rats were divided midsagittally. Half of each brain was immersed in Bouin's solution, and the remaining half was prepared for simultaneous histofluorescence-immunocytochemistry (McNeill and Sladek, Science 200: 72-74, 1978). To eliminate possible artifacts due to immersion fixation, additional animals were perfused with Bouin's solution. Also, whole brains were prepared by freeze-drying. Primary antisera used were #38 (T.M. Nett) and 4305B (L.A. Sternberger). Staining in the ME was similar with both antisera, and absorption with synthetic LHRH eliminated staining. The distribution of granular reaction product in freeze-dried tissue was similar to that found in perfused and immersed tissue, but granules were more numerous and more distinct in freeze-dried ME.

LHRH reaction product was observed in rostral ME in both the medial and lateral portions of the zona externa. Examination of CA on the same or adjacent section revealed that CA varicosities were located in a zone external to an area occupied by the majority of the LHRH reaction product. Occasional deposits of LHRH reaction product were found in the CA-containing zone, juxtaposed to portal capillaries. LHRH reaction product in the caudal ME was heavily localized in the region of the tuberoinfundibular sulcus, and, unlike the rostral ME, the CA zone was seen to overlap a major portion of the LHRH-containing region. These data suggest immunoreactive LHRH in the ME is better preserved in freeze-dried tissue than in tissue prepared in a more routine manner. Also, the morphological relationship of LHRH to CA differs in rostral and caudal ME in the rat. Supported by Postdoctoral Fellowship HD 05668.

840 MEASUREMENT OF SEROTONIN IN HYPOTHALAMIC NUCLEI WITHIN ADULT RAT BRAIN USING A MODIFIED BRAIN PUNCH TECHNIQUE. Ric I. Cone, Gary A. Davis* and Robert W. Goy. Wis. Reg. Primate Res. Ctr. and Neurosciences Training Program, Univ. Wisconsin, Madison, WI.

Endogenous levels of serotonin were measured in several hypothalamic nuclei within brain tissue from adult male Sprague Dawley rats using the radioisoenzymatic assay developed by Saavedra in conjunction with a modification of the Palkovits brain punch technique. In contrast to results reported by Saavedra and coworkers (1), we found considerably lower values for serotonin within the suprachiasmatic portion of the preoptic area (nucleus preopticus suprachiasmatis) and throughout a major portion of the arcuate nucleus. Levels in other areas of the hypothalamus were similar to those reported by Saavedra et al. Reasons for the differences are not clear, however, our results appear to be more consistent with results based on histofluorescent staining (2;3).

Modifications developed in our laboratory have improved the anatomical specificity of the brain punch technique. Maintaining sections at -10°C on a thermoelectric cold platform (Thermoelectric Unlimited, Inc.) instead of dry ice allows for better recognition of anatomical landmarks by minimizing frost build-up, and preventing disruption of the tissue section due to excessively low temperature during removal of the punched out areas. In addition, a reliable staining procedure for 300 µ thick, unfixed brain tissue has been developed using a modification of the cresyl violet stain. Sections can be processed directly on the slide with cytoarchitectonic resolution equal to 30 µ sections stained with cresyl violet.

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841 A MAP OF THE MIDBRAIN DOPAMINE SYSTEMS FOR INTRACRANIAL SELF-STIMULATION (ICSS): A FLUORESCENCE HISTOCHEMICAL STUDY. Dale Corbett* and Roy A. Wise. Dept. Psyc., Cen. Res. Drug Depen., Concordia Univ., Montreal, Quebec, Canada, H3G 1M8

Neurochemical destruction or pharmacological blockade of the brain dopamine (DA) systems results in the dramatic reduction or abolition of ICSS. These data suggest that ICSS from the region of the substantia nigra and certain prosencephalic structures is due to activation of the midbrain DA containing cell groups (A8-A10) or their efferent projections. Several questions still remain regarding the nature of the DA involvement in ICSS. First of all, despite marked anatomical differences among the nigrostriatal, mesolimbic and mesocortical DA systems, it is not known whether all of these systems participate in ICSS. Secondly, the disruption of ICSS by DA receptor blocker or DA lesions may be attributed in certain circumstances to an interference with sensory-motor abilities. Finally, it may be that a DA system located several synapses distal to the ICSS electrode is the critical DA system and not the DA fibers beneath the electrode tip. In order to answer some of these questions the A8-A10 cell groups and their efferent projections were mapped for ICSS using a moveable electrode (Wise, 1976). In 24 of the 50 rats used in this study fluorescent histochemical methods (Battenberg & Bloom, 1975) were employed to verify electrode placements.

ICSS thresholds decreased and response rates increased as electrodes were lowered ventrally towards the A10 cell group as defined by Lindvall and Bjorklund. Electrodes lowered through A10 into the interpeduncular nucleus ceased to support ICSS. The rostro-medial portion of the A9 cell group supported ICSS but only at relatively high current intensities. ICSS was not obtained from the A8 cell group, the caudal portion of the A10 cell group nor from the lateral A9 cell group. In the hypothalamus ICSS sites were distributed along the route of the DA fiber systems. Since these DA fiber systems are in close proximity to one another it is difficult to attribute ICSS to a single system.

These data clearly indicate differences within the DA cell groups with respect to ICSS. The A10 cell group supports ICSS whereas the A8 cell group does not. The present data question the view that the nigrostriatal DA system supports ICSS, since only the rostro-medial portion of the A9 cell group yielded ICSS. While it may be that the higher ICSS thresholds from the A9 region are due to the medial flow of the A9 fibers it is also possible that the ICSS instead results from current spread to the medially located A10 cell group. This latter possibility awaits further investigation.

842 ELECTROPHORETIC CHARACTERIZATION OF MONOAMINE OXIDASE IN CULTURED CELLS WITH A AND B ACTIVITIES. Maria R. Castro Costa*, Morris Hawkins, Jr.* and Xandra O. Breakefield. Department of Human Genetics, Yale University School of Medicine, New Haven, CT 06510.

Two types of monoamine oxidase (MAO, EC 1.4.3.4) activity can be distinguished on the basis of their substrate specificity and drug sensitivity. Using 5-hydroxytryptamine, phenylethylamine and tryptamine with varying concentrations of clorgyline and deprenyl, we have confirmed the presence of both types of MAO activity in living cells, homogenates and crude mitochondria from two cell lines. Rat hepatoma line MH_1C_1 expresses both A and B types of activity, while human sarcoma line Bu25 has predominantly B type activity. MH_1C_1 cells deaminate both 5-hydroxytryptamine (A substrate) and phenylethylamine (B substrate); these activities are blocked by low concentrations of clorgyline and deprenyl, respectively. Deamination of tryptamine (A and B substrate) is inhibited by clorgyline in a biphasic manner corresponding to 80% A and 20% B activity. No metabolism of 5-hydroxytryptamine can be detected in Bu25 cells; deamination of phenylethylamine is inhibited by low concentrations of deprenyl. A monophasic curve of clorgyline inhibition is seen with tryptamine.

Pargyline binds specifically and irreversibly to monoamine oxidase (Chuang et al., J. Biol. Chem. 249:2381, 1974). We have used [3-H]pargyline to label this enzyme in crude mitochondrial preparations from both cell lines. Following binding of [3-H]-pargyline, mitochondrial proteins are solubilized in sodium dodecyl sulfate (SDS) and separated by SDS polyacrylamide gel electrophoresis and isoelectric focusing (Ames and Nikaido, Biochem. 15:616, 1976). The location of labelled proteins is detected by autoradiography via fluorography. Several labelled proteins can be identified and their labelling is blocked by 0.4 mM clorgyline. The migration pattern of these proteins is similar in MH_1C_1 cells with A and B activity and Bu25 cells with only B activity. Thus the presence of A and B types of MAO activity does not appear to correlate with specific protein species.

843 EFFECT OF LOCUS COERULEUS STIMULATION ON REGIONAL BLOOD FLOW IN MICE. Richard L. Delanoy*, Wickliffe C. Abraham, Steven F. Zornetzer, and Adrian J. Dunn. Dept. Neuroscience, Univ. of Fla. College of Medicine, Gainesville, Fla., 32610.

The effects of unilateral locus coeruleus (LC) stimulation on regional cerebral blood flow were examined in mice using [^{14}C] antipyrene uptake as an index of perfusion. Both anatomical and physiological evidences have suggested that the LC is involved in the regulation of cerebral blood flow. This experiment was designed to investigate this association of LC with cerebral blood flow in various regions of unanesthesized, unrestrained mice.

Bipolar nichrome wire electrodes were stereotaxically implanted bilaterally in the region of LC in male Swiss mice. Two to three weeks following electrode implantation, a silastic cannula was inserted into the right jugular vein and threaded into the right atrium of each mouse. On the following day, each mouse was given 10 μCi of [^{3}H]2-deoxyglucose by means of a tubing extension connected to the jugular cannula, and forty minutes later, 1-5 μCi of [^{14}C]antipyrene. Concurrent with the antipyrene injection, the LC was stimulated unilaterally (75 μa, biphasic pulses for 20 sec.). These stimulus parameters were effective in altering behavior in another experimental situation. The heart was stopped with 150 ul of saturated KCl just prior to the termination of stimulation. Each animal was perfused with 0.9% saline and each forebrain hemisphere was dissected into 14 regions, in which radioactivity for both labels was determined individually by scintillation counting. The brainstem was histologically prepared for verification of electrode placement. The ratio of [^{14}C] uptake between the two hemispheres provided an estimation of differences in hemispheric blood flow consequent on LC stimulation. [^{3}H]2-deoxyglucose uptake, which was not temporally associated with LC stimulation, provided a means of normalizing the uptake of [^{14}C]antipyrene. Based on the histology, animals were divided into 3 groups: stimulated in LC (n=10), stimulated outside of LC (n=9), and sham-stimulated controls (n=6).

In no region examined were any changes in cerebral blood flow found due to the electrical stimulation. Furthermore, a comparison of the two hemispheres as a whole revealed that LC stimulation, using this paradigm, had no effect on hemispheric blood flow greater than 4% at a 95% confidence interval. These data will be discussed with regard to the existing literature describing LC modulation of cerebral blood flow.

844 HISTOFLUORESCENCE STUDY OF THE CATECHOLAMINERGIC INNERVATION OF THE AVIAN FOREBRAIN. L. Dubé* and A. Parent (SPON: L.A. Larochelle). Lab. Neurobiol., Fac. Méd., Univ. Laval, Québec,Canada.

The catecholaminergic (CA) innervation of the forebrain of birds was studied in chickens (Gallus domesticus) of 3 days to 1 month of age. Some of the animals were killed by decapitation and their brains processed either according to the Falck-Hillarp paraformaldehyde method (Falck et al., '62), or to the cryostat glyoxylic acid procedure of de la Torre and Surgeon ('76). Other birds were anaesthetized and perfused with a solution containing glyoxylic acid and a high concentration of magnesium ions. The brains of these animals were then processed according to the combined formaldehyde-glyoxylic acid method of Lorén et al. ('76).

In the diencephalon of the chicken, numerous varicose CA fibers are coursing in the lateral hypothalamus, along the medial aspect of the fasciculus prosencephali lateralis (FPL). These fibers give off collaterals to various diencephalic structures. Among those are the dorsomedial and dorsolateral anterior thalamic nuclei and the dorsomedial posterior thalamic nucleus. A very large number of CA varicosities is also found within the hypothalamic periventricular gray, especially at the level of the nucleus periventricularis magnocellularis and of the nucleus tuberis. A well-developed paraventricular organ consisting of numerous, closely-packed, CSF-contacting CA cells is also present along the ependymal wall of the hypothalamus. This cell group also contribute to the CA innervation of the hypothalamus.

In the rostral telencephalon, various structures such as the olfactory tubercle, the nucleus accumbens septi, the lateral septal nucleus and the so-called lobus parolfactorius receive a strickingly massive CA innervation. Numerous very fine CA varicosities also occur in the paleostriatum augmentatum, especially within its dorsomedial portion. In contrast, the paleostriatum primitivum contains only a few scattered varicosities although numerous patches of fluorescent material are present along its medial border, intermingled among the fibers of the FPL. The neostriatum, the ectostriatum and the hyperstriatum ventrale contain few varicose linear CA profiles that are evenly distributed. These linear profiles are much more numerous within the hyperstriatum accessorium and the hippocampal area where they are often lying just beneath the pial surface. In the caudal half of the telencephalon, the number of CA varicosities increases significantly in the neo- and hyperstriatum. In the neostriatum the CA varicosities are closely surrounding the non-fluorescent cell bodies. In the archistriatum, numerous fine CA varicosities are present in the pars dorsalis whereas the pars ventralis contains a large number of serotonin-type varicosities. (Supported by grant MT-5781 of the Medical Research Council of Canada).

845 DEVELOPMENT OF MONOAMINERGIC NEURONS IN THE ENTERIC NERVOUS SYSTEM OF THE CHICK EMBRYO. Miles L. Epstein and Michael D. Gershon, Dept. Anat., Univ. of Wisconsin, Madison,Wi.53706 and Dept Anat.,Columbia Univ., Coll.Phys. & Surg.,N.Y.,N.Y. 10032.

Both 5-hydroxytryptamine(5-HT) and norepinephrine (NE) have been found in neurites in the mammalian enteric nervous system. 5-HT has been found in intrinsic neurons while NE is the transmitter of neurons whose cell bodies lie extrinsic to the gut. In order to understand the role played by various enteric neurons in regulating the development of the system, it is important to know the order of neuronal development and the generality of this order among species. The early appearance of monoaminergic neurites can be detected by measuring the specific uptake of the respective transmitters. We have used the development of transmitter uptake as a means of detecting the appearance of incipient monoaminergic neurons in the enteric nervous system of the chick. Cells taking up 5-HT were detected by radioautography following incubation of embryonic gut in-vitro with ^{3}H-5-HT. Labeling was considered specific if it was prevented by incubation in the cold or in the presence of Lilly 11014 (50 uM). Adrenergic neurites were detected by formaldehyde-induced histofluorescence with and without prior incubation with α-methyl norepinephrine (α-M-NE). Amine uptake was correlated with fine structural observations of the enteric nervous system. Embryos were examined at 7 to 20 days of incubation. Little uptake of ^{3}H-5-HT was found prior to 9 days. At that time, and through day 13, ^{3}H-5-HT was found in cells surrounding myenteric ganglia and within the ganglionic neuropil. After 13 days ^{3}H-5-HT is confined to processes within the enteric nervous system. With the electron microscope, cells can be seen between days 9 and 13 at the periphery of the myenteric plexus apparently extending processes into the plexus. These observations suggest that cells able to take up 5-HT appear in the embryonic chick gut by 9 days and migrate into the myenteric plexus between days 9 and 13. Upon entering the plexus, uptake is observed only in cell processes. In contrast, adrenergic neurites which were rare at first could only be detected by fluorescence in tissue incubated with α-M-NE and not until days 11-12. Endogenous NE was not detected before days 13-14. This study indicates that serotonergic neurons are probably present in chicken as well as in mammalian gut. As in mammalian gut the 5-HT innervation appears before extrinsic adrenergic neurites reach the gut. In adrenergic development the transmitter uptake mechanism precedes detection of endogenous amine stores. The similarity of avian and mammalian enteric ontogeny suggests the pattern may be a general one. Supported by NIH grants #NS12969 and NS07062 and ADAMHA grant DA01772 and Univ. Wisconsin Graduate School grant.

846 RAPHE NUCLEI IN THE RAT: EFFERENT PROJECTIONS TO FOREBRAIN STUDIED USING THE HORSERADISH PEROXIDASE-RETROGRADE TRANSPORT (HRP) METHOD. J. H. Fallon and R. Y. Moore. Dept. of Neurosciences, U. Calif. at San Diego, La Jolla, CA. 92093

The ascending projections of the midbrain raphe nuclei were analyzed in the rat using the HRP technique. Injections (0.04-0.3 µl, 30% HRP) were made into basal forebrain, neostriatum, hippocampus, cerebral cortex or thalamus and HRP reaction product was localized in cell bodies of the nucleus dorsalis raphe (DR), nucleus centralis superior (CS), nucleus reticularis tegmenti pontis (NTP) and nucleus raphe pontis (NRP).

The results of this study demonstrate three principal features of the ascending projections of midbrain raphe neurons. First, DR has the most extensive projection showing heavy labeling after injections into all areas of neocortex, neostriatum, amygdala, piriform cortex, and lateral geniculate nuclei. Heavy labeling in CS occurs principally after injections in cingulate cortex, septum and hippocampus. NTP and NRP show only scattered labeled cells even after very large forebrain HRP injections. Second, although the DR, CS and NRP appear to be single, midline nuclei, they project as paired nuclei. That is, after a unilateral HRP injection, labeling is nearly exclusively found in cells in the half of the nucleus ipsilateral to the injection. Third, raphe efferents contain, in part, an amine-containing system whose distribution is intermediate in organization between the diffuse, widespread ascending and descending projections of locus coeruleus neurons and the highly topographically organized mesotelencephalic dopamine neuron projections (Fallon and Moore, 1978, J. Comp. Neur. 180 (3)). This study was supported by USPHS Grant NS-12080.

847 INTRANEURONAL FLUORESCENCE INTENSITY AS A MEASURE OF NEURONAL ACTIVITY IN THE NUCLEUS LOCUS COERULEUS. Charles Flicker*, Mark A. Geyer, and Arnold J. Mandell. Dept. Psychiatry., Sch. Med., UCSD, La Jolla, CA 92093

The Falck-Hillarp technique for the demonstration of catecholamines can be used as an index of neural activity in the norepinephrine (NE)-containing cells of the nucleus locus coeruleus (LC). Similar microspectrofluorimetric methods have been applied to serotonin-containing neurons of the midbrain raphe (Geyer et al., J. Pharmacol. Exp. Ther., in press) and to the dopaminergic neurons of the substantia nigra (Lichtensteiger et al., Brain Res. 117: 85, 1976).

Two-mm thick slices of rat brainstem are freeze-dried under vacuum for 3 weeks and then treated with paraformaldehyde vapor, which converts NE to a fluorescent derivative. Samples are embedded in paraffin after which sections 8-µm thick are cut on a microtome and mounted on slides. Slides are viewed with a Leitz microscope by red-light phase-contrast at 1150 x in order to align the 5 µm measurement aperture over the cytoplasm of LC cell bodies. Intensity readings are taken by illuminating a 40 µm diameter concentric area with light from a 1000 watt Xenon lamp, at a wavelength of 410 nm. The light emitted by the tissue specimen, filtered with a monochromator to 512 nm, is measured with a photometer.

Drugs that affect NE turnover also alter fluorescence intensity levels in LC. Pargyline, a monoamine oxidase inhibitor which increases NE levels throughout the brain, produces a dose-dependent (25, 50, and 100 mg/kg) increase in intraneuronal fluorescence intensity. Amphetamine, 2.0 mg/kg and 5.0 mg/kg, which increases release and blocks reuptake of NE, decreases intraneuronal fluorescence. Clonidine (30 µg/kg, 100 µg/kg, and 1 mg/kg), an α-adrenergic agonist which depresses LC neuronal activity presumably by activation of presynaptic "autoreceptors" (Svensson et al., Brain Res. 72: 291, 1975), increases LC neuron fluorescence. Piperoxane (0.5, 2.5, and 10.0 mg/kg), an α-adrenergic antagonist which increases impulse flow from the LC (Cedarbaum and Aghajanian, Brain Res. 112: 413, 1976), decreases intraneuronal fluorescence there.

These and related studies suggest that intraneuronal fluorescence intensity in the LC is dependent upon a transmitter pool which is increased by synthesis and reuptake of NE and decreased by release and metabolism of NE, and may therefore be considered a sensitive measure of LC neuronal activity.

This work is supported by DA-00265-07.

848 BEHAVIORAL AND ELECTROENCEPHALOGRAPHIC CORRELATES OF LOCUS COERULEUS NEURONAL DISCHARGE ACTIVITY IN THE UNANESTHETIZED SQUIRREL MONKEY. Stephen L. Foote, Floyd E. Bloom, and Andrew Schwartz*, Center for Behavioral Neurobiology, The Salk Institute, La Jolla, CA 92037. *St. Elizabeths Hospital, NIMH, Washington, D.C. 20032.

Discharge activity of individual locus coeruleus (LC) neurons was recorded extracellularly in the awake monkey. Animals were seated in a chair which allowed arm, leg, and head movements. Well-isolated action potentials were recorded through movable metal microelectrodes. EOG and cortical EEG were recorded simultaneously. Discharge activity was recorded under the following conditions: 1) sequential presentation of an array of sensory stimuli, 2) spontaneous or elicited movement, especially during orienting, 3) periods of 20 min to 1.5 hr with no imposed sensory stimulation, and 4) presentation and consumption of food. A maximum of four microelectrode penetrations was made in each hemisphere to allow unambiguous histological identification of each recording descent. Microlesions were made during the course of each penetration to facilitate accurate localization of recording sites. Unitary action potentials were considered to have arisen from an LC neuron only when the recording site was within the boundaries of the compact portion of the nucleus. LC was defined as encompassing both A4 and A6, the two large norepinephrine-containing neuronal populations in the pons (Hubbard and DiCarlo, JCN, 1973).

LC neurons responded with bursts of increased discharge activity to many auditory and visual stimuli, including the sight of food. During periods with no experimenter-induced sensory stimulation, mean discharge rate (averaged over periods of 5-30 seconds) increased during episodes of EEG desynchronization. When the monkey exhibited behavioral and EEG signs of drowsiness, LC neurons typically slowed to 0.1 to 0.5 Hz. During slow-wave sleep, periods of 5-30 seconds without any discharge activity were common. During these periods of decreased activity, presentation of a stimulus of sufficient strength or novelty to elicit EEG desynchronization and orienting movements usually resulted in a burst of 2-5 action potentials over a period of 200-700 msec. If the stimulus produced sustained alertness, LC neurons typically continued to discharge at 0.2 to 2 Hz until the animal again became drowsy or inattentive.

These preliminary observations suggest that, in squirrel monkey, LC may participate in controlling environmental surveillance. Studies supported by NIAAA Grant AA 03504.

849 CHANGES OF PROTEIN CARBOXYMETHYLASE ACTIVITY IN THE SUPERIOR CERVICAL GANGLION DURING POSTNATAL DEVELOPMENT AND AFTER AXONAL INJURY. G.M. Gilad, C. Gagnon* and I. J. Kopin, Laboratory of Clinical Science, NIMH, Bethesda, Maryland 20014

The activity of protein carboxymethylase (PCM) which catalyses the transfer of a methyl group from S-adenosyl-L-methionine to carboxyl side chains of proteins to form labile protein-methyl esters (Diliberto & Axelrod, Proc. Natl. Acad. Sci., USA, 71: 1701-1704, 1974), was examined in the rat superior cervical ganglion (SCG) and iris during postnatal development. In the SCG, PCM activity, 3.3 ± 0.3 (pmole methanol/10 min per ganglion) at birth, gradually increased reaching adult levels 28.3 ± 3, by the 30th postnatal day; an 8-fold increase which parallels the increase in protein. In the iris, one of the target tissues innervated by SCG neurons, PCM activity, 3.9 ± 0.09 at birth, has increased only about 4 fold , to 12.5 ± 0.75, during development, reaching adult levels by 40 d. The source of PCM activity in the iris is mainly in tissues other than sympathetic terminals, since 10 d after SCG removal, only 15% reduction could be detected in the ipsilateral iris as compared to contralateral controls. One day after postganglionic nerve injury (the nerve was crushed at a distance of about 5 mm from the ganglion) PCM activity has increased to 126% of control within the proximal nerve stump, suggesting an accumulation due to blockade of axonal transport or sprouting. In SCG no change in PCM activity could be detected at that time. However, 5 d after the injury, PCM activity fell to 62% of control, a decrease comparable in magnitude to that seen in the catecholamine synthesizing enzyme tryosine hydroxylase. Death of chromatolytic neurons may have contributed to the observed changes, but this is unlikely, since at 5 d postoperative protein content of the SCG has increased to 123% of control, presumably because of the net increase in (structural) protein synthesis known to occur during chromatolysis and regeneration. We conclude: (a) during postnatal development PCM activity in the SCG is gradually increased with growth to achieve adult levels 30 d after birth: (b) PCM may be localized in neurons of the SCG and transported down their axons; (c) PCM activity is reduced during the reaction to axonal injury as are other enzymes important in neuronal function, suggesting that PCM serves an active role in neuronal function.

850 ESTROGEN DEPENDENT ALTERATIONS IN TYROSINE HYDROXYLASE, GLUTAMATE DECARBOXYLASE AND LORDOSIS BEHAVIOR IN SEPTAL LESIONED MALE RATS CHRONICALLY EXPOSED TO ESTROGEN DURING THE POST LESION PERIOD. J. H. Gordon, D. M. Nance, C. J. Wallis* and R. A. Gorski. Dept. Anat. & Brain Res. Inst., UCLA, Los Angeles, CA 90024.

Septal lesions (SL) in female rats result in an increased sensitivity to the behavioral effects of acute estradiol benzoate (ACUTE-EB; 2 ug/day X 3) as measured by the lordosis quotient (LQ; number of lordotic responses X 100/number of mounts). Intact or castrated male rats do not show an enhanced LQ following ACUTE-EB unless they are chronically treated with 2 ug EB/day for 2-4 weeks immediately following a SL. The present study was undertaken to examine possible neurochemical alterations which could account for the enhanced behavioral sensitivity seen following ACUTE-EB treatment in the SL males chronically treated with EB during the post lesion period (SL-EB). The results of the LQ test (2 months post lesion, 1 month post chronic EB treatment) showed an enhanced response following ACUTE-EB in the SL-EB group (LQ = 44±6, n = 21) compared to that of SL males chronically treated with oil (LQ = 10±5, n = 16) or normal males (LQ = 0±0, n = 20). Six weeks following the LQ test the three groups were subdivided and treated with ACUTE-EB or OIL and decapitated. The brains were removed, frozen and stored at -50°C prior to dissection and assay of enzymatic activities. Tyrosine hydroxylase (TH) activity was assayed in the dopamine (DA) rich areas of the forebrain (striatum, STR; nucleus accumbens septi, ACB; and olfactory tubercle, OLT). The TH activity in the SL-EB given ACUTE-EB was significantly suppressed in both the STR and ACB relative to all other groups. The glutamate decarboxylase (GAD) activity in both the substantia nigra (SN) and ventral tegmentum (VTR) was significantly increased in the SL-EB given ACUTE-EB relative to all other groups. In summary, the SL-EB given ACUTE-EB showed 1) enhanced LQ, 2) decreased TH activity (DA terminals), and 3) increased GAD activity (DA cell bodies). These data correspond to those of SL females given ACUTE-EB which show 1) enhanced LQ, 2) decreased DA turnover, and 3) increased GAD activity, relative to sham animals given ACUTE-EB. Thus, a decrease in DA activity in both males and females is associated with an increased GAD activity in the region of the DA cell bodies (perhaps indicating greater inhibitory tone on the DA cell bodies). Since pharmacological and neurochemical data indicate that dopamine is inhibitory on the display of lordosis, it may be that the SL-EB animals acutely treated with EB have less inhibitory dopamine activity to overcome in order to display lordosis and thus, are capable of showing an enhanced behavioral response to the acute administration of EB. (Supported by HD 01182.)

851 IMMUNOHISTOFLUORESCENCE STUDY OF THE DISTRIBUTION OF EPINEPHRINE IN THE RAT CNS. L. Harvey, G.M.Brown, L. Grota. Dept. Neurosciences, McMaster Univ., Hamilton, Ont., Canada.

There is evidence in the literature that epinephrine (E) is both localized and synthesized within several regions of the CNS. This evidence stems from studies involving assay of E in tissue-punches from various brain regions and from studies on the distribution and activity of its synthetic enzyme phenylethanolamine-N-methyl transferase (PNMT).

In view of the major role played by the other catecholamines (CA) in CNS function, the potential importance of E in the CNS should not be overlooked. Unfortunately the methods currently available for visualizing the CA within nervous tissue (the Falck Hillarp & glyoxylic acid techniques) either work poorly for E or do not adequately distinguish it from norepinephrine. We are reporting an immunohistofluorescence technique which shows possibilities in this direction.

An antibody to synephrine, the mono-hydroxylated analogue of E has been prepared in our lab and has been found to cross-react with E and metanephrine but not with the other CA, including norepinephrine, and their derivatives.

We are now using this antibody in an immunohistofluorescence study of the distribution of E in the rat CNS using a double antibody technique. Rats (200-300 g., male or female) are killed by decapitation and the brains quickly removed onto dry ice. After cooling for 1 - 1½ hr., they are mounted in a cryostat and sectioned at -15°C, 10-12 μ thickness. Sections are picked up onto glass slides pre-cleaned in methanol. Slides are then incubated 30 min. with rabbit antiserum containing antibody to synephrine, washed, fixed briefly in 0.5% w/v paraformaldehyde, washed again, then incubated a further 30 min. with an antibody to rabbit γ-globulin tagged with FITC.

As controls, adjacent brain sections are incubated with normal rabbit serum at the same dilution as that containing the antibody or with antiserum pre-saturated with E.

Consistent staining has been found in several regions of the CNS on slides incubated with antiserum but not on control slides. Among them are several of the hypothalamic nuclei, the nucleus interstitialis of the stria terminalis, nucleus septi lateralis, amygdala, hippocampus, cingulate cortex and cerebellum. These results 1) tend to confirm the distribution of E as reported in the literature, and 2) do not correspond to the distribution of norepinephrine.

852 EVIDENCE FOR β-ADRENERGIC RECEPTORS IN CEREBRAL CAPILLARIES. Timothy J. Herbst*, Marcus E. Raichle and James A. Ferrendelli. Dept. of Pharmacol. and Dept. of Neurol. and Neurol. Surg. (Neurol.), Wash. Univ. Med. Schl., St. Louis, MO 63110.

There is ample evidence that adenylate cyclase is closely linked to or is a component of adrenergic and other receptors in several tissues and that cyclic AMP mediates some of the actions of neurotransmitters at specific receptor sites. Recent data indicate that cerebral capillaries are innervated by CNS adrenergic neurons. In addition, catecholamines and vasopressin modify CNS capillary function. To determine whether cerebral capillaries contain a catecholamine- and/or vasopressin-sensitive cyclic AMP system, we have studied the effects of these compounds and other drugs on cyclic AMP levels in isolated cerebral microvessels.

Microvessels were isolated from rat cerebral cortices by a standard procedure involving disruption of the neuropil, centrifugation through 25% albumin, and filtration on a bed of glass beads. The final tissue fraction, suspended in Krebs-Ringers bicarbonate buffer, contained mostly capillaries and accompanying pericytes, with slight contamination by muscular vessels, but with virtually no neural elements, as confirmed by darkfield or phase-contrast microscopy for each preparation. This tissue suspension was then incubated in the presence of various drugs, and cyclic AMP levels were measured by radioimmunoassay.

Basal levels of cyclic AMP in isolated microvessels were 2 - 3 pmoles/mg protein. Incubation of the tissue with 100 μM norepinephrine (NE) caused a 2- to 8-fold elevation of cyclic AMP levels within 5 min. In contrast, vasopressin (0.02 and 2.0 I.U.) had no effect. The effect of NE was dose-dependent with an ED_{50} of 3 μM. The phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (40 μM) increased basal levels of cyclic AMP by 50% but did not alter the response to NE. Epinephrine and isoproterenol also elevated cyclic AMP levels in cerebral microvessels, but phenylephrine, dopamine, serotonin and histamine did not. Propranolol (100 μM) completely blocked the response of NE, whereas an equal concentration of phentolamine did not alter its effect.

These results indicate that cerebral capillaries contain a cyclic AMP system which is regulated via β-adrenergic receptors and support the contention that cerebral capillary function is under neural control. The data also suggest that the effect of vasopressin on cerebral capillary function is not directly mediated by a cyclic AMP system.

(Supported in part by USPHS Grants NS-09667, NS-11059, NS-06833 and HL-13851).

853 RAPHE UNIT ACTIVITY IN FREELY MOVING CATS: CORRELATION WITH LEVEL OF BEHAVIORAL AROUSAL. Barry L. Jacobs and Michael E. Trulson. Prog. in Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08540.

Single unit activity (n=51) was recorded from the dorsal raphe nucleus of freely moving cats (n=12) by means of movable 32μ or 62μ dia nichrome wires. Virtually all the cells discharged with the slow regular pattern that characterizes raphe neurons recorded in vivo and in vitro. Across the sleep-waking cycle, these cells displayed a strong positive correlation with level of arousal, as defined by both behavioral and electrographic criteria. From a mean baseline firing rate of 2.82 spikes/sec during quiet waking, the mean discharge rate of these cells phasically increased by 112% in response to an auditory stimulus. This effect rapidly habituated with repeated stimulus presentations. During waking accompanied by movement, unit activity was significantly increased by 22% as compared to quiet waking, but there was no correlation between unit activity and gross body movements. Raphe unit activity showed a significant decrease of 17% during drowsiness (first appearance of EEG synchronization) as compared to quiet waking, and then progressive decreases during the early (-34%), middle (-52%), and late (-68%) phases of slow wave sleep. During all phases of slow wave sleep, the occurrence of sleep spindles was correlated with a significant decrease in unit activity. Raphe unit activity showed decreases of 81% during pre-REM (the 60 sec immediately before REM onset) and 98% during REM, as compared to quiet waking. Unit activity reappeared 3.2 sec before the end of REM, with significant increases in unit activity of 83% and 17% during the first sec and first 10 sec, respectively, as compared to quiet waking. These data, in conjunction with a large behavioral literature, indicate that the role of CNS serotonin may be compensatory to behavioral arousal, and furthermore, that it may play a modulatory, rather than a mediative role in behavioral and physiological processes. (Supported by NIMH grant MH-23433).

854 ONTOGENY OF UPTAKE AND RELEASE OF ^{3}H-CATECHOLAMINES IN BRAINS OF RATS NEONATALLY EXPOSED TO LEAD. Kathryn M. Jason* (Spon: B. Weiss). University of Rochester, Rochester, NY 14627.

In vitro uptake and release studies were carried out in 15 and 35 day old rats that had been exposed to 0, 25, or 75 mg/kg lead acetate on postnatal days 2 through 14 by oral intubation. Striatal and cortical minces were incubated with 10^{-7} M ^{3}H-dopamine (^{3}H-DA) or ^{3}H-noradrenaline (^{3}H-NA), respectively. Lead treatment had no effect on uptake of ^{3}H-NA in the cortex. However, uptake of ^{3}H-DA in the striatum was altered by lead at both 15 and 35 days. At 15 days, less ^{3}H-DA was present in striatal tissue from the high lead group after 5 or 20 minutes incubation, indicating lead-induced changes in the properties of uptake and retention processes. At 35 days, more ^{3}H-DA was present in tissue of the high lead group animals than controls after 20 minutes incubation and may be the result of decreased competition from lower DA levels in that group. Lead treatment had no effect on potassium-induced release of tritiated amines. In the absence of calcium, however, there was a lead-related increase in spontaneous release of ^{3}H-NA from the cortex, resulting in decreased evoked release. Lead may alter intracellular binding sites for NA, or may act to replace the calcium necessary for spontaneous release.

(Supported in part by NIMH grant MH-11752, and NIH grants NS 10777, ES 01247, and ES 01248)

855 CHOLINERGIC, DOPAMINERGIC AND GABA-ERGIC INTERACTIONS IN THE NUCLEUS ACCUMBENS AND GLOBUS PALLIDUS AFFECTING AMBULATORY ACTIVITY. D. L. Jones, M. Wu* and G. J. Mogenson, Dept. Physiol. Fac. Med., Univ. Western Ontario, London, Canada N6A 5C1.

The nucleus accumbens (N.Ac.) receives dopaminergic projections from the ventral tegmental area (VTA) of the midbrain which have been implicated in ambulatory activity. Increased ambulatory activity is produced by injections of amphetamine, L-DOPA or dopamine (DA) into the N.Ac., and destruction of the VTA-N.Ac. pathway attenuates amphetamine stimulated hypermotility. The N.Ac. also receives cholinergic projections and contains cholinergic and GABA-ergic neurons. There is recent evidence of an efferent GABA-ergic projection to the globus pallidus (GP). The following experiments investigated the interaction of cholinergic and dopaminergic synapses within the N.Ac. in the control of ambulatory activity. The experiments also investigated the contribution of the suggested GABA-ergic pathway to the GP to ambulatory activity.

The administration of DA (10 and 20 µg) into the N.Ac. increased ambulatory activity in an open field test. The combined administration of DA and carbachol (cholinergic agonist, 5 and 10 µg) into the N.Ac. elicited an increase in ambulatory activity which was more rapid in onset than that elicited by DA alone. However, the administration of atropine (cholinergic-muscarinic blocker, 5 and 10 µg) together with DA into the N.Ac. did not attenuate the DA-stimulated increase in ambulatory activity. Ambulatory activity was increased when picrotoxin (GABA blocker, 0.225 and 0.75 µg) was administered into the GP. The administration of GABA (2.25 µg) into the GP together with DA into the N.Ac. attenuated the DA-stimulated increase in ambulatory activity.

These data suggest the following conclusions. (1) There does not appear to be a cholinergic interneuron between the N.Ac. - DA neuron and the effector pathway responsible for the DA-stimulated ambulatory activity. (2) Cholinergic synapses within the N.Ac. appear to modulate the DA-stimulated ambulatory activity, perhaps through an inhibitory interneuron. (3) The GP plays a role in locomotor behavior and may be part of the effector pathway. (4) The N.Ac. may communicate with the GP via a GABA-ergic pathway.

(Supported by the Medical Research Council of Canada. DLJ is the recipient of a Medical Research Council Fellowship).

856 LOCUS COERULEUS NEURONS IN FREELY BEHAVING RATS EXHIBIT PRONOUNCED ALTERATIONS OF FIRING RATE DURING SENSORY STIMULATION AND STAGES OF THE SLEEP-WAKE CYCLE. G. Jones*, S.L. Foote, M. Segal[1] and F. Bloom. A.V. Davis Ctr. for Behav. Neurobiology, The Salk Inst., La Jolla, CA 92037.

The nucleus locus coeruleus (LC) in the rat is a highly compact group of noradrenergic neurons from which a uniquely divergent efferent system of axons arises. Pharmacological, physiological and behavioral observations have generated many hypothetical functions of these neurons which can best be evaluated in the freely behaving animal. We are currently investigating the changes in discharge rate and pattern in neurons of the unrestrained albino rat LC in relation to three variables: 1) stimulation of specific sensory modalities; 2) spontaneous sleep-wake cycle alterations; and 3) freely occurring behaviors.

Single and multiple cell recordings were obtained from freely moving rats chronically implanted with cortical EEG leads, subdermal neck EMG leads, and a stimulating electrode in the ipsilateral cingulum bundle. As reported by others and confirmed in our preliminary experiments, some LC cells can be antidromically activated from the ipsilateral cingulum bundle; however the most useful criteria we have found for tentative LC neuron identification include their unique spontaneous firing and sensory response patterns. All recordings sites were confirmed by subsequent histology.

Both multi-unit and single unit data indicate that LC neurons exhibit pronounced increases in spike rate from basal resting levels of about one to ten H_z, with a latency usually between twenty and fifty msec. in response to gross auditory or visual stimulation; qualitative tactile stimulation experiments give similar results. Most LC cells showed multi-modal responsivity. The sensory evoked activation was usually followed by a transient absence of spikes with a gradual return to basal rates, over the next 200 to 1000 msec. LC cells showed no obvious correlations with overt motor behavior. Qualitative observations indicate these responses do habituate, usually beginning within five presentations of the same stimulus.

During recordings which persisted throughout sleep waking cycles, discharge rates were highest during the transitions between either slow wave sleep (SWS) and waking (W) or between rapid eye movement sleep (REM) and W, with rate increases preceding the SWS to W transition, but not the REM to waking transition. Spontaneous rates in waking rats otherwise were highest, with only occasional spikes during SWS and virtually no firing during REM.

These preliminary observations indicate the feasibility of testing hypotheses of LC function based upon studies of discharge frequency during manipulations of the freely moving rat. (Supported by NIAAA Grant AA 03504; [1]Weizmann Institute, Rehovot, Israel.)

857 INHIBITION OF THE SYMPATHETIC PREGANGLIONIC NEURONS BY CATECHOLAMINES. K. Kadzielawa. University of Florida, College of Medicine, Dep. Pharmacol. Therap., Box J-267, Gainesville, Fla 32610.

The purpose of this study was to assess the effects of catecholamines: norepinephrine (NE), epinephrine (E), dopamine (DA) and α-methylnorepinephrine (α-MNE) on the activity of the sympathetic preganglionic neurons (SPGN) located in the thoracic (T2-T4) segments of the spinal cord. These neurons are the main central sympathetic output station regulating heart rate and blood pressure.

Experiments were performed on cats anesthetized with a mixture of chloralose and urethane as well as in the cerveau isolé preparation. SPGN were identified with antidromic stimulation of the white rami of the stellate ganglion. The rate of the spontaneous or D,L-homocysteate induced firing of these neurons was analyzed. Catecholamines were applied by means of microiontophoresis from seven barrel micropipettes.

The activity of some of the SPGN was attenuated by NE, E and DA. α-MNE demonstrated a clear-cut, delayed and prolonged inhibitory action on the majority of spontaneously firing neurons and decreased the response to the excitant amino acids (homocystate and glutamate) in some of them. This inhibitory action of α-MNE may contribute to the antihypertensive action of α-methyldopa, which was shown recently to inhibit spinal sympathetic reflexes (Baum, T. & Shropshire, A. T., Eur. J. Pharmacol., 1977, 46, 259-263). Fuxe and his group (Hökfelt, T. et al., Brain Res., 1974, 66, 235-251) have demonstrated a dense net of axon terminals exhibiting PNMT activity in the areas of location of SPGN. Further study will indicate whether E may serve a role of an inhibitory neurotransmitter released from the terminals of descending axons of brain stem neurons.

The early report by DeGroat, W.C. and Ryall, R.W. (Exper.Brain Res., 1967, 3, 299-305) indicated that serotonin demonstrates excitatory action while NE inhibits the activity of some of PGN.

858 IMMUNOHISTOCHEMICAL DEMONSTRATION OF CATECHOL-O-METHYLTRANSFERASE IN MAMMALIAN BRAIN. Gary P. Kaplan*, Boyd K. Hartman and Cyrus R. Creveling*. Depts. of Psychiatry and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110 and Laboratory of Chemistry, NIAMDD, Bethesda, MD 20014.

The immunofluorescent demonstration of catechol-o-methyltransferase (COMT) (EC 2.1.1.6) was obtained in rat liver and kidney and in several areas of rat, chinchilla, and bovine brain. Tissues were fixed for 5 to 10 days in 4% formaldehyde followed by equilibration with a 15% sucrose solution. A specific antiserum prepared against rat liver COMT was used on frozen tissue sections in an indirect immunofluorescent method. Optimal immunofluorescence was obtained after a 48 hour incubation of the tissue sections in a 1:500 dilution of the antiserum, followed by incubation with fluorescein isothiocyanate conjugated anti-rabbit globulin. Controls consisted of sections prepared as described above, with normal rabbit serum replacing the specific antiserum in the first incubation. Control sections showed only trace amounts of non-specific fluorescence.

In the brain, all specific fluorescence was present in extraneuronal cellular elements. Ventricular ependymal cells and cells of the choroid plexus exhibited the greatest intensity of immunofluorescence. Glial immunofluorescence appeared most prominently in large fiber tracts including the corpus callosum and internal capsule. Interfascicular and perineuronal satellite oligodendrocytes as well as fibrous astrocytes were immunoreactive, though immunofluorescence of myelinated axons was not seen. In addition, Bergmann's glial cells in the cerebellum stained brightly for COMT. The presence of small quantities of COMT in neurons cannot be excluded. However, the pattern of localization observed in the extraneuronal elements suggests that this enzyme may function as a barrier to free diffusion of catechol compounds within the central nervous system.

859 SYNAPTIC STRUCTURES IN THE INTERMEDIOLATERAL NUCLEI OF THE THORACIC SPINAL CORD AND THE DORSAL MOTOR NUCLEUS OF VAGUS OF THE RAT, WITH SPECIAL REFERENCE TO MONOAMINERGIC ELEMENTS. Makoto Kato*, Yuji Yaku*, and Tanemichi Chiba (SPON: S. Kawamura). Dept. Anat., Sch. Med., Chiba University, Chiba 280, Japan.

Intermediolateral nuclei(IML) of the thoracic spinal cord and the dorsal motor nucleus of vagus(DMV) were examined by glyoxylic acid induced monoamine fluorescence and electron microscopy. IML and DMV are thought to be the site of localization of preganglionic sympathetic and preganglionic parasympathetic neuronal somata respectively. Both IML and DMV are well known as one of the nuclei which are densely innervated by catecholaminergic neurons and are also known as the final station of the cardiovascular reflex pathway in the central nervous system.

In the present study, synaptic structure was analysed in the following four groups of adult rats. 1) normal control 2) treated with 5-hydroxydopamine 3) treated with 6-hydroxydopamine 4) fixed by 3% potassium permanganate.

In the IML, axon varicosities with flat synaptic vesicles occupied 27.8 ± 1.0% of the total number of axon varicosities in 3,400 sq. μm area. Serial synapses were encountered in some of which the first presynaptic side was identified as catecholaminergic. Catecholaminergic axon varicosities often showed typical synaptic contacts to both dendrites and soma. Fluorescence microscopy revealed that catecholaminergic nerve bundles are seen to extend both laterodorsally and medially from the nucleus.

In the DMV, axon varicosities with flat synaptic vesicles were rarely encountered. Serial synapses were also rare. Catecholaminergic axons were found to be about 12% of total number of axons in 3,400 sq. μm area. The population of catecholaminergic nerve terminals was larger in DMV than in the nucleus tractus solitarius(2.40 ± 0.09%) in accordance with the fluorescence microscopic observations. Most catecholamine neurons(A2) were located in the latero-caudal portion of the nucleus tractus solitarius.

From the present results, it was confirmed that a considerable number of catecholaminergic axons form synapses in both IML and DMV. Modulatory(most probably inhibitory) function of catecholamine neurons to IML and DMV could be suggested as presynaptic and postsynaptic respectively from the present observations.

860 THE BEHAVIORAL EFFECTS OF AMPHETAMINE ARE CORRELATED WITH ITS EFFECTS ON cAMP IN DIFFERENT BRAIN REGIONS. Linda A. Kennedy* and Michael J. Zigmond (Sponsor: B. Dixit). Department of Pharmacology, School of Pharmacy, and Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

In the rat, low doses of amphetamine produce behavioral activity characterized primarily by increased locomotion, while high doses produce stereotyped behavior consisting of intense sniffing and licking in a restricted area of the cage. Both of these behavioral effects appear to result from the release of dopamine (DA) since they are prevented by pretreatment with DA receptor antagonists. The two types of behavioral responses appear to result from the stimulation of dopamine receptors at two different sites in the CNS, locomotion from DA release in nucleus accumbens and stereotypy from DA release in striatum. DA stimulates adenylate cyclase in vitro, and DA agonists have been shown by some investigators to elevate endogenous cAMP levels in vivo. We therefore attempted to determine whether cAMP levels would increase in brain following amphetamine administration and, if so, whether the regional distribution of this increase would correlate with the presumed regional distribution of DA release after low and high doses of the drug. Rats injected with amphetamine were sacrificed 15 min later by 4-sec microwave irradiation (2.5 kW, 2.45 mHz). Striatum and frontal cortex were dissected from the brain and cAMP was measured using a protein-binding assay. Frontal cortex was analyzed since it receives a dopaminergic innervation from the same mesencephalic cell group as nucleus accumbens, but consists of a larger tissue mass. Following 3 mg/kg amphetamine, the concentration of cAMP in frontal cortex was increased by 27% ($p<.01$), while striatal content was unaffected. At 10 mg/kg, amphetamine increased the cAMP content of both frontal cortex (39%, $p<.01$) and striatum (32%, $p<.01$). Treatment with fluphenazine (0.5 mg/kg, s.c.) 30 min prior to amphetamine prevented the cAMP increase induced by 10 mg/kg amphetamine in both brain regions. Additional animals were given identical amphetamine treatments and behavior was monitored 30-60 min later. Rats given 3 mg/kg amphetamine showed an increase in locomotor behavior while rats given 10 mg/kg showed an increase in stereotyped behavior. Both behaviors were blocked by fluphenazine (0.5 mg/kg, s.c.) pretreatment. Thus, locomotor behavior was correlated with elevated cAMP levels in frontal cortex, while stereotypy was associated with elevated cAMP levels in the striatum. Both the cAMP increase and the behavioral effects were blocked by pretreatment with fluphenazine. These findings are consistent with the hypothesis that the two types of behaviors elicited by amphetamine may involve different brain regions. They further suggest that fluphenazine-sensitive elevations in cAMP may be useful as an index of DA release.

(Supported, in part, by USPHS grants MH00058 and MH29670).

861 FLUORESCENCE MICROSCOPIC MAPPING OF SUBSTANTIA NIGRA DOPAMINE SOMATA AND THEIR DENDRITES: RELATION TO DOPAMINE AND NON-DOPAMINE THIONIN-STAINED CELLS IN IDENTICAL VIBRATOME SECTIONS. Haing-Ja Kim and Aryeh Routtenberg. Cresap Neuroscience Lab. Northwestern University, Evanston, Illinois 60201.

Substantia nigra (SN) dopamine cells (A9 group) have been implicated in various behavioral functions including the motor syndrome of Parkinson's disease. Distribution of these A9 cells and their dendritic varicose processes was therefore studied by taking advantage of the formaldehyde-Vibratome fluorescence method (Hökfelt and Ljungdahl, 1972). In order to identify dopamine (DA) cells amongst all SN cells, sections from various coronal levels of SN were first treated for catecholamine fluorescence and subsequently Nissl-stained as described previously (Collier and Routtenberg, 1977). This formaldehyde-Vibratome method successfully demonstrated the fluorescence of both DA cell bodies and their fine varicosity-containing processes in rats pretreated with nialamide (300 mg/kg). That the fine fluorescent varicose processes originate from A9 DA cells was clearly seen in both normal and SN-6 OH DA-treated (with desmethylimipramine pretreatment) material. Comparison of fluorescence and thionin photomicrographs prepared from identical sections disclosed that many fine fluorescent varicose processes seen in the pars reticulata (SNR) were indeed dendritic, as opposed to axonal. In the anterior third of SN, cells which stained with thionin were densely distributed in the pars compacta (SNC), while SNR cells were rather sparse. The majority of those SNC cells were dopaminergic and their ventrolaterally oriented processes formed dendritic bundles. As SN expanded caudally, there was a noticeable increase in SNR cells. SNC-DA cells tended to divide into two subgroups, namely medial and lateral. At the level of the exit of the oculomotor nerve, a considerable number of non-DA cells were observed among the medial SNC-DA cells and immediately below the DA cell layer. At this level, the medial SNC-DA cells could no longer be delineated from A10 DA cells. Those A9 DA cells, however, tended to lie parallel to the transverse course of SNC. Hence, their fine dendritic varicose processes were seen to run first dorsolaterally towards the lateral SNC cells. Some of these processes, then, turned ventrally as if to bathe small non-DA cells, presumably interneurons, which lie below the A9 DA cells. Perforating through this layer of small neurons, these dendritic processes further extended adjacent to the large deeply-stained (Nissl) non-DA cells present in the middle or ventral layer of SNR. The present results thus suggest a close association between the A9 cells and non-DA pars reticulata neurons via the long dendritic varicose processes emanating from A9 DA cells in SNC. (Supported by MH 25281 and NSF 19388 to A. R.)

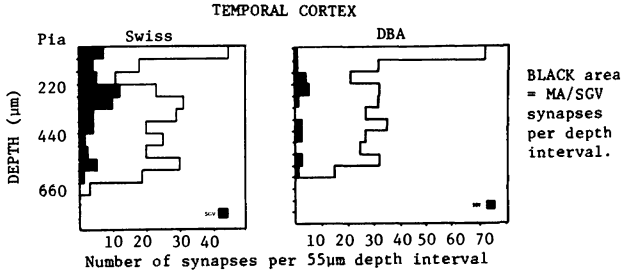
862 A SERIAL ULTRATHIN SECTION ANALYSIS OF MONOAMINERGIC NERVE FIBERS IN THE SUPERIOR CERVICAL GANGLION OF THE RABBIT. Hisatake Kondo* (SPON: G.D. Pappas). Dept. Anat., Sch. Md., Tohoku Univ., Sendai, JAPAN and Dept. Anat., Coll. Md., Univ. Illinois, Chicago Illinois 60680.

It is known that fluorescent varicose fibers are present in mammalian superior cervical ganglion (SCG). Some authors consider these fibers to arise from the ganglionic SIF (small intensely fluorescent) cells and to make synapses onto the principal neuron, which participate in the generation of slow IPSP. However, this consideration has not been confirmed in electron microscopy. In the SCG of rabbits pretreated with 5-hydroxydopamine, thickened neuronal profiles packed with numerous small granular vesicles (45 nm in mean diameter) characteristic of the monoaminergic nerve fibers were found to make synapses onto principal neurons, mostly on their processes but occasionally on their soma. The proportion of synapses formed by these neuronal profiles to those by cholinergic preganglionic neuronal profiles were about 3:19 in random sections covered about 40,000 μm^2 in which about 15 principal neurons sectioned through the nucleus were present. Monoaminergic neuronal profiles and cholinergic neuronal profiles were frequently enclosed by a common Schwann cell and both formed synapses in series onto a single process of the principal neuron. Occasionally these two neuronal profiles were directly apposed to each other without any membrane specializations. These monoaminergic neuronal profiles survived the preganglionectomy. Five monoaminergic neuronal profiles were followed up to 100 μm in maximum length using 300-500 serial ultrathin sections. Following the thickened vesicle-containing portions (1.0-1.5 μm in diameter and 1.5-2.0 μm in length), the neuronal profiles changed their diameter abruptly into 0.1-0.2 μm and remained thin except for a few thickened portions along their course. The thin portions contained several neurotubules and filaments but few, if any, granular vesicles. No ribosomes were encountered in any of the monoaminergic nerve fibers. They sent no branches and received no synaptic inputs along their course and eventually extended beyond the long series of serial sections. No cytoplasmic processes showing features of SIF cell processes were seen in the present materials.

These results suggest that the monoaminergic nerve fibers are derived not from ganglionic SIF cells but from principal neurons (possibly their axons) and that these fibers are more likely candidates for the generation of slow IPSP than the ganglionic SIF cells.

863 MONOAMINERGIC CORTICAL CIRCUITRY IN AUDIOGENIC SEIZURE-PRONE MICE: A MORPHOLOGIC STUDY. Donald A. Kristt and Mark S. Shirley*. Dept. Pathol., The Johns Hopkins Univ. Sch. Med., Baltimore, Md. 21205.

Audiogenic seizures in DBA/2J mice begin postnatally (pn) and are associated with decreased brain MA levels (mostly norepinephrine, NA). We studied synapses in temporal cortex (TC), presumed primary auditory area, and somatosensory cortex (SC) at 6 dpn, when MA synapses reach (relative) peak numbers. All synapses were counted and depth recorded in 2.5μ panels extending from pia to white matter. Swiss and C57 mice were used as controls. MA synapses were identified with a cytochemical marker, 5OH-dopamine, which produces small granules in MA synaptic vesicles (SGV). MA synapses in TC were decreased in number (3-10% of total vs. 20% in controls) and lacked a specific laminar distribution as seen in DBA-SC and control TC and SC; SC MA synapses were comparable to controls (15-20% of total). In DBA the laminar distribution of all counted synapses was comparable to controls and total number of synapses was within normal limits, in both TC and SC (v. Figs.). Since systemically administered 5OHDA does not mark cortical synapses in older animals, glyoxylic acid induced histofluorescence was used to study 21 dpn (most sensitive to auditory stimuli) and adult (generally resistant) mice. In DBA the number of catecholamine fibers was dramatically reduced in most neocortical fields (caudal > rostral) at 21 dpn,but by adulthood it was comparable to controls. The neostriatum which predominantly receives a dopaminergic innervation, was equivalent to controls at all ages. Hence, there is evidence for abnormal MA (probably NA) cortical circuitry which is apparent by 6 dpn and which persists for several weeks; substantial "recovery" is seen by adulthood. We propose that since MA synapses generally inhibit neuronal excitability, the paucity of MA innervation and synapses in TC (or impaired MA uptake/storage in MA terminals) may play an important role in the susceptibility of DBA mice to audiogenic seizures. (Support by NIH NS00279, TIA.)



864 **PITFALLS IN ASSESSING BRAIN MONOAMINE METABOLISM IN MAN.** C.R. Lake, M.G. Ziegler* and M.H. Ebert.* NIMH, Bethesda, MD 20014

Abnormal central nervous system (CNS) monoamine neurotransmission is implicated in several neurological and psychiatric illnesses. In an attempt to evaluate the brain activity of some of these amines, lumbar spinal taps are performed and metabolites of the amines measured, often utilizing the probenecid technique. There are several potential sources of error in interpretations of some of these studies. Recently one of these neurotransmitters, norepinephrine (NE), has been measured in cerebrospinal fluid (CSF). Probenecid, used to block active transport of acid metabolites of dopamine (DA) and serotonin (5-HT) from CSF to blood, caused a significant elevation in NE ($p < 0.005$). This implies an effect of probenecid on the synthesis, release, or metabolism of NE itself, and raises a question of the validity of the method when applied to NE or its metabolites.

There is a gradient in CSF of NE which increases 31% from the 1st 4cc out of the lumbar tap needle to the third 4 ml aliquot. However, there is no significant further rise after the 1st 12cc. For comparison of NE levels in CSF, careful control of volumes of CSF removed is required.

There were no differences between CSF levels of NE from the same individuals whether on a low- or high-monoamine diet.

Since NE levels in CSF and plasma correlate positively with age, it is important to control this variable by either age matching or age correction of the NE values.

There is a circadian rhythm in levels of NE in CSF from rhesus monkeys with significantly higher levels from 12 noon to 6 p.m. daily than from 9 p.m. to 9 a.m.

Thus when analyzing CSF for monoamine neurotransmitters or their metabolites, age of subjects, stress factors, time of removal, and volume of CSF removed must be adequately controlled.

865 MONOAMINERGIC PRECURSOR INFLUENCES ON INTERMALE AGGRESSION IN MICE. S. M. Lasley, J. B. Thurmond, and J. W. Brown*. Neuropsychopharm. Program, Univ. of Louisville, Louisville, Ky. 40208

The monoamine precursors L-tyrosine, L-phenylalanine, and L-tryptophan were administered by diet and measures of aggressive behavior, locomotor activity, and endogenous levels of the monoamines and precursors recorded. Male CF-1 mice were maintained on a semi-synthetic 12 percent casein protein diet for 2 weeks, then switched to diets modified by the addition of a 1, 2, or 4 percent L-amino acid supplement, or 0 or 4 percent casein (controls). Measures of aggressive behavior and locomotor activity were obtained before and after the dietary supplements were administered. Resident mice fed high supplements of L-tyrosine displayed a marked increase in the number of attacks on intruders and shorter attack latencies compared to controls, but their locomotor activity was unaffected. High L-phenylalanine supplements, alone or in combination with L-tyrosine reduced the latency to attack and increased motility but did not affect the number of attacks. As a whole, the group of animals fed L-tryptophan showed no changes in aggression or motility. Thus, the dietary regimens, which were designed to enhance catecholaminergic and serotonergic functioning, were found to differentially affect the territorial-induced attacks in mice.

Three days after the second aggression test half the animals in a group were sacrificed for whole brain analyses of norepinephrine, dopamine, serotonin, and the three L-amino acids in order to determine the amount of the precursor available to the monoaminergic neurons and any changes in monoamine concentration resulting from the diet treatment. Dopamine was found to be the most labile amine, increasing significantly with high supplements of tyrosine or phenylalanine and decreasing significantly with high supplements of tryptophan. Brain precursor levels generally reflected: 1) the level administered in the diet; 2) the effect of competitive uptake into the neurons as a result of having a common carrier protein. It appears then that some measures of functional activity (synthesis, turnover) may provide important additional information. (Supported in part by NIMH grant no. MH26677.)

866 DEVELOPMENTAL ORGANIZATION OF SEROTONIN-CONTAINING CELL GROUPS WITHIN THE BRAINSTEM RAPHE OF THE RAT. Pat Levitt* and Robert Y. Moore, Dept. Neurosciences, Univ. Calif., San Diego, La Jolla, Ca. 92093.

Recent autoradiographic and horseradish peroxidase studies from our laboratory (Fallon and Moore, this volume) have indicated that some cells of the fused midline raphe nuclei in the mesencephalon project ipsilaterally to forebrain structures. These same nuclei contain serotonergic neurons. Fluorescence histochemical studies were undertaken to determine whether the serotonin-containing neuron groups extending from the mesencephalon through the medulla develop as bilateral structures, and the time at which the midline cell groups eventually fuse.

A sensitive perfusion-freeze dry technique (Loren, I. et al, 1976, Brain Research 117, 313-318) was used for histochemical analysis of rat fetuses and pups. The procedure does not require drug pretreatment of the animals for the visualization of an intense serotonin fluorophore. Serotonergic cells first fluoresce on embryonic days 12-14 (Olson, L. et al, 1972, Z. Anat. Entwickl. -Gesch. 137, 301-316). At embryonic day 17, all the serotonin cell groups are arranged as paired nuclei. Most of the neurons are tightly packed and their long axis is oriented in a dorsoventral or mediolateral direction. Cell groups B3, B6, and B9 (Dahlstrom and Fuxe nomenclature) remain bilaterally oriented into adulthood, although the neurons become more loosely packed through postnatal week one. Those midline groups which are unpaired in adulthood (B1, B2, B4, B5, B7, B8) fuse in a rostrocaudal gradient. On postnatal day one (P1), the B7 group is completely fused, B8 is partially fused, while the caudal pontine and medullary cells remain paired. On P3, all the midline groups have fused except B1 and B2, which have undergone a partial fusion. The two caudal groups fuse by P6.

The results reveal that all the fused adult serotonergic neuron groups develop as bilateral structures, undergoing a primary migration from the ventricular zone to the midline prenatally, and a secondary migration to form single midline cell groups postnatally. This migration occurs in a rostrocaudal direction, comparable to the gradient of the first observable serotonin fluorescence. The bilateral origins may be maintained in the adult, as expressed by the apparent ipsilateral projections of some of the raphe neurons. Supported by USPHS Grant NS-12080.

867 EFFERENT PROJECTIONS OF THE LOCUS COERULUS. A.D. Loewy and C.B. Saper, Depts. Anat. & Neurobiol. and Medicine, Wash. Univ. Sch. Med., St. Louis, MO 63110

Although different facets of the projections of the locus coerulus (LC) have been studied using autoradiographic, immunohistochemical, and fluorescence histochemical techniques, no attempt has been made to characterize the total efferent output of this nucleus. We have placed small injections of ^{3}H amino acids in and around the LC in rats and after 5 to 7 days survival the brains and spinal cords were processed using the autoradiographic method. Ascending fibers travel primarily through the dorsal noradrenergic bundle to innervate a variety of structures in most respects similar to that described by Jones and Moore ('77). Descending fibers travel in the bundle of Probst in the dorsal part of the reticular formation ventral to the vestibular nuclei and turn ventrolaterally along the medial edge of the spinal trigeminal nucleus to descend further in the ventrolateral medulla. Other labeled fibers are seen in the paramedian reticular formation and raphe magnus nucleus, and immediately lateral to the area postrema and dorsal to the central canal. Descending fibers reach the spinal cord primarily via the lateral funiculus and seem to end mostly in the ventral horn. The projections of the LC correspond with many, but not all, of the dopamine-beta-hydroxylase containing pathways described by Swanson and Hartman ('75). Two notable exceptions are the noradrenergic projections to the supraoptic nucleus of the hypothalamus and the dorsal accessory olive which do not appear to arise from the LC. (Supported by USPHS grant #12751 and American Heart Association grant #77 797)

868 CENTRAL SEROTONINERGIC AND DOPAMINERGIC MODULATION OF RESPIRATORY DRIVE. D.B.A. Lundberg*, G.R. Breese and R.A. Mueller. Depts. Anesthesiol., Psychiat. and Pharmacol., Biological Sciences Research Center, Univ. of North Carolina Sch. Med., Chapel Hill, NC 27514.

Armijo and Flórez have reported (Neuropharmacol. 13:977, 1974) that 5-hydroxytryptophan (5HTP) depresses respiration in cats, and that this effect can be blocked by the inhibition of central aromatic amino acid decarboxylase activity. The present investigations attempted to assess the importance of central serotonin and dopamine containing neurones in the control of ventilation.

Sprague-Dawley rats, 250-300g, were lightly anesthetized with ether to permit tail artery and tracheal cannulation, and were placed in a whole body plethysmograph to permit recording of respiratory rate and depth. Anesthesia was maintained with 0.7% halothane in oxygen.

Graded doses of 5HTP (3.1-25mg/kg, i.p.) in pargyline pretreated rats (50mg/kg 40 min before 5HTP) produced a progressive depression of tidal volume and minute respiration with little change in respiratory frequency. These effects were antagonized by the administration of 10mg/kg methysergide. Pretreatment of newborn rats with 5,7-dihydroxytryptamine and pargyline to selectively reduce CNS serotonin content magnified the degree of respiratory depression produced by 5HTP administration when adult.

Apomorphine (1-10mg/kg, i.p.) produced a dose dependent increase in respiratory frequency and minute ventilation and a slight decrease in tidal volume. Haloperidol (2mg/kg, i.p.) was able to antagonize the apomorphine-induced increase in respiratory frequency. Neonatal treatment with 6-hydroxydopamine to reduce brain dopamine and norepinephrine content produced an increased response to apomorphine when adult.

These results suggest that central dopaminergic and serotoninergic neurones may modulate respiratory activity by influencing the respiratory frequency and tidal volume of ventilation.

869 2-PHENYLETHYLAMINE UPTAKE BY RABBIT RED BLOOD CELLS. Michael F. Mason* and Aron D. Mosnaim. Dept. of Pharmac., Univ. of Health Sciences/Chicago Med. Sch. & Sch. of Grad. and Postdoctoral Studies, Chicago, IL 60612.

2-Phenylethylamine (PEA), a biogenic amine postulated to play a role in central synaptic transmission, readily crosses the blood-brain barrier. Thus, variations in its free plasma concentration could result in significant changes in its brain levels and be ultimately reflected in central adrenergic and/or phenylethylaminergic mechanisms (Non-catecholic PEA's. Marcel Dekker, Inc., New York, 1978). In this work, we investigated the factors governing the PEA content of red blood cells (RBC). Rabbit blood, drawn from the carotid artery into heparin, was centrifuged (220 x g, 15 min.), the RBCs were washed with Ca^{++} free modified Ringer's and resuspended in modified Ringer's. The resultant mixture was incubated (37°C) with varying concentrations of TLC purified ^{3}H-PEA ($1x10^{-9}M$-$1x10^{-2}M$). After incubation, the RBCs were rapidly separated, digested with 70% perchloric acid, decolorized with 30% H_2O_2 and counted. The buffer fraction was also assayed for ^{3}H and ^{14}C. In all the experiments C^{14} sucrose was added as a plasma marker; all values were corrected to hematocrit of 50%. Percent uptake of PEA was relatively constant, 58% of the total recovered radioactivity over the range of 10^{-9} to $10^{-4}M$ (n=86). Lysis occurred at higher concentrations. Uptake was constant at 2', 10' and 30' incubation, showing some decrease at 180'. RBC lysates (freeze-thaw method) exhibited a $conc._{RBC}/conc._{Medium}$ ratio of 1 (n=26). In Na^{+} free medium where the Na^{+} was replaced by glucose, the percentage of recovered radioactivity in the erythrocytes was lowered to about 44% (c_{RBC}/c_{Me}=0.79). Preincubation with $10^{-5}M$ (n=38, 30 min.) iodoacetamide or $10^{-7}M$ 2,4-Dinitrophenol (n=6, 30 min.) had no effect. In a glucose free (sucrose substituted) medium the c_{RBC}/c_{Me} was unchanged. Uptake showed no temperature dependence (6° incubation compared to 37°C). In a similar manner, uptake of ^{3}H-d-amphetamine ($1x10^{-5}M$) was about 60% (n=4). This work suggests that PEA is rapidly taken up into erythrocytes by passive diffusion with a small component of Na^{+} dependent uptake. It also indicates that erythrocytes play an important role in regulating the levels of free plasma PEA. Further studies are necessary to elucidate the possible physiological importance of these findings. Supported in part by NIH General Research Support Grant FR-3566 and by Univ. of Health Sciences/Sch. of Grad. & Postdoc. Studies.

870 NORADRENALINE - ACETYLCHOLINE INTERACTIONS IN BRAIN: BEHAVIOURAL FUNCTIONS. Stephen T. Mason* & Hans C. Fibiger, Dept. Psychiatry, Univ. British Columbia, Vancouver, Canada.

It is well established that a noradrenergic-cholinergic interaction occurs in the peripheral nervous system and biochemical evidence suggests that such may also exist in the central nervous system. To elucidate the behavioural function of this interaction forbrain noradrenaline (NA) was severely depleted by intracerebral injection of 4 micrograms of the catecholamine neurotoxin 6-hydroxydopamine into the fibres of the ascending NA systems in the mesencephalon. The behavioural responses to cholinergic drugs were then assessed. The cholinergic agonists, arecoline and pilocarpine, produced a cataleptic state of behavioural immobility which was almost completely blocked by prior depletion of forebrain NA. The cholinergic blocking agents, atropine and scopolamine, induced locomotor stimulation which was potentiated by NA depletion. This was shown to be muscarinic in nature since the nicotinic antagonist, mecamylamine and the agonist nicotine were not altered in their behavioural actions after NA depletion. It is concluded that a noradrenaline -acetylcholine interaction in the central nervous system may have a behavioural function in arousal processes, with catalepsy at one extreme and locomotor activation at the other.

871 THE RAPID ACTIVATION OF ADRENAL TYROSINE HYDROXYLASE (TH) BY ELECTROCONVULSIVE SHOCK (ECS) AND ITS SUBSEQUENT IN VIVO DEACTIVATION. Joseph M. Masserano and Norman Weiner. Dept. Pharm., Univ. Colo. Med. Center, Denver, CO 80262.

Previous work in our laboratory has demonstrated that various stresses (decapitation, pain, immobilization and cold) will rapidly activate rat adrenal TH. In the present study we have examined the effects of ECS on activation of adrenal TH. Rats were shocked with a 500 mA current applied transorbitally for 0.2 seconds. This produces a consistent tonic-clonic seizure lasting approximately 30 seconds. Following ECS the animals were injected with pentobarbital (60 mg/kg) at various time intervals and the adrenals were removed surgically under anesthesia to avoid adrenal TH activation by decapitation. Approximately an 80% activation of TH was obtained at 5 minutes following ECS By one hour TH activity had decreased to 20% above control values. There was a ten-fold increase in adrenal medulla cyclic AMP twenty minutes following ECS, which rapidly declined to near control values by one hour. ECS also produced an increase in the proportion of cyclic AMP-independent protein kinase in the adrenal medulla, which slightly preceded the maximal increase in cyclic AMP. It thus appears that the activation of adrenal TH following ECS occurs rapidly and is maintained for a period of about one hour in vivo. These results suggest a possible association of tyrosine hydroxylase activation with activation of cyclic AMP-dependent protein kinase.

This research was supported by USPHS grants NSO7927, and NSO9199.

872 THE DEVELOPMENTAL COURSE OF THE RAT BLOOD BRAIN BARRIER To RO 4-4602 Clyde B. Mathura, John Marshall*, William C. Curtis* and Henry Mitchell* Dept. Psych. Howard University, and Dept. Chem., UDC, Washington, D.C. In an effort to determine the developmental course of the blood-brain-barrier to the penetration of a peripheral DOPA decarboxylase inhibitor, 4 and 10-day old rat pups were injected intraperitoneally with either RO 4-4602 (50 mg/kg) or saline in equal volumes. At 60 min. after injections, the animals were immersed in liquid CO2 and their brains excised under near freezing conditions. Whole brains were analysed for the measurement of nanogram quantities of the acidic metabolites of dopamine, homovanillic acid (HVA) and dihydroxphenylacetic acid (DOPAC). A combined GC- Mass Fragmentography assay technique (Karoum, et al.,1975) was used. The technique involved sequential HCL and ethyl acetate extraction, evaporation under N^2, methylation in lipopure methanol, derivatization of the acidic methabolites, and finally the Me/PFP derivative being injected into the apparatus, a Finnigam Model 3000 D Quadropole GC- Mass Spec employing an 8ft 1/8 inch i.d. 3% SE-54 steel column.
Preliminary data have shown both HVA and DOPAC levels to be significantly reduced in the 4 day-old but not in the 10 day-old pups. It appears as though the 4 day-old blood brain barrier is not selectively inhibiting the activity of the enzyme. Additional data on differential regional permeabilities and other developmental age periods will also be reported.
These preliminary findings have implications for the use of L-DOPA as a precursor to dopamine in providing purely central effects in the developing brain. The use of pharmacological tools (e.g. L-DOPA+RO 4-4602) established with adult animals is not readily applied to developing animals because of the immature blood brain barrier.

Supported by MBS grant number RR-0816-08.

873 STRAIN DIFFERENCES IN RAT ADRENAL BIOSYNTHETIC ENZYMES AND STRESS INDUCED INCREASES IN PLASMA CATECHOLAMINES. R. McCarty, G.M. Gilad, V.K. Weise* and I.J. Kopin. Laboratory of Clinical Science, NIMH, Bethesda, Maryland 20014.

In an earlier study (McCarty and Kopin, Physiol. Behav., 1978), we compared the changes in plasma norepinephrine (NE) and epinephrine (EPI) of 5 normotensive rats strains following exposure to 5 min of footshock stress. The most reactive strain (Wistar-Kyoto, WKY) and the least reactive strain (Brown-Norway, B-N) were selected for additional study. A catheter was inserted into the tail artery of each rat to allow for repeated sampling of blood in conscious, unhandled rats. Two days later, basal plasma levels of NE and EPI did not differ between the two strains. However, following footshock stress, plasma levels of NE and EPI were twice as high in WKY rats as in B-N rats (Table).

Enzyme activities and catecholamine (CA) content of the paired adrenals of unstressed rats of the two strains were also examined. Activities (nmoles product/pair/hr) of tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) but not phenylethanolamine-N-methyl transferase (PNMT) were significantly higher in B-N rats. In addition, the adrenal content of NE but not EPI was higher in the B-N strain (Table).

The adrenal catecholamine biosynthetic enzyme activities and CA content of these two strains were inversely related to the plasma NE and EPI levels during stress. These results suggest prominent strain differences in the rate of release of CA from the adrenal medulla and/or the rate of removal of CA from the circulation.

	Wistar-Kyoto	Brown-Norway
Plasma CA (pg/ml)		
NE basal	438 ± 79	376 ± 40
stressed	2210 ± 230**	1080 ± 57
EPI basal	354 ± 46	337 ± 78
stressed	1590 ± 68**	758 ± 70
Paired adrenals		
TH	56.4 ± 2.6	69.1 ± 5.1*
DBH	429 ± 21	580 ± 31**
PNMT	38.7 ± 1.8	34.6 ± 2.2
NE (µg)	9.0 ± 0.6	16.4 ± 1.3**
EPI (µg)	43.1 ± 2.7	43.7 ± 2.7

*P < 0.05; **P < 0.01.

874 COMPARATIVE CATECHOLAMINE-NEUROPHYSIN MORPHOLOGY IN THE RAT SUPRAOPTIC AND PARAVENTRICULAR NUCLEI. Thomas H. McNeill and John R. Sladek, Jr. Dept. of Anatomy, Univ. Rochester, Rochester N.Y. 14642

The correlative morphological distribution of catecholamine (CA) varicosities and peptide-containing (oxytocin, vasopressin) perikarya in the supraoptic (SON) and paraventricular (PVN) nuclei of the rat was examined using a simultaneous histofluorescence-immunocytochemical technique. This technique allows for the simultaneous visualization of peptides and neurotransmitters in either the same section or in adjacent sections of a single tissue block. Six adult male Wistar rats (250-300gm) were prepared for simultaneous histofluorescence-immunocytochemistry following the technique of McNeill and Sladek (Science 200:72-74, 1978). Immunocytochemical analysis for peptide-containing perikarya employed antisera generated against bovine neurophysin I (NP)(provided by Dr. E. A. Zimmerman).

Only a few NP-containing perikarya appeared to be contacted by CA varicosities in the rostral SON. The heaviest innervation of CA varicosities was seen ventral to the nucleus in a zone occupied by NP-containing processes. In contrast, almost all NP-containing perikarya in caudal SON appeared contacted, often by varicosities which completely surrounded perikarya. The densest concentration of CA varicosities in the PVN was located periventricularly in the parvicellular portion of the nucleus. Periventricularly located immunoreactive perikarya were contacted by numerous CA varicosities in this part of the PVN. Some perikarya in the magnocellular portion of the PVN also were contacted by CA terminals. Many cells which did not stain positively for NP were ringed by CA varicosities in the magnocellular portion of the nucleus.

These data suggest that the major CA innervation to the SON and PVN do not coexist with the major distribution of magnocellular perikarya. It may be suggested that CA influences of PVN neurons may be via an axodendritic mechanism or possibly a parvicellular interneuronal pool. Evidence is also presented that suggests that a possible subcompartmentalization of the SON for CA-peptide interrelationships may exist. Investigations using specific vasopressin antisera are currently underway and will be presented.

Supported by NS 11642 (JRS)

875 BRAIN-STIMULATION REWARD ASSOCIATED WITH THE VENTRAL TEGMENTAL AREA IS ATTENUATED BY MICROINJECTIONS OF SPIROPERIDOL INTO THE NUCLEUS ACCUMBENS BUT NOT INTO PREFRONTAL CORTEX. G. J. Mogenson, M. Wu* and M. Takigawa*. Department of Physiology, University of Western Ontario, London, Canada.

Brain-stimulation reward was observed with electrodes in the ventral tegmental area (VTA) confirming previous findings. The role of dopaminergic neurons, which project from the VTA to the nucleus accumbens septi and the medial prefrontal cortex, was investigated by injecting spiroperidol, a dopamine antagonist, into these two forebrain sites while rats were self-stimulating the VTA. Injections of spiroperidol (1 µg in 1 µl) into the ipsilateral nucleus accumbens significantly attenuated self-stimulation of the VTA compared to microinjections of the drug vehicle. In most animals self-stimulation was reduced 1 or 2 min after the injection and was completely suppressed for 5 to 10 min; the total number of responses for brain-stimulation reward was reduced by more than 50 per cent during a 15 min test period. Spiroperidol injected into the contralateral nucleus accumbens, as a control for possible motor or non-specific effects, did not reduce self-stimulation of the VTA. These observations provide additional evidence that dopaminergic (A_{10}) neurons projecting from the VTA to the nucleus accumbens contribute to brain stimulation reward. Spiroperidol (1 µg in 1 µl) microinjected into the ipsilateral or contralateral medial prefrontal cortex did not reduce self-stimulation of the VTA. There was also no reduction in rate of VTA self-stimulation when the dose of spiroperidol into the ipsilateral and contralateral prefrontal cortex was increased to 2 µg (in 1 µl). These observations appear to be inconsistent with previous studies implicating dopamine in self-stimulation of the medial prefrontal cortex and additional investigation is needed to clarify the discrepancy. (Supported by the Medical Research Council of Canada)

876 LOCUS COERULEUS STIMULATION POTENTIATES PURKINJE CELL RESPONSES TO AFFERENT SYNAPTIC INPUTS. Hylan C. Moises, Barry Waterhouse, and Donald J. Woodward. Dept. Cell Biology, Univ. Tx. Health Sci. Ctr., Dallas, Tx. 75235.

We previously reported that excitatory and inhibitory responses of rat cerebellar Purkinje (P) cells, produced both synaptically and by microiontophoresis of putative amino acid neurotransmitters, are enhanced during norepinephrine (NE) iontophoresis. Moreover, it was shown that NE, released synaptically during activation of the noradrenergic pathway from the locus coeruleus (LC) to cerebellum, can modulate P cell responsiveness to iontophoretic applications of gamma aminobutyric acid, the basket and stellate cell neurotransmitter. In this study, we examined whether endogenously released NE could exert similar modulatory effects on P cell responsiveness to afferent synaptic inputs. Inputs to the Purkinje cell were activated by suprathreshold current stimulation (3 shocks at 500 Hz with 1-3 sec repetition rates) of the rat sensorimotor cortex and mossy or climbing fiber responses recorded extracellularly using glass micropipettes. Post-stimulus time histograms were used to quantitate the response evoked by an input when tested before and at various time intervals after preconditioning stimulation of the LC. Climbing fiber (CF) and mossy fiber (MF) evoked excitations and pure, "off beam" inhibitions were tested in 28 neurons following cerebral cortical stimulation. In 8 of 11 cells, complex spike excitations evoked by activation of CF inputs were increased (from 0.90 spikes/stimulus to 1.24 spikes/stimulus) when preceded by an LC conditioning stimulation (3 shocks of 0.1 msec duration at 100 Hz) intensity determined to be subthreshold for directly affecting P cell discharge. In 4 of 7 neurons, subthreshold LC stimulation enhanced simple spike excitations (from 0.74 to 0.88 spikes/stimulus) elicited via MF afferent input. Post-excitatory inhibitions observed after simple and complex spike excitations of the P cell were also augmented by LC stimulation in 6 of 6 and in 6 of 9 cells examined, respectively. In 5 additional neurons, pure inhibitory responses evoked by cortical stimulation, presumably mediated via MF activation of the local cerebellar inhibitory interneurons, were greatly potentiated in both duration and magnitude when preceded by LC stimulation at currents which alone elicited no depression of spontaneous discharge. Typically, this facilitating influence of LC activation on all inputs was observed when conditioning was applied 100-400 msec prior to a cerebral cortical stimulation. Stimulation in areas outside LC did not alter in 2 cells and reduced evoked synaptic responses in 3 cells. These data suggest that tonic noradrenergic input may act to facilitate the transmission of excitatory and inhibitory afferent inputs to P cells in the cerebellar cortex. (Supported by NSF BNS77-00174 to DJW).

877 EFFECT OF CHOLINERGIC AGENTS ON CATECHOLAMINE TURNOVER IN HYPOTHALAMIC REGIONS. William W. Morgan. Dept. Anat., Univ. Texas Hlth. Sci. Ctr., San Antonio, TX 78284.

Adult Sprague-Dawley female (150-175 gms) or male (130-170 gms) rats were treated with intraperitoneal (i.p.) injections of physostigmine sulfate. Because of the short term action of this compound, physostigmine was given at hourly intervals for a total of 1 to 4 hours. Control animals received a comparable number of saline injections. In all studies the rats were sacrificed by decapitation between 15:30 and 17:30. The brain of each animal was rapidly removed and immediately frozen on dry ice. The anterior hypothalamus, arcuate-median eminence and anterior telencephalon were subsequently dissected from each brain using clearly defined anatomical landmarks. The remaining brain was discarded. The contents of noradrenaline (NA) and dopamine (DA) in these areas were determined with a highly sensitive radioisotope-enzyme assay or a spectrofluorometric procedure. The decline in the content of these amines 1, 2 or 4 hours after the administration of alpha methyl-paratyrosine (αMPT, methyl ester; 250 mg/kg; i.p.) was used as an indirect method of measuring NA or DA turnover. The levels of these two amines were expressed as nanograms NA or DA per tissue or per mg protein before statistical evaluation by the analysis of variance. In male rats physostigmine (1 mg/kg) produced a statistically significant increase in NA turnover in the anterior hypothalamus within 1 hour ($p<0.05$). Statistically greater increases in NA turnover in this brain area were observed after 2 ($p<0.005$) or 4 hours of physostigmine treatment ($p<0.001$). Dosages of physostigmine of 0.5 mg to 1.0 mg per hour were equally effective. The effect of physostigmine (1 mg/kg) adminisitered for 1 or 2 hours was not blocked by atropine sulfate (40 mg/kg) given 30 minutes before the first injection of physostigmine. On the other hand, mecamylamine HCl (10 mg/kg) also given 30 minutes before the first dosage of physostigmine (1 mg/kg) blocked the effect on NA turnover in the anterior hypothalamus. Dopamine turnover in the anterior hypothalamus and the content and turnover of NA and DA in the other two brain areas were not affected by physostigmine treatment or by mecamylamine or atropine. Noradrenaline turnover was increased in the telencephalon of female rats after 4 hours of treatment with physostigmine (1.5 mg/kg), a dosage which was frequently fatal to male rats of the same weight. The data suggest that the activity of noradrenergic neurons with terminals in the anterior hypothalamus are particularly sensitive to cholinergic input probably via nicotinic receptors. Supported by NSF Grant #PCM 76-03876 and Research Scientist Development Award # 5 K02 MH00028-03.

878 CHANGES IN CATECHOLAMINE UPTAKE IN BRAIN AREAS OF THE SPONTANEOUSLY HYPERTENSIVE RAT. M.M. Myers*, E. Sukiennik and E.D. Hendley (SPON: G.D. Webb) Dept. Physiology and Biophysics Coll. Med., Univ. Vermont, Burlington, VT 05401.

The functional activity of catecholamine(CA) neurons in the central nervous system is thought to be involved in the processes responsible for central regulation of blood pressure. A number of workers have considered the possible relationship between alterations in these CA pathways and hypertension. Most research has concentrated on differences in amine content or enzyme activities in brain stem areas known to be associated with blood pressure regulation between the Spontaneously Hypertensive Rat(SHR) and the normotensive Wistar Kyoto(WKy). In a previous report we found that norepinephrine(NE) uptake in the cerebral cortex was greater in the SHR and suggested that these changes may be related to the behavioral hyperactivity which was found in the SHR(Fed.Proc. 36: 1047, 1977). In the present study we have further characterized the changes in CA uptake in three brain areas known to receive innervation from either NE neurons(cerebellum), dopamine(DA) neurons(striatum), or both NE and DA(anterior cingulate-frontal cortex).

Rates of both NE and DA uptake were measured in sucrose homogenates prepared from all three brain areas in 5 pairs of 6-8 week old SHR and WKy males. Homogenates were incubated for 4 min at 37°C in Krebs-Ringer bicarbonate buffer with either 80nM ^{3}H-NE or 80nM ^{3}H-DA. The rate of uptake into NE neurons was defined as the difference between NE accumulation with and without 400nM desmethylimiprimine(DMI). Uptake into DA neurons was measured as the difference in DA accumulation in the presence of 400 nM DMI minus the accumulation not blocked by 40uM cocaine.

In the cerebellum where no significant DA accumulation could be measured, the rate of NE uptake was greater in the SHR which is in agreement with our previous findings in the cerebral cortex. In contrast, DA uptake decreased in the striatum of the SHR. Consistent with these results, NE uptake was increased and DA uptake decreased in the anterior cingulate-frontal cortex of the SHR with the ratio of NE to DA uptake being about 50% higher in the hypertensive animals.

These data show that in the SHR 1)there are alterations in CA pathways projecting to areas of the brain not usually associated with blood pressure regulation, and 2)that although changes in both NE and DA uptake can be demonstrated they are in opposite directions such that the ratio of NE to DA uptake within an area is a more sensitive measure of the CA change than either measurement alone.

Supported by a Grant-In-Aid from the Vermont Heart Association and USPHS grant R01-25811.

879 CENTRAL CATECHOLAMINES (CAs) IN GENETICALLY OBESE MICE (obob): EVIDENCE FOR A RESERPINE (RES)-RESISTANT POOL. R. Olsauskas*, J.E. Comaty*, A.J. Vazquez, and G.A. Oltmans. Dept. Pharm., Chicago Med. School, Chicago, IL 60612.

Genetically obese mice (obob) have significantly higher levels of brain norepinephrine (NE) than their lean littermates (Lorden et al., Br. Res., 131:162, 1977). The effects of pharmacological manipulation of central CAs have not been studied in the obob. We administered saline (SAL), RES, or RES and α-methyl-para-tyrosine (α-MPT) to obob and lean littermate controls. Hypothalamic (HT) and telencephalic (TEL) NE and dopamine (DA) levels were measured after drug treatment. Although RES (2.5 mg/kg, 40 and 16 hours prior to sacrifice) reduced NE levels in the HT and TEL areas of obob, NE levels remained significantly higher than in lean controls. To determine if this persistent NE excess was newly synthesized NE, the tyrosine hydroxylase inhibitor α-MPT (300 mg/kg, 4 hours prior to sacrifice) was combined with the RES treatment. Although significant additional decreases in DA levels were produced in the areas studied (indicating that α-MPT treatment was effective in inhibiting tyrosine hydroxylase), this latter treatment did not decrease obob brain NE content to control levels. Data for the HT sections are presented in the following table ($\bar{X}$, μg/g±S.D.):

Group	Amine	SAL	RES only	RES + α-MPT
obob	NE	2.315±.248*(n=6)	.131±.038*(6)	.115±.043*(6)
lean	NE	1.930±.214 (10)	.066±.041 (9)	.050±.021 (10)
obob	DA	.634±.047 (6)	.082±.024 (6)	.034±.021**(5)
lean	DA	.671±.090 (10)	.090±.057 (10)	.038±.015**(10)

*Differs from lean, p <.01 **Differs from RES only, p <.01

In general, drug effects in the TEL paralleled those in the HT. These results indicate that in obob there is a reserpine-resistant NE pool. This pool may be the result of tighter vesicular binding of NE in the obob. The results provide additional evidence for the presence of an altered central CA function in the obob. Such abnormal CA function might contribute either directly or indirectly to the abnormal characteristics of the obob such as impaired thermoregulation, overeating, and altered pituitary function. We are currently studying the relationship of the central CAs to several of these abnormalities. (Supported by NIH grant 1RO3MH30179 and RR-05366).

880 PLASMA CATECHOLAMINES IN GOLDBLATT DOGS. Juan Parra*, A. James Blair, Jr.*, Marla Huber-Smith*, William Boyle*, Daisy S. McCann, Dept. Med., Wayne County Gen. Hosp., Eloise, Mi. 48132 and U.of Mich., Ann Arbor, Mi. 48104.

Cause and effect relationships between catecholamines and essential hypertension remain tenuous. Large amounts of norepinephrine (NE) or epinephrine (E) released from a tumor are the etiological agents for hypertension due to pheochromocytoma. Central nor-adrenergic and/or dopaminergic tracts have been implicated in the initiation, but not maintenance, of many experimental hypertension models including the Goldblatt dog, DOCA-Na induced hypertension and genetic animal hypertension. High plasma NE levels have been associated with high renin, but not normal or low renin, hypertension in humans.

We followed the pattern of plasma NE and E levels in one kidney Goldblatt dogs. Base levels obtained prior to nephrectomy, for the parameters studied were: mean blood pressure (MAP) 92 ± 2.0*; heart rate 103 ± 4; plasma NE 219 ± 25 pg/ml; E 122 ± 20 pg/ml; aldosterone 94 ± 6 pg/ml; plasma renin activity (PRA) 2.0 ± 0.1; serum sodium (Na^+) 145 ± 1.3 meq/l; plasma potassium (K^+) 4.6 ± .8 meq/l.

Of six dogs submitted to unilateral nephrectomy, 2 served as controls in whom the Goldblatt clamp was applied but not tightened; the remaining 4 developed chronic hypertension within 3 days after blood supply to the remaining kidney was reduced. The chronic MAP increase ($p<.001$) was accompanied by a chronic, statistically significant increase in NE ($p<.05$). PRA and aldosterone rose sharply immediately after application of the clamp. Both gradually returned to normal levels over the next two weeks. Pulse rate after an initial drop, rose over a two week period to stabilize at 118 ± 1 ($p<0.01$). Chronic E values did not change significantly; K^+ and Na^+ remained stable throughout the eight weeks of the study.

The data supports the concept that NE has a role to play in the development and possibly maintenance of hypertension in the Goldblatt dog.

*SEM

881 NEUROANATOMY OF CATECHOLAMINERGIC SYSTEMS IN RHESUS MONKEY AND MAN. J. Pearson, L. Brandeis* and M. Goldstein. Dept. Path., Sch. Med., NYU Med. Ctr., New York, N.Y. 10016.

An antibody to tyrosine hydroxylase isolated from a human pheochromocytoma is being used with the peroxidase-anti-peroxidase immunohistochemical technique of Sternberger to map distribution of simian and human catecholaminergic neuronal perikarya and fiber tracts. Cell bodies of dopaminergic and adrenergic neurons consistently stain with greater intensity than those of noradrenergic neurons. Antibodies against other catecholamine synthetic enzymes further increase precision in neurotransmitter identification.

Catecholaminergic fibers extend backwards above the anterior part of the corpus callosum to enter superior parts of the frontal cortex. Other fibers and neurons are present in limbic cortex associated with olfactory tracts. Clustered terminals are found in the tracts. TH fibers from substantia nigra form dense bundles in the globus pallidus before spreading diffusely within the caudate and putamen. Others form a layer external to the putamen. There are periventricular and supraoptic aggregates of small TH positive neurons. Hind brain fiber tracts are clearly shown as are noradrenergic and adrenergic nuclear groups.

Dimensions and neuronal population densities of the stained structures are being determined for rhesus monkey using serial sections cut in three planes.

Knowledge of catecholamine neuroanatomy in man is crucial to the understanding of neurologic disease. A survey of the neuropathology of a variety of conditions has been initiated. Sympathetic ganglia in familial dysautonomia have approximately 90% reduction in neuron population yet present normal TH activity per ganglion by biochemical methods. TH immunohistochemistry indicates an abnormally high proportion of TH rich neurons which stain more intensely than in controls. Differential survival of a select neuronal population or functional hypertrophy of persistent neurons may occur. Increase in neuron size supports the latter possibility.

882 ANTAGONISM BY UPTAKE INHIBITORS OF α-METHYL-m-TYROSINE DEPLETION OF EPINEPHRINE, NOREPINEPHRINE AND DOPAMINE BUT NOT SEROTONIN IN RAT BRAIN. Kenneth W. Perry* and Ray W. Fuller. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46206.

Epinephrine (E), norepinephrine (NE) and dopamine (DA) were measured in rat brain regions by a high pressure liquid chromatographic method with electrochemical detection [Biochem. Pharmacol. 26, 2087 (1977)]. E was detected in hypothalamus, midbrain and brain stem but not in the cerebral hemisphere. All three of the catecholamines (CA) were depleted by α-methyl-m-tyrosine (100 mg/kg, s.c.) at 6 hrs after injection. DA depletion was greater in the cerebral hemisphere (60% depletion) than in other regions, while the depletion of NE (80%) and E (50%) was about the same in all regions. The CA depletion after α-methyl-m-tyrosine (αMMT) injection was antagonized by pretreatment with various CA uptake inhibitors but was not completely prevented. Administration of an uptake inhibitor 6 hrs after αMMT injection, a time when the catecholamines were already depleted, partially reversed the depletion. However, serotonin depletion by αMMT was not altered by pretreatment with fluoxetine, an inhibitor of uptake into serotonin neurons. Apparently part of the CA (but not serotonin) depletion after αMMT injection is due to the action of amine products (α-methyl-m-tyramine and metaraminol) that enter the neuron *via* the membrane pump and whose presence within nerve terminals is maintained by the uptake pumps. Since metaraminol (MA) is localized primarily in NE neurons and α-methyl-m-tyramine (MTA) in DA neurons [Dorris & Shore, J. Pharmacol. Exp. Ther. 179, 10 (1977)], a NE uptake inhibitor would be expected to decrease MA concentration in rat brain after αMMT injection whereas a DA uptake inhibitor would be expected to decrease MTA concentration. MA & MTA were measured by high pressure liquid chromatography with fluorometric detection [Perry & Fuller, Society for Neuroscience Abstract #809, vol. III, p. 256 (1977)] 6 hrs after administration of αMMT alone or in combination with mazindol, a CA uptake inhibitor. Mazindol decreased MA concentration in brain by 90% but decreased MTA concentration only 20%, indicating that it inhibited uptake into NE neurons more than into DA neurons. This finding agrees with the order of selectivity for mazindol in antagonizing the depletion of CA by αMMT, which was NE>DA>E. Other uptake inhibitors studied and their order of selectivity were: desipramine, NE>E>>DA; imipramine, E>NE>>DA: nortriptyline, NE>E>>DA; and amitriptyline, E>NE>>DA. These studies show that αMMT is a useful tool for evaluating the selectivity of CA uptake inhibitors in vivo.

883 LITHIUM PREVENTS DEVELOPMENT OF DOPAMINE RECEPTOR SUPERSENSITIVITY. Agu Pert, Jack E. Rosenblatt*, Candace B. Pert and William E. Bunney, Jr. Biological Psychiatry Br., NIMH, Bethesda, MD 20014.

Long-term treatment of rats with haloperidol produces an increased sensitivity to the locomotor and stereotypic effects of apomorphine (APO). This behavioral dopaminergic supersensitivity is also accompanied by increased [^{3}H]spiroperidol binding in the striatum. The purpose of the present study was to assess the effects of lithium on the development of dopaminergic supersensitivity as measured both behaviorally and by striatal dopamine receptor binding. Rats were treated for 21 days with either haloperidol (1 mg/kg), haloperidol and lithium, saline or saline and lithium. Seven days after discontinuation of all treatments, some animals from each group were tested for APO (0.5 mg/kg) induced locomotor activity and stereotyped behavior. The rest of the animals were sacrificed for assay of striatal dopamine receptor binding. Freshly dissected caudates were homogenized and washed in Tris buffer. The final suspension was incubated in triplicate at 37°C with tritiated spiroperidol and either (+) or (-) butaclamol. APO-induced locomotor activity and stereotyped behavior were increased in haloperidol pretreated rats but not in rats that had been pretreated concurrently with both lithium and haloperidol. Specific binding of [^{3}H]spiroperidol was increased in caudates of haloperidol pretreated rats but not in rats that had been pretreated with both lithium and haloperidol. Lithium treatment alone did not affect behavioral sensitivity to APO or striatal [^{3}H]spiroperidol binding. Lithium also did not affect levels of haloperidol in whole brain or the caudate. Lithium was also found to prevent the development of "presynaptic" dopamine receptor supersensitivity as assessed by the ability of low doses of APO (0.05 mg/kg) to depress locomotor behavior. In addition, supersensitivity to amphetamine following depletion of catecholamines with chronic administration of α-methyl-p-tyrosine was also prevented by lithium. These findings suggest that lithium's ability to prevent recurrent manic-depressive episodes may be related, at least in part, to it's ability to stabilize dopaminergic receptor sensitivity. Work is presently in progress to ascertain whether chronic lithium also interferes with the development of catecholaminergic subsensitivity, as well as supersensitivity of other neurotransmitter systems.

884 THE EFFECTS OF BIOGENIC AMINES ON THE OCCIPITAL (VISUAL) CORTEX OF THE CAT. T.A. Reader. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada.

Several evidences in favor of specific afferent pathways containing the biogenic amines dopamine (DA), norepinephrine (NE) and serotonin (5-HT) have been reported in the mammalian CNS. In an attempt to determine their functional role in cerebral cortex we examined the effects of DA, NE and 5-HT upon visually-driven neurons in the visual (occipital) cortex of the cat ("encéphale isolé" preparation). We also compared the effects of these biogenic amines to those produced by the neurotransmitters acetylcholine (ACh) and γ-amino-n-butyric acid (GABA). The amines DA, NE and 5-HT were found to inhibit the visually-evoked activity of a majority of cortical neurons. This inhibition, obtained with ejection currents of 50-100 nA during 20 to 30 s was usually of prolonged duration, lasting 4-6 min and often associated with spike hyperpolarization. The amino acid neurotransmitter GABA inhibited and hyperpolarized all the neurons tested. This effect (ejection currents of 5-30 nA) was rapid in onset and termination lasting only as long as GABA was ejected. The neurotransmitter ACh (40-60 nA for 5-15 s) increased the visually-evoked activity of the majority of cells samples below 1000 μ and this excitation could be blocked or reduced by GABA or by the biogenic amines. The blockade of the ACh-induced excitation by GABA was rapid in onset and ceased as soon as the ejection of GABA was terminated, the cell recovering immediately its sensitivity towards ACh. In contrast the blockade caused by the biogenic amines on the ACh-induced increase in evoked firing was of long duration, lasting for several minutes. The present results show that the evoked activity of a great number of visual cortical cells may be inhibited by DA, NE and 5-HT and that this inhibitory response differs markedly from that induced upon the same cells by GABA. The long duration of the effects of these amines, when compared to GABA, on the visually-evoked activity and on the ACh-induced excitations tends to rule out a simple balance between excitations and inhibitions to explain the reduction in firing and the suppression of the sensitivity towards ACh. Although the molecular mechanisms underlying biogenic amine and ACh interactions are currently in debate the interactions here reported probably call upon mechanisms closely related to the direct postsynaptic actions of these putative neuromodulators. It should also be recalled that presynaptic muscarinic and nicotinic receptors have been postulated to regulate the release of DA and NE (Reader et al., Brain Res., 111 (1976) 95). Thus different levels of interaction may coexist in cerebral cortex and account for opposite effects of biogenic amines and ACh on overall neuronal excitability. (Supported by the MRC, the CRSQ and CAFIR)

885 NUCLEAR ORGANIZATION OF THE ISTHMUS OF THE RAT: A GOLGI ANALYSIS. J. N. Riley and R. Y. Moore. Dept. Neurosciences, University of Calif. at San Diego, La Jolla, CA. 92093.

Intense interest in the organization of the isthmus has been generated by Nauta's formulation of the concept of a "limbic midbrain region" and the discovery that nuclei in the isthmus contain neurons with identified neurotransmitters. These include the nucleus locus coeruleus (LC), nucleus raphe dorsalis (RD) and nucleus central superior (CS). A Golgi analysis of the organization of these nuclei, in particular the organization of dendritic fields and relationships among nuclei would seem worthwhile to better understand the anatomical organization of this important region.

Golgi-Kopsch material was prepared from the brains of male and female Sprague-Dawley rats, ages 16-90 days old.

In the raphe nuclei, two types of neurons can be distinguished. In RD, smaller neurons located in the ventral part of the nucleus have dendrites oriented in a dorsal-ventral axis. A significant portion of neurons in ventral RD have dendrites that bifurcate in the dorsal region of the nucleus, extending into the lateral wings of the nucleus. Dendrites of cells located in the medial and dorsal regions are arranged in a less regular fashion. Some neurons in the dorsal and medial parts of the nucleus have dendrites extending for considerable distances into the central gray and dorsal tegmental nucleus (DTN). In CS, the larger neurons tend to be located nearer the midline and have a significant portion of their dendrites oriented perpendicular to the dorsal-ventral axis of the nucleus. Smaller neurons, tending to be located at the more lateral edges of the nucleus, usually have their dendrites oriented parallel to the dorsal-ventral axis of the nucleus.

The DTN appears to be composed of a relatively homogeneous population of neurons. Most of the dendrites of these neurons are strictly confined to the limits of the nucleus, in striking contrast to other isthmic nuclei. Neurons of DTN lateralis have a similar morphology to DTN neurons but have dendrites extending into DTN, RD, LC, and ventrally into the mesencephalic reticular formation. Some neurons located dorsal to RD and DTN have dendrites that run parallel and adjacent to the fourth ventricle.

Our observations on LC neurons are consistent with the descriptions of Swanson (1976). In addition, observations on the central gray, nucleus cuneiformis, and parabrachial regions will be presented. Supported by USPHS Grants NS-12080 and NS-05732.

886 AGE-RELATED DIFFERENCES IN BRAIN NOREPINEPHRINE NEURON FUNCTION IN STRESSED AND NONSTRESSED RATS. Sue Ritter and Nancy L. Pelzer*. Coll. Vet. Med., Wash. State Univ., Pullman, WA 99164.

Previous work in our laboratory has suggested that the response of brain noradrenergic (NE) neurons to severe stress is altered by age or some age-related variable. NE depletions after 6 hours of cold stress were much greater in 7-month-old than in 3-month-old rats in both hypothalamic and telencephalic regions. Hypothalamic NE concentrations immediately after cold stress were reduced to 61.5 ± 3.2% of control in older rats but to only 79.6 ± 2.3% of control in younger rats ($p<.01$). Likewise, telencephalic NE was reduced to 62.3 ± 4.6% of control in the older rats, but only 82.1 ± 3.6% of control in the younger rats ($p<.01$). If the magnitude of the NE depletion observed in these experiments immediately after stress exposure represents the size of the functional pool of NE, then our results suggest that the size of the functional pool may be altered during the aging process.

In order to investigate these possibilities in more detail, 3-month- and 7-month-old rats were subjected to 1 hour of footshock stress. We hoped to determine whether the differences in NE function observed in the two age groups after cold stress would also be present after a different type of stress. The results obtained after footshock were similar to those obtained after cold stress. Older rats sustained larger NE depletions in both brain regions analyzed than did the younger rats when compared to nonstressed controls of the same age. Hypothalamic NE was 65.6 ± 3.6% *vs* 79.2 + 2.1% of control in older and younger rats, respectively ($p<.01$). Telencephalic NE concentrations after footshock were 85.7 ± 2.7% and 99.6 ± 4.1% of control, respectively, in older and younger rats ($p<.05$). We also compared the resting levels of NE in the hypothalami and telencephalons of nonstressed rats of these two age groups. Hypothalamic NE content did not differ in the two groups but telencephalic NE concentration was 27.3 ± 1.7% higher in the older rats than in the younger rats ($p<.001$).

These results demonstrate that age-related increases in stress-induced NE depletion are not specific to a particular kind of stress but represent a general change in the function of NE neurons. Futhermore, our demonstration of elevated resting cortical NE levels in older animals, despite their greater susceptibility to depletion, suggests the possibility that the readily releasible NE pool of old rats may be increased in size in order to compensate for its increased lability. Finally, the absence of elevated resting levels of NE in the hypothalami of older animals suggests that these neurons respond differently to age-related functional changes of NE utilization than do cortical NE neurons.

887 PERSISTANT BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF HYPOXIA IN RATS. M. K. Shellenberger, S. Norton* and C. L. Littlejohn*. Dept. Pharmacol. and Ks. Center for Ment. Retard. Res., Kansas Univ. Med. Center, Kansas City, Ks. 66103.

Animals were exposed to carbon monoxide (CO) at 0.52% in air to a point near respiratory failure as a model of slow onset hypoxia in which asphyxia does not occur. There are considerable data which indicate that prolonged hypoxia of this type damages predominately telencephalic structures. While there are a number of published studies concerning the acute neurochemical effects of hypoxic-anoxic episodes, little information is available concerning possible residual neurochemical alterations. Groups of male and female adult rats were used in these studies. The residual behavioral effects were evaluated in single animals over 24 hr periods in a residential maze, utilizing photocell counts, 4-6 wks after exposure.

Analysis of the data showed that as a group the female rats were significantly more active than controls during the nocturnal period and in total activity for the 24 hr period. However, it was also found that activity in the CO-exposed animals was positively correlated to the duration of exposure for both males ($r = .918$) and females($r = .946$). Females were found to tolerate longer periods of hypoxia (mean exposure = 100.3 min ± 10.5 S.E.) than males (mean exposure = 60.8 min ± 7.2 S.E.). This factor probably accounts, at least partially, for the greater number of hyperactive females.

Following behavioral evaluation the brains were taken, sampled and analyzed for dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT). The factors noted above led to selection of animals with longer exposures for initial chemical analysis. Female rats (mean exposure = 146.7 min ± 1.5 S.E., N = 3) were found to have significant reductions in striatal DA and midbrain NE. On the other hand, male rats (mean exposure = 100 min ± 2.7 S.E., N = 3) did not show reduced striatal DA although midbrain NE was significantly lower than in controls. The data clearly indicate that periods of hypoxia, in excess of 60 min, produce behavioral alterations and neurochemical deficits which persist over long periods of time. Whether the neurochemical changes are causally related to the altered behavior cannot be determined at present.

Supported by USPHS Grant MH 27739.

888 NORADRENERGIC PROJECTIONS TO THE LOCUS COERULEUS. Michael A. Silver*, William G. Soden* and David M. Jacobowitz (SPON: L. K. Y. Ng). Lab. Clin. Sci., NIMH, Bethesda, Md. 20014.

The locus coeruleus (LC) has been described as receiving a noradrenergic (NA) innervation from the caudally situated A_1, A_2 and A_5 cell groups. Horseradish peroxidase retrograde flow studies have described cell bodies in the vicinity of these NA cell groups projecting to the LC, however, this technique does not offer neurotransmitter specificity. A retrograde flow mapping technique (in press) that is specific for dopamine-β-hydroxylase (DβH) containing neurons, has recently been developed, based upon the phenomenon of specific uptake and retrograde flow of antibody to DβH (ADβH) by noradrenergic neurons. This study reports the application of this highly sensitive and specific technique to the elucidation of the origin of adrenergic fibers terminating within the LC.

Eight adult male rats received stereotaxic injections of ADβH into the right caudal LC via a glass micropipette in volumes of 0.1 - 0.2 µl over a 15-30 minute period. Control rats received equivalent injections of pre-immune serum (PIS). The rats were decapitated after 24 hours and cryostat sections processed for immunofluorescence visualization of intraneuronal ADβH. Proper injection placement was verified in thionin-stained sections.

Following unilateral locus injection, ADβH was visualized within cell bodies of the contralateral LC, and ipsilateral A_2 NA groups. Substantial numbers of cells were labelled, bilaterally within the A_1, A_4 and A_5 groups. No other cell bodies were visualized by this technique and none were seen in the brains of PIS injected subjects. Since epinephrine releasing neurons situated within the vicinity of the A_1 and A_2 cell groups have been reported to project to the LC, it is not known whether ADβH labelled neurons within the A_1 and A_2 groups are epinephrine and/or norepinephrine releasing neurons. ADβH-containing fibers arising from the contralateral LC were seen to decussate ventral to the dorsal tegmental nuclei. Caudal to the LC, fibers were observed in and radiating from the ipsilateral ventral noradrenergic bundle. Ascending noradrenergic fibers of the ipsilateral dorsal bundle could be followed rostrally through the mesencephalon indicating anterograde transport of ADβH. This study represents the first indication that NA fibers arising from distant perikarya may innervate the NA neurons of the locus coeruleus. Since all NA cell groups were not found to innervate the LC, the circuitry of NA projections may serve as the anatomical basis for a functional organization.

889 SIMULTANEOUS VISUALIZATION OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) AND CATECHOLAMINES IN THE GUINEA PIG BRAIN. A.I. Silverman and J.R. Sladek, Jr. (SPON: J.R. Currie). Dept. Anat., Columbia Univ., New York, NY 10032 and Univ. Rochester, Rochester, NY 14642.

There is considerable reason to believe that catecholamines (CA) and LHRH interact functionally in the reproductive cycle. Morphological studies using immunocytochemical and histochemical methods have demonstrated the existence of both substances within similar hypothalamic regions. However, these studies did not determine point to point relationships between CA and LHRH. A recently introduced method (McNeill and Sladek, Science 200: 72-74, 1978) allows the simultaneous visualization of monoamines and neuropeptides and has been used in the present study to morphologically characterize the distribution of CA and LHRH.

Adult male guinea pigs were prepared for Falck-Hillarp CA histofluorescence by freeze-drying and hot formaldehyde vapor perfusion. Serial sections were made at 10 µm and adjacent sections to those chosen for fluorescence were stained for LHRH using the unlabeled antibody enzyme technique. The primary antiserum was #4305B at a dilution of 1:500 (donated by L.A. Sternberger). The distribution of CA and LHRH was analyzed with the use of a microscope comparator bridge. Positively stained LHRH terminals and CA varicosities were observed throughout the median eminence. The distribution of LHRH terminals closely paralelled that reported previously (Endocrin. 99:30-41, 1976) in Bouin's fixed tissue. CA varicosities were seen in heaviest densities in the contact zone of the median eminence both laterally and medially. Superimposition of the CA and LHRH terminal fields revealed that the majority of CA varicosities were located external to the bulk of LHRH terminals. This finding was most prominent in caudal median eminence. Rostral median eminence was characterized by a small degree of overlap. Occasional strings of LHRH terminals appeared to penetrate the outer CA zone at all levels. LHRH containing perikarya were observed in the medial preoptic and septal regions. In the majority of instances CA varicosities appeared in juxtaposition to these perikarya at somatic and dendritic surfaces. These data indicate that LHRH and CA coexist in the median eminence in separate zones, with a small degree of overlap and that most positively stained LHRH perikarya appeared to be contacted by CA varicosities. Supported by HD 10665.

890 STIMULUS-DEPENDENT CHANGES IN PAIN-SENSITIVITY AFTER CENTRAL MONOAMINE DEPLETION: HYPERALGESIA ON THE HOT-PLATE ASSOCIATED WITH NOREPINEPHRINE DEPLETION AND IN FLINCH-JUMP WITH SEROTONIN DEPLETION. K. J. Simansky and J. A. Harvey. Dept. of Psychol., Univ. Iowa, Iowa City, IA 52242.

Electrolytic lesions which deplete forebrain serotonin (5-HT) in the rat are associated with hyperalgesia as measured by the hot-plate and flinch-jump techniques (e.g., Yunger & Harvey, JCPP, 1973, 83, 173). However, these lesions generally damage fibers of the noradrenergic (NE) and dopaminergic (DA) systems as well. The relative importance of 5-HT, NE, and DA depletion in hyperalgesia was assessed in rats given intraventricular injections of 5,7-dihydroxytryptamine (DHT), 6-hydroxydopamine (HDA), or vehicle (VC). Doses of neurotoxin employed were: 0 (VC), 120, 360, and 1080 nmol. All rats (n=8/group) were tested for both jump thresholds and paw-lick latencies by the presentation of ascending and descending intensities of shock or heat. Telencephalic 5-HT, NE, and DA content and brainstem and spinal cord 5-HT and NE content were determined.

HDA produced a dose-related decrease in paw-lick latencies but no significant change in the jump threshold. DHT produced a dose-related hyperalgesia to shock ($p < .01$) but only the highest dose of DHT lowered paw-lick latencies. The magnitude and pattern of central monoamine depletion suggested a role for NE in the hot-plate effect and for 5-HT in the lowered jump thresholds. For example, the 360 nmol dose of HDA and the highest dose of DHT both depleted telencephalic NE by 65% and DA by about 20% but the HDA failed to affect 5-HT (-7%) while DHT produced a 93% fall. Behaviorally, both groups showed equivalent decreases in paw-lick but only the DHT group had a lowered jump threshold. In a separate experiment, animals were treated with vehicle or the highest dose of DHT or HDA following pre-treatment with desipramine (DMI). DMI attenuated the NE depletion associated with each neurotoxin (e.g., HDA, no DMI -94% NE in telencephalon vs. -27% after DMI; DHT, -65% vs. -17%) and also prevented the hyperalgesia on the hot-plate without altering the magnitude of the effect on the jump threshold after DHT. These data suggest a dissociation in the central monoaminergic mediation of reactivity to "painful" stimuli that is related to the nature of the test stimulus. Supported by USPHS Grant No. MH 16841 and MH 10641.

891 CORRELATIVE DISTRIBUTION OF NEUROPHYSINS AND CATECHOLAMINES IN RHESUS MONKEY HYPOTHALAMUS. J.R. Sladek, Jr. and E.A. Zimmerman. Dept. Anatomy, Univ. Rochester, Rochester, N.Y. 14642 and Dept. Neurology, Columbia Univ., New York, N.Y. 10032.

Independent analyses using catecholamine (CA) fluorescence and neurophysin immunocytochemistry have placed both neurochemicals in similar hypothalamic loci. Physiological data indicate a possible interaction between CA and magnocellular peptides. Concurrent examination of both substances is now possible with the use of a simultaneous visualization technique which allows immunocytochemical staining of neuropeptides on tissue sections previously prepared for Falck-Hillarp fluorescence (McNeill and Sladek, Science 200:72-74, 1978). This technique was applied to the problem of morphological interrelationships between CA and neurophysin-containing neurons.

Rhesus monkey hypothalami were freeze-dried, treated with hot formaldehyde vapors, paraffin-embedded and serially sectioned at 10uM. Pairs of adjacent sections through the paraventricular (PVN) and supraoptic (SON) nuclei were examined for CA fluorescence and either human estrogen-stimulated (ESN) or nicotine-stimulated (NSN) neurophysin following PAP immunocytochemistry. Antisera to ESN and NSN appear to stain oxytocin and vasopressin neurons, respectively. Neuroanatomical maps were prepared of the combined ESN, NSN, and CA distribution patterns.

Numerous CA varicosities appeared in juxtaposition to positively-stained ESN and NSN perikarya and dendrites in the SON and PVN. Dense fields of CA varicosities were seen in PVN and SON, but the patterns of highest density were located slightly peripheral to the bulk of positively stained perikarya in both loci. A greater degree of overlap of CA fields and ESN/NSN perikarya was seen in rostral rather than caudal SON. Rostral PVN contained a high (4+) density CA field just medial to the ESN/NSN perikarya and a medium (3+) density field within the area occupied by the ESN/NSN perikarya. The 4+ field was reduced markedly at the caudal and rostral poles of PVN as the nucleus attenuated.

These observations provide evidence that CA varicosities in part overlap ESN and NSN-containing perikaryal fields. In many instances juxtaposition appears to exist between CA varicosities and neurophysin perikarya and may form the morphological basis for functional interactions. It is interesting to speculate that axo-dendritic interactions might account for the large number of CA varicosities located just to the periphery of PVN and SON, but this needs to be explored further.

Supported by NS11642 and NS11008.

892 METHIONINE ENKEPHALIN AND LEUCINE ENKEPHALIN INCREASE DOPAMINE IN THE CNS OF MYTILUS EDULIS. George B. Stefano and Edward J. Catapane. Dept. Natural Sciences, Medgar Evers College, C.U.N.Y., Brooklyn, N.Y. and The East Coast Neuroscience Foundation, Inc., Northport, N.Y.

The CNS of the marine bivalve mollusc Mytilus edulis is composed of a pair of cerebral, visceral and pedal ganglia, which have been shown to be partially composed of serotonergic and dopaminergic neurons. A variety of monoaminergic pharmacological agents have been shown to effect the metabolism of these substances in a manner similar to that demonstrated in mammalian systems. In mammals narcotic analgesics and the endogenous pentapeptide enkephalins are known to stimulated dopamine turnover and this effect can be blocked by naloxone. While the mechanism of action is as yet unknown, an opiate receptor is considered to be involved in mediating the effect. Due to the recent interest of the interactions of enkephalins with biogenic amines we investigated the interrelationships among methionine enkephalin, leucine enkephalin, dopamine, serotonin and the specific opiate receptor antagonist naloxone in the CNS of the mollusc M. edulis. Intracardiac administration of 10 µl of 5, 10, 40 and 80 µg of methionine enkephalin and leucine enkephalin were followed at 30, 90 and 180 min with assays of the CNS for dopamine and serotonin. Both enkephalins produced statistically significant increases in endogenous dopamine at the 90 min interval. 80 µg of methionine enkephalin caused a 30% increase while 80 µg of leucine enkephalin caused a 26% increase. No changes were detectable in serotonin levels as a result of these treatments. The effects of naloxone were studied to determine if an opiate specific receptor might be responsible for the effects of the enkephalins. Coadministering 10 to 80 µg of naloxone prevented the change in dopamine concentrations, while naloxone by itself produced no effects during the time course of the experiments. While high affinity opiate receptor binding has yet to be demonstrated in the invertebrates, this study suggests the possible presence of an opiate receptor in the CNS of M. edulis.

This work was supported in part by grant 1-T32GM07641-01 of the M.A.R.C. Program of N.I.G.M.S.

893 EFFECTS OF ELECTRICAL STIMULATION OF RAPHE DORSALIS AFFERENTS ON ACTIVITY OF RAPHE UNITS AND ADJACENT MIDLINE CELLS IN THE ANESTHETIZED RAT. Warren C. Stern, Allen Johnson* and Peter J. Morgane. D. Dix Hosp., Raleigh, NC 27610 and Worcester Fndn. Exptl. Biol., Shrewsbury, MA 01545.

Recent anatomical studies in the rat using horseradish peroxidase have shown major afferents to the raphe dorsalis (RD) from the lateral habenula (LHb) and substantia nigra (SN). In the present series of experiments we examined the effects of electrical stimulation of these afferents on the firing rates of RD units and adjacent cells in the midline. Rats were anesthetized with chloral hydrate and implanted with a concentric stimulating electrode in the LHb (n=28) or SN (n=6) and with a glass micropipette recording electrode (filled with 3M KCl and fast green dye) into the region of the RD. Stimulation consisted of trains of bipolar pulses, 0.1 msec per pulse, 0.2-1.0 mA, 1 or 10 Hz for 10-300 sec. Cellular firing rates were recorded on paper using a window discriminator and/or on FM tape. At the end of each recording the brains were perfused for histology to determine placement of stimulating and recording electrodes.

The table below summarizes the results obtained for LHb stimulation:

	Mean ± se Firing Rate			% Cells Showing*		
	Baseline	1 Hz	10 Hz	Dec.	No Change	Inc.
Raphe (n=19)	2.5±.3	1.9±.4	1.4±.3	73	27	0
Slow Units (4-10/s, n=26)	6 ± .4	6 ± .7	5 ± 2	54	27	19
Moderate Units (10-20/s, n=28)	14 ± .6	12 ± 1	13 ± 2	46	36	18
Fast Units (>20/s, n=15)	31 ± 2	25 ± 3	17 ± 4	67	33	0

* A change of 25% or greater from baseline was required for a cell to be categorized as dec. or inc. firing.

Two populations of units showed consistent inhibition after stimulation of the LHb--RD cells and midline cells with a fast baseline rate. For many RD cells the duration of inhibition following individual 1 Hz pulses was about 250 msec. After cessation of 10 Hz trains the inhibition often lasted for up to 20 sec or longer. SN stimulation also produced substantial inhibition of RD activity with less consistent effects on non-raphe units (n=14).

Thus, LHb stimulation decreases activity not only of raphe cells but also fast firing neurons in the midline area. (Supported by NSF Grant BNS 77-16512 and funds from Burroughs Wellcome Co.)

894 SPECIFIC INHIBITORS OF SEROTONIN BINDING TO SBP (SEROTONIN BINDING PROTEIN) AFFECT PAIN THRESHOLD AND BRAIN SEROTONIN LEVELS. Hadassah Tamir, Stephen E. Karpiak, Michael D. Gershon, and Meir Wilchek*, Div. Neurosci., N.Y. State Psychiatric Inst., Depts. of Anat. and Psychiatry, Columbia Univ., New York, N. Y. 10032, and Weizmann Inst. of Sci., Rehovot, Israel.

We reported[1,2,3] the presence of a protein in the 100,000 g supernatant of both CNS and myenteric plexus homogenates with high affinity for serotonin. This protein (SBP) is found in serotonergic neurons[4], binds newly synthesized serotonin, and the binding of serotonin is inhibited by reserpine. We now report strong inhibition by synthetic derivatives of 5-HTP, in particular 5-HTP-5-HTP-amide and N-acetyl-5-HTP-5-HTP-amide. In vitro, 50% inhibition is found with 0.2 and 1.0 μM, respectively. Sites of interaction of serotonin with membrane receptors were not affected by these dipeptides. Uptake of serotonin by synaptosomes was only slightly inhibited (9% at 10 μM). 5-HTP decarboxylase and tryptophan hydroxylase[5] were not affected up to 10 μM. The N-acetyl derivative was not hydrolyzed by homogenates of brain or myenteric plexus. The ^{14}C labelled peptide was taken up by an osmotically sensitive compartment of synaptosomes in a saturable manner. This peptide apparently is not transported by the serotonin carrier, since neither serotonin nor drugs affecting serotonin uptake affected the uptake of the dipeptide. Intraventricular injection of N-acetyl dipeptide into rats caused a biphasic effect depending on dose. The lower dose (10 nanomoles) induces a 40% decrease of serotonin in whole brain. The higher dose (~300 nanomoles) causes an increase of 95% in serotonin brain levels. The maximum decrease is seen at 90 min after injection. Injection of the N-acetyl dipeptide (1 nanomole) either into the periaqueductal gray or intraventricularly caused a 90% increase in pain threshold lasting for several hours, as determined by a flinch-jump test. Since N-acetyl-5-HTP-5-HTP-amide specifically affects SBP in vitro, its in vivo effects suggest that SBP is involved in serotonin storage.

Supported by NIH grant NS-12506.

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895 THE INVOLVEMENT OF CYCLIC AMP AND POTASSIUM DEPOLARIZATION IN THE INDUCTION OF TYROSINE HYDROXYLASE IN NEUROBLASTOMA CELLS. A. W. Tank* and Norman Weiner. Dept. Pharm., Univ. Colo. Med. Center, Denver, CO 80262.

Considerable evidence has implicated the involvement of cAMP in the induction of tyrosine hydroxylase (TH) in neuroblastoma cells. The induction is elicited by analogs of cAMP and compounds which raise intracellular cAMP levels, such as prostaglandin E_1 (PGE_1) or the phosphodiesterase (PDE) inhibitor 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (RO-20-1724). In the presence of the latter two compounds, intracellular cAMP levels are elevated persistently and TH activity is increased 2-3 fold over controls 48 hours after treatment is begun. The dependence of the induction on the duration of increase in cAMP levels was investigated by incubating the cells in the presence of RO-20-1724 for 2, 6, and 12 hours. When the medium containing RO-20-1724 is removed at the times indicated and replaced with fresh medium lacking the PDE inhibitor, the elevated cAMP levels decrease to those seen in controls within 15 minutes. These transient increases in cAMP levels elicit only a 25-35% increase in TH activity measured 48 hr after the initial treatment. However, if the cells are treated with the PDE inhibitor in the presence of 56 mM KCl for 12 hours, and the medium is then replaced with that containing normal (3 mM) KCl and no PDE inhibitor, TH activity is elevated approximately 2-fold 48 hours after the initial treatment. Under these circumstances, the levels of cAMP are increased to approximately the same extent as that seen with the PDE inhibitor alone. TH activity increases by about 20% 48 hours after a 12 hour treatment with 56 mM KCl alone. We conclude that, although changes in cAMP levels may be involved in the induction of TH in neuroblastoma cells, a transient elevation in the level of cAMP is not sufficient to elicit a large increase in TH activity. However, a 2-fold induction of TH is elicited when cAMP levels are elevated in the presence of a depolarizing concentration of KCl for 12 hours.

This research was supported by USPHS grants NS07927, NS09199, and USPHS Research Fellowship Award NS05567.

896 NEUROCHEMICAL CORRELATES OF ISOLATION-INDUCED AGGRESSION IN DISCRETE BRAIN AREAS OF THE MOUSE. Y. Tizabi*, V. J. Massari* and D. M. Jacobowitz (SPON: R. W. Colburn). Dept. of Pharmacology, Howard Univ., Washington, D.C. 20059; and Lab. Clin. Sci., NIMH, Beth., Md. 20014.

Prolonged isolation has been shown to induce a syndrome characterized by increases in general reactivity, response to painful stimuli, vocalization and development of compulsive aggressive behavior. This study was undertaken to determine the neurochemical correlates of isolation-induced aggressive behavior. Adult male mice of Swiss-Webster (NIH) strain were kept in metallic isolation cages for 6 weeks. After this period they were tested for aggressiveness as follows: on 2 occasions 2 days apart, each isolated mouse was removed from its home cage and was introduced into a plastic cage; 3 minutes later a naïve group-housed mouse of BALB/CN strain, (the intruder), was placed in the same plastic cage. If on both occasions the isolated mouse attacked the intruder within 1 minute and continued the attack for 2 minutes, it was considered an aggressor. The isolated mice that did not initiate any attack on either occasion were used as controls. Two days after the second test period aggressors and controls were injected with 400 mg/kg i.p. of alpha-methyl-p-tyrosine, or saline. All mice were sacrificed 2 hours after injection, their brains removed, frozen on dry ice and sliced in a cryostat and 17 discrete areas were microdissected and assayed for norepinephrine (NE) and dopamine (DA). Using a 2-way analysis of variance, the NE steady state level was found to be significantly lower in olfactory tubercle and substantia nigra (zona compacta) and significantly higher in the septum of the aggressive mice. There was an increase in the turnover of NE in the A10 cell body region (ventromedial tegmentum) of the aggressive mice. The DA steady state of the aggressors showed a significantly lower concentration only in the olfactory tubercle. DA turnover was significantly reduced in the olfactory tubercle and increased in the caudate-putamen compared to the controls. It is suggested that NE inhibits the DA cells in the substantia nigra and A10 which project to the caudate-putamen and olfactory tubercle, respectively. Thus, isolation-induced aggression is correlated with an increased NE turnover in the A10 region which may produce decreased DA levels and turnover in the olfactory tubercle. A decline in NE levels in the substantia nigra may increase DA turnover in the caudate-putamen. The changes in DA turnover in the caudate-putamen and olfactory-tubercle further suggest DA involvement in motor and olfactory mechanisms associated with isolation-induced aggression.

897 EFFECTS OF LSD ON BEHAVIOR AND RAPHE UNIT ACTIVITY IN FREELY MOVING CATS. Michael E. Trulson and Barry L. Jacobs. Prog. in Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08540.

Single unit activity (n=44) was recorded from the dorsal raphe nucleus of freely moving cats (n=9) via chronically implanted nichrome wires, while simultaneously quantifying LSD-induced behavioral changes (e.g., limb flicking and abortive grooming). LSD at a dose of 10 μg/kg i.p. produced a mean decrease in raphe unit activity of 15% from an active waking baseline, and produced a mean of 12 limb flicks per hour, while a dose of 50 μg/kg decreased raphe unit activity by 45% and elicited 45 limb flicks per hour. Following the 50 μg/kg dose of LSD, raphe unit activity returned to baseline levels within 4-6 hours, while the behavioral changes persisted for at least 8 hours. Twenty-four hours after the initial dose of LSD (50 μg/kg), a second injection of the same dose was virtually without behavioral effect (i.e., tolerance), but produced a 63% decrease in raphe unit activity. Administration of saline or brom-LSD (a non-hallucinogenic congener of LSD) produced no behavioral changes and no significant change in raphe unit activity. Control cells outside the dorsal raphe nucleus showed no significant change in activity following LSD administration. Although the present data provide support for a causal relationship between LSD's inactivation of central serotonergic neurotransmission and the behavioral effects of the drug, three important dissociations between raphe unit activity and behavioral changes were observed: 1) low doses of LSD produced only small decreases in raphe unit activity, but produced highly significant behavioral changes; 2) LSD-induced behavioral changes outlast the depression of raphe unit activity; and 3) raphe neurons are at least as responsive to LSD during tolerance as compared to the non-tolerant condition. (Supported by NIMH grant MH-23433).

898 Effects of Phenylalanine and Tyrosine Depletion on Serotonin Synthesis in the Mouse Brain. Thomas W. Vickroy* and Kenneth M. Johnson. Univ. Tex. Med. Branch, Galveston, TX 77550.

It has been established that the synthesis of brain serotonin is dependent on the availability of its precursor tryptophan (try) which is, in turn, dependent on the ratio of plasma try to the sum of large-neutral amino acids (including tyr and phe) which compete with try for uptake into the brain. Previously, it was demonstrated that administration of 100 units/kg of the enzyme phenylalanine ammonia-lyase (PAL, an enzyme which catalyzes the deamination of phe and tyr) to rats depletes plasma phe and tyr to trace levels by 4 hrs and they remain so for at least 24 hrs, in addition, brain 5-HT levels were tripled at 4 hrs, elevated by 42% at 8 hrs, and not different from control by 24 hrs. The purpose of the study reported here is to examine the effects of several doses of PAL on the conversion of a tracer dose of ^{3}H-try to ^{3}H-5-HT in the mouse brain during a 20 min pulse period.

Male albino mice were administered either saline, 25, 50, or 100 U/kg PAL and sacrificed by decapitation either 4 or 8 hrs later. Radioactive and endogenous try and 5-HT were separated by ion-exchange chromatography and aliquots were taken for estimation of tritium by LSC and for fluorometric determination of try and 5-HT. The specific activity (SA) of try was also estimated in liver and plasma and the incorporation of ^{3}H-try into protein was measured in both brain and liver. An accumulation index (AI) was used to estimate the synthesis rate of 5-HT in the brain. AI equals dpm ^{3}H-5-HT/g/try SA.

Four hrs after PAL administration dose - responsive increases were observed in the dpm ^{3}H-try/g and try SA in brain; however, since there was not a concomitant increase in dpm ^{3}H-5-HT/g, there was a dose-responsive decrease in the AI, suggesting a compensatory decrease in the conversion rate of try to 5-HT. We observed an approximate tripling of 5-HT levels at 25 and 50 U/kg but no change at 100 U/kg. Protein synthesis was significantly decreased in the brain but not in the liver. Liver try levels were increased at all doses of PAL, being the greatest at 25 U/kg.

Eight hrs after PAL no changes were observed in brain 5-HT or try levels, 5-HT SA, or plasma try; however, try SA was increased and the AI for 5-HT remained decreased as did brain protein synthesis. We observed a significant increase in liver try levels and a decrease in liver protein synthesis.

This study has shown that PAL is a tool which can be used to explore the effects of phe and tyr depletion on try disposition and metabolism in brain and liver and should aid our understanding of how the brain responds to daily variations in circulating levels of these important biogenic amine precursors. (Supported in part by a PMA Research Starter Grant to Kenneth M. Johnson.)

899 EFFECTS OF DOPAMINE ANALOGS ON TYROSINE HYDROXYLATION IN THE RAT STRIATUM USING AN IMPROVED ASSAY FOR TYROSINE HYDROXYLASE. W.G. Waggoner* and H.J. Leighton* (SPON: C.N. Jones). Wellcome Res. Labs., Dept. of Pharmacology, Research Triangle Park, N.C. 27709.

The rate-limiting step for the synthesis of catecholamines is tyrosine hydroxylation. An improved assay for tyrosine hydroxylase (TH), based on the formation of ^3H_2O from 3,5-^{3}H-tyrosine, has been employed to study the effects of agents on tyrosine hydroxylation in the rat striatum. The method for separation of ^3H_2O from the reactants involves a freeze-drying modification, where the product, ^3H_2O, is sublimed, collected and quantitated. This has allowed for a one-step isolation procedure from the enzyme-reaction tube to the counting vial. The technique eliminates the usual requirement for separation of ^3H_2O by column chromatography and improves the reproducibility and sensitivity of the assay. With the use of only a high efficiency vacuum pump and disposable culture tubes, approximately 60 samples/hour can be analyzed. Using this technique, derivatives of 5,6-dihydroxy-2-aminotetralins (AT), (i.e. N,N-di-n-propyl, N,N-diethyl and N,N-dimethyl) were compared with apomorphine (AP), dopamine (DA) and bromocryptine for their ability to inhibit TH in rat striatal synaptosomes. Two distinct types of TH inhibitors were identified. One type, consisting of AP, bromocryptine, and the above mentioned N-substituted AT, was antagonized by 10^{-6}M neuroleptics (N) (haloperidol, pimozide, chlorpromazine, sulpiride and fluphenazine) but not by the DA uptake inhibitor benztropine (BZ), suggesting that their major mechanism of action involves activation of dopamine receptors on the plasma membrane of the synaptosome. The second type of TH inhibitor, consisting of DA, epinine, and AT lacking amino substitution, was antagonized by BZ but not by N. The latter type of TH inhibitor was weakly antagonized by N in the presence of BZ, suggesting that these compounds have a weak affinity for the plasma membrane receptor as well as a primary mechanism of action within the synaptosome. The data suggest that TH in the rat striatum can be modulated at the local level by activation of presynaptic DA receptors. Complex neuronal feedback mechanisms are not necessarily required to observe receptor mediated inhibition of TH.

900 EFFECT OF GABA-MIMETIC AGENTS AND INHIBITORS OF GABA CATABOLISM ON THE ACTIVITY OF SUBSTANTIA NIGRA (SN) DOPAMINE (DA) NEURONS. J.R.Walters, J.M.Lakoski, N.Eng*, NINCDS, NIH, Bethesda, MD. 20014

One model which has been used to explain effects of DA receptor stimulating and blocking drugs on DA activity has postulated a striato-nigral feedback loop which tonically regulates the activity of DA neurons in the SN. GABA has been considered a possible transmitter in this feedback loop since the demonstration that some striato-nigral neurons contain glutamic acid decarboxylase. These considerations have lead to the expectation that drugs which potentiate GABA-mediated neurotransmission might attenuate the activity of SN DA neurons. Studies with γ-hydroxybutyric acid (GHB) or its precursor, γ-butyrolactone (GBL), a possible GABA agonist, and with Na valproate (VAL), an inhibitor of GABA metabolism, have tended to support this expectation. GBL has been shown to inhibit the activity of SN DA neurons in both control and haloperidol (HAL)-treated rats (Walters & Roth, N.S. Arch. Pharmacol., 1976). Administration of VAL (400 mg/kg, 45 min.) increases GABA levels by 46% in the striatum and 31% in the nigra, while significantly attenuating in vivo striatal DA synthesis (determined by following DOPA accumulation after administration of a DOPA decarboxylase inhibitor) by 30% in control rats and 40% in HAL-treated rats.

However, studies with a more active and better defined GABA agonist, muscimol, and a more effective inhibitor of GABA catabolism, aminooxyacetic acid (AOAA) have cast doubt on the idea that GABAergic mechanisms are involved in the effects of GHB and VAL on DA activity, and have suggested that the tonic activity of DA cells may not be particularly sensitive to attenuation by GABA-active agents. Thus extracellular single unit recording techniques have shown that muscimol (3.5 mg/kg, i.p.) does not inhibit the activity of SN DA neurons, and actually increases the activity of these cells when administered to gallamine-paralyzed rats. This dose also increased striatal in vivo DA synthesis by 83%. In addition, AOAA had no effect on DA synthesis nor on the single unit activity of SN DA neurons in doses (50 mg/kg, 45 min. or 5 hrs.) which caused considerably greater increases in GABA levels in the striatum and nigra (232% and 173% of control at 45 min., 482% and 331% at 5 hr., respectively) than did VAL.

On the other hand, the HAL-induced (1 mg/kg, 45 min.) increase in in vivo striatal DA synthesis was reduced to 78% of the HAL control by muscimol (3.5 mg/kg, 60 min.) and to 47% of the HAL control by AOAA (50 mg/kg, 5 hr.). AOAA also attenuated the effect of HAL on single unit activity of DA cells. Thus these agents do have some attenuating effects on the DA system. The results caution, however, against using the changes seen in HAL-treated animals as a basis for predicting how a drug might alter DA activity in the absence of neuroleptic treatment.

901 SUPERSENSITIVITY TO SEROTONIN IN THE AMYGDALA AND THE VENTRAL LATERAL GENICULATE IN 5,7-DIHYDROXYTRYPTAMINE PRETREATED RATS. Rex Y. Wang, Claude deMontigny, and George K. Aghajanian, Depts. Psychiat. and Pharmacol., Yale Univ. Sch. Med., New Haven, CT 06508.

A dense and uniform serotonergic (5-HT) input to the amygdala (AMYG) and the ventral lateral geniculate nucleus (vLGN) from the midbrain raphe has been previously demonstrated by histofluorescence and horseradish peroxidase methods; physiological and pharmacological studies have supported these findings. The aim of this study was to investigate the phenomenon of denervation supersensitivity of 5-HT receptors in these two areas. 5,7-Dihydroxytryptamine (5,7-DHT), a relatively selective neurotoxin for 5-HT neurons, injected either intraventricularly or directly into the ascending 5-HT pathway in the ventromedial tegmentum (VMT) resulted in the disappearance of 5-HT terminals in the above two areas. The response of cells to microiontophoretically applied 5-HT, norepinephrine (NE) γ-aminobutyric acid (GABA) and d-lysergic acid diethylamide (LSD) in 5,7-DHT pretreated and control (injected with either vehicle or 6-OHDA into the VMT 5-HT pathway) rats were determined by extracellular recording. The equieffective dose of these compounds was evaluated by the I·T50 values, i.e., the product of the current (I) and the time (T50) required to obtain a 50% depression from the baseline firing rate.

Enhanced sensitivity of AMYG cells to the inhibitory effect of 5-HT began to develop within 24 hours and was nearly complete between 5 to 7 days after 5,7-DHT was injected directly in the VMT. There was a marked increase in sensitivity to 5-HT and its analog LSD (which is resistant to the 5-HT uptake system) in the AMYG and the vLGN after injection of 5,7-DHT directly into the VMT and the lateral ventricle respectively. In the AMYG the sensitivity of cells to NE and to a lesser extent GABA was also increased; concurrent iontophoresis of any combination of 5-HT, LSD, NE and GABA resulted in a marked synergistic effect in 5,7-DHT pretreated rats but not in controls. Concurrent iontophoresis of chlorimipramine (which blocks 5-HT uptake) and 5-HT caused a prolongation of the inhibition by 5-HT in control rats but not in 5,7-DHT pretreated rats; presumably this is due to the fact that in the latter the 5-HT terminals and their uptake system have already been destroyed.

In conclusion, denervation supersensitivity to 5-HT has been demonstrated in the AMYG and the vLGN. The supersensitivity induced by 5,7-DHT was relatively nonspecific in the AMYG. Our results also suggest that both pre- and postsynaptic mechanisms contribute to the development of the denervation supersensitivity. (Supported in part by USPH Grants MH-17871, MH-14459, and Can. Med. Res. Council).

902 THE PRODUCTION OF MYOCLONUS BY INTRACEREBROVENTRICULAR INFUSION OF INDOLES FOLLOWING INTRACISTERNAL 5,7-DIHYDROXYTRYPTAMINE AND ATTENUATION BY SEROTONIN RECEPTOR ANTAGONISTS. John D. Warbritton III*, R. Malcolm Stewart and Ross J. Baldessarini. Mailman Laboratories for Psychiatric Research, McLean Division of Massachusetts General Hospital, Harvard Medical School, Belmont, Massachusetts, 02178; and Department of Neurology, University of Texas Health Science Center at Dallas, Dallas, Texas, 75235.

Serotonin (5-HT) is implicated as a neuromodulator in the central nervous system (CNS) with important inhibitory behavioral effects. The present work investigated the excitatory behavioral effects of intracerebroventricularly infused indoleamines in rats lesioned with a serotonin neurotoxin, 5,7-dihydroxytryptamine given after desmethylimipramine. Continuous (90 min.) intracranial infusion of 5-HT into the right lateral ventricle of male rats through a chronically implanted cannula produced intermittent jerking of head and limbs (myoclonus) as recorded with a Stoelting electronic activity monitor. This effect was dependent on the dose of 5-HT infused, over a range of 2-16 µg/min. At the higher doses toxic effects included generalized seizures and death. The approximate half-maximally effective dose (ED50) of 5HT was 6.7 µg/min. Infusion of structurally related indoles including tryptamine, indoleacetaldehyde (IAAld) and 5-hydroxyindoleacetic acid (5-HIAA) were also made. Myoclonus was not seen after tryptamine. At doses comparable on a molar basis to the ED50 of serotonin, IAAld and 5-HIAA produced similar behavioral effects to 5-HT. Acute intraperitoneal administration of the putative serotonin receptor blockers cyproheptadine and methiothepin strongly inhibited the myoclonic effects of 5-HT but only slightly inhibited 5-HIAA myoclonus. Infusion of norepinephrine 1.3 µg/min. (dose) after lesions with 5,7-DHT increased locomotor activity no more than in control animals and did not produce myoclonus. These studies suggest that indoles other than 5-HT may have strong effects on the CNS but that the effects of deaminated indoles may not be mediated by serotonin receptors.

(This work was supported in part by U.S. PHS (NIMH) Grants MH 16674 and MH 22515, and Career Research Scientist Award MH 74374, a fellowship from the Massachusetts General Hospital (to J. Warbritton), a BRSG Award (from the University of Texas Health Science Center 5-507 RR-5426-16.

903 NORADRENERGIC MODULATION OF SOMATOSENSORY CORTICAL NEURONAL RESPONSES TO IONTOPHORETICALLY APPLIED PUTATIVE NEUROTRANSMITTERS. Barry D. Waterhouse, Hylan C. Moises and Donald J. Woodward. Dept. Cell Biol., Univ. Tx. Health Sci. Ctr., Dallas, Tx. 75235.

In the present study we have examined whether norepinephrine (NE) in low doses can alter responsiveness of somatosensory cortical neurons to putative neurotransmitter substances. Extracellular activity of cortical neurons was recorded from halothane-anesthetized albino and hooded rats using multibarrel micropipettes. Responses to microiontophoretic pulses (8-10 sec. duration at 45-50 sec. intervals) of acetylcholine (ACH) and gamma-aminobutyric acid (GABA), putative cerebral cortical neurotransmitters, were examined before,during and after NE iontophoresis (2-40 nanoamps). Post-drug response histograms were used to quantitate effects of NE on spontaneous and drug-evoked activity.

In 21 of 25 cells, NE exerted differential effects on spontaneous activity and ACH-induced excitation. ACH responses of 8 cells were potentiated above control levels with doses of NE which had little or no effect on spontaneous rate. In 13 cells, ACH-evoked activity was preserved relative to NE suppression of background discharge such that signal to noise ratios were enhanced. These effects often lasted from one to several minutes following NE application. The ability of dopamine to enhance ACH was weak or absent in 5 of 7 cells for which NE did modulate ACH.

In 7 of 11 cells, NE at low doses augmented GABA inhibition, and this effect often persisted for one to several minutes after cessation of NE iontophoresis. Four cells showed potentiation of GABA inhibition with doses of NE which caused little or no change in spontaneous activity.

In summary, these findings suggest that NE enhances the responsiveness of cortical neurons to both excitatory and inhibitory putative neurotransmitters. These data are consistent with other evidence reported by this laboratory which suggests that NE may act in cerebellum to modulate Purkinje cell responsiveness to synaptically and iontophoretically released transmitter agents. (Supported by NIH 1 F32 NS05699-1 to B.D.W. and NSF BNS77-01174 to D.J.W.).

904 HIGH PLASMA CATECHOLAMINES PREDICT STRESS-INDUCED LETHALITY. Barton G. Weick,* Sue Ritter, Robert C. Ritter (SPON: T.A. McKean). Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, College of Veterinary Medicine, Washington State University, Pullman, WA 99164

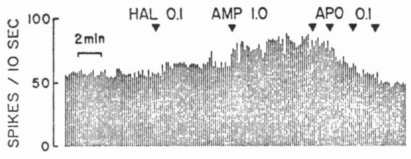
Plasma catecholamines (CA) and blood glucose concentrations were measured before, during and after footshock stress in male Sprague-Dawley rats. Adrenal CA's, liver glycogen, and splenic weight and norepinephrine (NE) content were also measured at the time of death. Of the rats tested with footshock, 60 percent died spontaneously within 5 hours after footshock (spontaneous lethality, L). Plasma CA concentrations were significantly higher in L's than survivors (S) at the termination of footshock (10.57 ± 2.07 ng/ml, L; 3.70 ± .52 ng/ml, S; $p < .05$) and 2 hours after shock (21.88 ± 8.12 ng ml, L; 3.73 ± .60 ng/ml, S; $p < .05$). The peak plasma CA levels during stress were significantly higher in L's than S's (13.72 ± 2.72 ng/ml, L; 4.91 ± .69 ng/ml, S; $p < .05$). After stress the peak plasma CA level was also significantly higher in L's than S's (27.34 ± 5.10, ng/ml L; 6.90 ± 1.69 ng/ml, S; $p < .01$). Adrenal CA content of L's was indistinguishable from that of S's (L, 60 percent of control, $p < .05$; S, 45 percent of control, $p < .01$) indicating that stress-induced lethality was not due to sympatho-adrenomedullary exhaustion. Furthermore, the fact that plasma CA levels of L's were three to four times greater than those of S's suggests that elevated CA levels may be responsible for some deleterious effects which contribute to the lethal outcome of stress. Blood glucose levels for L's and S's were not different during stress. However, blood glucose levels of L's were significantly lower than S's before and after stress. This result suggests that glucostasis in L's was impaired. Both L's and S's were similarly depleted of liver glycogen after stress ($p > .1$). Therefore, the failure of glucostasis in L's was probably not due to depletion of glycogen. S's had lower spleen weights suggesting an impairment of splenic contraction in L's. More complete splenic contraction in S's may be of adaptive value during stress and may also indicate greater sensitivity of the target organ to catecholamines.

Our results show that rats which are more susceptible to stress-induced lethality exhibit extraordinarily high plasma CA concentrations, but may be relatively refractory to beneficial CA mediated effects, such as maintenance of glucostasis and splenic contraction. Plasma CA levels appear to be predictive of susceptibility to stress-induced lethality. Furthermore, high plasma CA concentrations or reduced target organ sensitivity to CA may play a role in the pathogenesis of stress-induced lethality.

905 AMPHETAMINE-INDUCED INCREASE IN SUBSTANTIA NIGRA FIRING RATE AFTER PRETREATMENT WITH HALOPERIDOL. James J. Welch*, Jeffrey M. Liebman and Jeffrey K. Saelens. Research Dept., Pharmaceutical Div., CIBA-GEIGY Corp., Summit, NJ 07901.

Systemic administration of apomorphine (APO) or d-amphetamine (AMP) reliably reduces spontaneous firing by single cells in the zona compacta of the substantia nigra, and this effect can be reversed by subsequent administration of haloperidol (HAL). These findings are consistent with presumed local and/or neuronal loop feedback regulatory mechanisms acting upon dopaminergic neurons in this region (Bunney et al., J. Pharmacol. Exp. Ther. 185:560, 1973; Groves et al., Science 190:522, 1975). We have noted, however, other effects which cannot be attributed to presumed regulatory feedback mechanisms.

Extracellular single unit activity was recorded from chloral hydrate-anesthetized Wistar rats through single barrel micropipettes. Cell bodies in the substantia nigra, zona compacta, which are presumed to be dopaminergic, were identified by standard criteria and subsequent histological verification. Following recording of baseline firing rate, successive intravenous drug injections were administered at 5-10 min intervals. After pretreatment with HAL (0.1 mg/kg), which by itself increased firing rate slightly in most cells, AMP (1 mg/kg) did not reduce firing rate, but increased it reliably by an average of 36% over baseline, an effect which was reversed by APO (see fig.).



An even greater average increase followed injection of 5 mg/kg AMP in HAL-pretreated rats. After HAL pretreatment, APO (0.1-0.5 mg/kg), unlike AMP, consistently reduced firing rate. In non-pretreated rats, AMP never increased, but instead reduced firing rate. At a dose which elicited profound sympathomimetic effects, L-norepinephrine bitartrate (1 µg/kg) failed to alter firing rate appreciably either before or after HAL injection. The enhancing effects of AMP on firing rate after HAL are not readily predicted by a negative feedback mechanism involving dopaminergic neurons. It may be that the concurrent norepinephrine releasing effect of AMP can modulate firing rate of substantia nigra neurons when dopamine receptor blockade prevents AMP-released dopamine from activating negative feedback mechanisms.

906 ROTATIONAL BEHAVIOR IN RATS FOLLOWING NEONATAL, UNILATERAL 6-HYDROXYDOPAMINE (6-OHDA) LESIONS OF THE CAUDATE OR MIDBRAIN.
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Adult rats with unilateral 6-OHDA lesions of the nigrostriatal (NS) dopamine (DA) pathway rotate in a direction ipsilateral to the lesioned side after d-amphetamine but contralateral after apomorphine. Our examination of the results of similar lesions in newborn rats suggests that maturation affects the rotation response. Rats received unilateral injections of 10 µg 6-OHDA in 1 µl ascorbic acid/saline solution in the caudate or 5 µg in 0.5 µl in the area of the substantia nigra between 12 and 24 hours after birth. They were tested for preferred direction of rotation after apomorphine (1.0, 2.5 or 5.0 mg/kg) or d-amphetamine (1.0, 5.0 or 10.0 mg/kg) 7 and 14 days after the 6-OHDA treatment. At least 8 animals were studied for each combination of drug dose, lesion site and age of testing. Beginning at 50 days of age, each rat was also tested for rotation to d-amphetamine (2.5 mg/kg) and apomorphine (2.5 mg/kg) with a one week interval between rats.

Rotational preferences at 7 and 14 days did not differ from chance. Preferences in the adult rats after both apomorphine or d-amphetamine were contralateral to the lesion side, regardless of lesion site. The extent of DA depletion in the lesioned caudate compared to the unlesioned caudate averaged 52% in animals that had received 6-OHDA in the caudate nucleus; relative DA depletion was 45% in rats that were lesioned in the area of the substantia nigra. Some rats were treated with apomorphine or d-amphetamine and sacrificed by focused microwave irradiation. In these animals striatal and forebrain cyclic nucleotide levels showed no consistant differences between lesioned and unlesioned sides. Furthermore, the expected increases in cAMP on the side contralateral to the direction of rotation were not found.

DA receptor supersensitivity, thought to underlie the rotational response to apomorphine, did not develop by 14 days. However, it was evident that supersensitivity had developed by 50 days. This suggests that supersensitivity develops more slowly in the neonatally lesioned rat than in the rat lesioned as an adult. Furthermore, the contralateral rotation observed after d-amphetamine is inconsistent with the hypothesis that presynaptic activation of NS neurons elicits rotation contralateral to the NS terminations with highest dopamine content. (Supported by USPHS Grants HD-10570, HD-03110 and MH-00013.)

907 ROLE OF THE TECTOSPINAL TRACT IN APOMORPHENE-INDUCED ROTATION.
David Wirtshafter, Karen E. Asin, and Ernest W. Kent. Dept. Psych. Univ. Il.-Chicago Circle, Chicago, Illinois 60680.

It is well known that unilateral 6-hydroxydopamine (6-OHDA) induced distruction of dopamine-containing neurons in the substantia nigra (SN) results in contralateral circling behavior in response to apomorphine (Apo). The recent work of Marshal and Ungerstedt (Sci.,198,1977) suggests that the striato-nigral projection may be crucially involved in this response. This projection is known to be partly GABAergic, and its importance in rotation is further substantiated by our current finding that Apo-induced rotation can be antagonized by picrotoxin.

If the projection from the caudate to the SN is importantly involved in Apo-induced rotation, the question arises as to which efferents from the SN are involved. Recent anatomical and electrophysiological evidence has indicated the existence of a projection from the SN which terminates, in part, on tectospinal motorneurons in the deeper layers of the superior colliculus (SC). Therefore, this study was designed to examine the possible involvement of the tectospinal system in Apo-induced rotation.

Since the tectospinal tract decussates in the midbrain (the dorsal tegmental decussation (DTD)) interruption of this system is possible by means of a saegittal knife cut made with a retractable wire knife. DTD knife cuts were made in rats with 6-OHDA unilateral lesions of the SN (8ug/4ul). Ten to twelve days following surgery the rotational response to .5 mg/kg Apo was examined in a circular tub. Animals with DTD transections exhibited a greater than 80% reduction in rotation relative to animals with SN lesions alone. This reduction could not be attributed to hypomotility, as animals with knife cuts alone showed spontaneous and Apo-induced photocell activity equivalent to unoperated controls. Additionally, there was a virtual absence of postual asymmetry in DTD + SN rats, compared to rats with SN lesions alone.

Since it is possible that these effects on rotation may have been due to damage to non-tectospinal decussating fibers, large electrolytic lesions were made in the SC ipsilateral to 6-OHDA SN lesions. When tested with .5 mg/kg Apo, these subjects showed a marked attenuation in rotation relative to animals with nigral lesions alone. SC lesions by themselves produced only mild ipsilateral rotation, suggesting that the attenuation of contralateral rotation seen in animals with SN lesions could not have been due to simple cancellation.

In conclusion, we have demonstrated that the tectospinal system plays a major role in the contralateral rotation induced by Apo in animals with unilateral 6-OHDA lesions of the SN. It appears that a striato-nigro-tecto-spinal pathway is involved in the production of postural asymmetries by dopamine agonists.

908 HISTOCHEMICAL AND CYTOCHEMICAL IDENTIFICATION OF AMINERGIC NERVE FIBERS IN THE HUMAN MALE PELVIC/PERINEAL VISCERA.
Joe Wood, J.A. McConnell and G.S. Benson*. Depts. of Neurobiol. and Anat., and Surgery, Univ. Texas Med. Sch., Houston, Texas 77025.

Specific cytochemical mapping of the nerve distribution to the human male pelvic/perineal viscera is not in agreement with pharmacological studies; plus the anatomical literature is not supportive of the various physiologic studies conducted on such organ areas. Many agents employed pharmacologically and with some degree of success in urologic therapy are lacking of basic anatomical, neurochemical, and physiologic verification due to lack of data on the normal nerve supply to the male pelvic area.

Tissues utilized in these studies were obtained during surgical procedures for open prostatectomy, vasectomy and transsexual gender reassignment procedures. The areas studied were corpus cavernosum, corpus spongiosum and penile urethra, in addition to anterior bladder body (dome), vas deferens and epididymis. Adrenergic fibers were demonstrated by glyoxylic acid (GA) histofluorescence and electron microscopically with gluteraldehyde-potassium dichromate (GDC). Results show varying numbers of adrenergic fibers in all tissues. Such fibers are most noticeable in the vas deferens and in the corpus cavernosum. The corpus spongiosum contains a small number of adrenergic fibers. Many of the fibers are seen in the vicinity of blood vessels; however, others are in close approximation to the smooth muscle cells.

These catecholamine containing fibers are easily demonstrated with histofluorescence and lead to a better pattern distribution elucidation of sites which can be followed electron microscopically. Areas of the GCD reaction can be visualized at the light microscopic level and then tissue can be processed for fine structural study. X-ray microanalysis shows a positive confirmation of the chromium (catecholamine deposits).

These studies show that the mapping of and realization of catecholamine fibers to areas not previously definitively studied can be accomplished with the GA technique, and indicate that at the electron microscopic level confirmation of biogenic amine sites can be accomplished with GCD. X-ray microanalysis then identifies specific nerve types heretofore not assigned to aminergic systems.

Sponsored in part by USPHS Grant NS10326 and AUA Research Scholarship Grant.

909 INTERACTION OF NOREPINEPHRINE WITH CEREBROCORTICAL ACTIVITY EVOKED BY STIMULATION OF SOMATOSENSORY AFFERENT PATHWAYS IN THE RAT.
Donald J. Woodward and Barry D. Waterhouse. Dept. Cell Biology, Univ. Tx. Health Sci. Ctr., Dallas, Tx. 75235

The present study was conducted to investigate the action of norepinephrine (NE) on transmission of information through somatosensory cortical neuronal circuits. Natural stimulation of afferent pathways was evoked by suprathreshhold mechanical stimulation (500 micron punctate deformation of glaborous skin at 1 Hz.) of the contralateral forepaw. Multibarrel micropipettes were used to apply drug iontophoretically and to record extracellular activity of forelimb somatosensory neurons in albino and hooded rats lightly anesthetized with halothane. Unit responses to forepaw stimulation were examined before, during and after iontophoresis of NE. NE induced changes in stimulus bound and spontaneous activity were quantitated using post-stimulus response histograms.

All cortical neurons studied were spontaneously active and had one of the following responses to forepaw stimulation: 1) short latency excitation, 2) inhibition or 3) short latency excitation followed by a period of inhibition. In 32 of 43 (74%) neurons, NE (10-50 nanoamps) modulated differentially both stimulus bound excitation and spontaneous activity such that signal to noise ratio increased approximately twofold. Evoked spiking in 12 cells was quantitatively potentiated above control levels during NE application. These effects often persisted for several minutes following NE administration.

In 27 of 33 (82%) cells, NE augmented stimulus bound inhibition or post-excitatory suppression of activity. Potentiation of inhibition was observed in 5 cells at levels of NE which caused minimal or no change in background discharge. These changes often lasted several minutes after cessation of NE application.

In summary, these results suggest that low levels of NE may facilitate the relay of afferent information within the cerebral cortical circuitry. These data are consistent with a modulatory as opposed to a specific information transfer role for NE in cerebral cortex.

(Supported by NSF BNS 77-01174 to D.J.W. and NIH 1 F32 NS05 699-1 to B.D.W.).

MOTOR SYSTEMS

910 PROGRAMMED RESPONSES IN HUMAN ANKLE FLEXORS AND EXTENSORS EVOKED BY FORCED ANKLE FLEXION. Gyan C. Agarwal and Gerald L. Gottlieb, Dept. of Physiology, Rush Presbyterian St.-Luke's Medical Center, Chicago, Illinois

Torques were applied to dorsiflex or plantarflex the ankles of seated human subjects. The subjects were instructed to resist the torque, assist it or to not to react at all.

Two phasic responses are evoked in the triceps surae and anterior tibial muscle EMG's. The earliest is the myotatic reflex and occurs in the stretched muscle at latencies of between 40 and 90 msec. This response is followed by the "programmed" response at a latency of between 100 and 200 msec following torque onset.

The programmed response may be characterized as follows: 1) Its latency is significantly shorter than that of a visual reaction time but comparable to a kinaesthetic reaction time. 2) The latency in a single muscle is slightly but statistically different with different instructions (ie "resist", "assist") but no one instruction always produces the shortest latency among different subjects. 3) Experiments in which the subject can anticipate neither the magnitude nor the direction of the torque do not show increases in latency. This is in marked contrast to visual reaction tasks with a simple vs choice paradigm. 4) The amplitude of the programmed response is proportional to the amplitude or the disturbing torque whenever the subject is instructed to resist and restore the foot to its initial position. The response amplitude is largely independent of the torque with most other instructions. 5) The amplitude of the programmed response is reduced by prior contraction of the extensors of flexors. 6) The instruction to not react suppresses the programmed response.

We conclude from the above that programmed EMG responses in the leg muscles at latencies between 100 and 200 msec evoked by ankle flexion are not reflex in nature. They are a form of voluntary reaction triggered by a mechanical stimulus which is dependent on prior instruction and also on peripheral input, if "appropriate".

Peripheral input is only useful in generating appropriate responses if the subject was instructed to generate proportional responses in opposition to the disturbing torque.

The relationship between the programmed responses seen in the leg and the apparantly "transcortical reflex" responses found in the upper limb is not clear at present.

(Supported by NIH grants NS-00196, NS-12877 and NSF grant ENG-7608754)

911 EFFECTS OF ALTERED GRAVITY ON RHYTHMICAL HOPPING IN MAN. S.B. Backman* and D.G.D. Watt. Dept. of Anaesthesia Res. and Aviation Med. Res. Unit, McGill University, Montreal, Quebec, Canada H3G1Y6

Previous investigations have demonstrated that human subjects asked to hop up and down without external cues chose their frequency with great accuracy and consistency. It was suggested that this results from at least 3 mechanisms: a pre-programmed landing, a stretch response on contact with the ground, and a vestibular otolith response to sudden weightlessness on taking off.

The present experiments were designed to assess possible vestibular contributions by rotating the gravity vector 90° and varying the inertial accelerations experienced during hopping. Subjects were suspended horizontally, substituting for gravity with bungee cords running from the waist to the wall. Hopping at the subject's preferred frequency in the normal vertical direction was compared with hopping on the wall (horizontal) at 3 simulated g levels.

Average hopping frequencies were: vert:1.86 hops/sec, horiz(1g):1.63, horiz(.67g):1.41, horiz(.33g):1.21. The lower frequency at 1g (horiz) resulted from inadequate tension in the bungees in some subjects. The lower frequency of hopping at decreased g levels was almost entirely due to more time spent off the ground.

In the vertical direction, emg activity in gastrocnemius-soleus started on average 120 msec before contact. In the horizontal position, it maintained its relationship to the previous take-off, and so started 157 msec before contact at 1g, 246 msec before at .67g and 334 msec before at .33g. Its amplitude became smaller as g levels decreased. This early emg activity peaked about the time of ground contact and then declined.

Following contact, 2 distinct bursts of activity could be identified. One started 40-50 msec after contact and lasted about 50 msec. A later and larger burst started 100 msec after contact and lasted until 250-400 msec. The latter activity was very similar at all 3 g levels (subject horizontal) but was always significantly larger when they were vertical.

These results confirm that emg activity during a hop is triggered by the previous take-off, possibly due to an otolith-spinal reflex. After landing, there is evidence for both segmental and long-loop stretch responses. Late activity is influenced by the direction of the gravity vector, also possibly through the otoliths.

(Supported by M.R.C. Canada Grant MA-5837)

912 THE CERVICO-OCULAR REFLEX IN NORMAL HUMAN ADULTS. David E. Barlow* and William Freedman. Dept. of Rehab. Med., Temple Univ., Phila., PA 19141

The cervico-ocular reflex (COR) was examined in ten normal adults, five male and five female, ranging in age from 21 to 32. To produce neck torsion without optokinetic or vestibular stimulation, each subject underwent body rotation in the dark while his head was held fixed with respect to the environment by means of a biteplate. A hydraulically-driven rotatable platform produced passive body rotations of ±0.4 radians in amplitude at five stimulus frequencies: 0.025, 0.05, 0.1, 0.2 and 0.4 Hz. During certain trials the subjects themselves produced active body rotation using their neck muscles while standing on a ball-bearing turntable. The subjects' shoulder movement was recorded by a potentiometer and eye movements were recorded using electrooculographic (EOG) techniques.

Each experimental trial consisted of between four and twenty cycles of rotation and was analyzed by a computer as follows: individual cycles of rotation were defined, overlaid and averaged. The resulting single cycle of average shoulder movement was considered the stimulus and the resulting cycle of averaged eye movement was considered the response. The 'gain' of the response was then defined by the ratio of the amplitude of the averaged eye movement over the amplitude of the averaged stimulating shoulder movement. The 'phase angle' of the response was defined by the displacement, measured in degrees, between the corresponding points in the averaged shoulder and eye position signals.

The experimental results indicate that:

1) The amplitude of the cervico-ocular response declines with increasing frequency of neck torsion. The average gain of the response fell from 18% at 0.025 Hz to 4% at 0.4 Hz.

2) The phase lag of the response increases with increasing stimulus frequency. The average phase lag of -115° at 0.025 Hz increased to -210° at 0.4 Hz.

3) Conditions which required subject concentration and alertness (e.g., responding quickly to an audio signal or trying to maintain fixation in the dark) yielded responses that were two to three times smaller than conditions which allowed the subject to relax.

4) The COR response was found to be very variable whether analyzed cycle by cycle within an individual trial, trial by trial within an individual subject, or subject to subject. In all three methods of analysis gain differences of up to a factor of four and phase differences ranging from 60° to 120° were observed.

5) The responses produced during active body rotation were similar, in both gain and phase, to responses observed during passive body rotation.

913 STUDIES OF DESCENDING PROJECTIONS FROM THE CAUDAL MEDULLA IN THE CAT. R. M. Bowker* and J. D. Coulter, Marine Biomedical Institute and Depts. of Physiology and Biophysics and Psychiatry and Behavioral Sciences, The University of Texas Medical Branch, Galveston, Texas 77550.

The few studies of the origins of descending projections from the caudal medulla in the cat have been limited to descriptions of the dorsal column nuclei (Burton and Loewy, 1977) and of the nucleus retroambiguus (Kuypers and Maisky, 1975). In this report we describe the topographical anatomy and neurophysiology of additional descending cell groups in the caudal medulla of the cat in particular those originating in the nucleus supraspinalis and adjacent reticular formation.

Large, multiple injections of horseradish peroxidase (HRP) were made into the upper cervical spinal cord, C_2-C_3, cervical enlargement, C_6-C_8, mid-thoracic cord, T_7, and lumbar enlargement. After a 3 day survival period the animals were perfused and the tissues were routinely prepared and reacted with tetramethyl benzidine (Hardy and Heimer, 1977). In physiological experiments with chloralose anesthetized cats, glass micropipettes were used to record from identified units in the caudal medulla antidromically activated from different spinal levels. Receptive fields were characterized for these neurons.

Following injections of HRP into the upper cervical cord, medium (20-40μm) and small (<20μm) cells were labeled in the nucleus supraspinalis and the medial nucleus reticularis ventralis (NRV) beginning at the spinomedullary junction and continuing rostrally. With HRP injections covering both the dorsal and ventral spinal grey matter labeled cells were seen bilaterally in these medullary cell groups, but mainly ipsilaterally. Injections of HRP placed in the dorsal spinal grey matter resulted in labeling of neurons in the ipsilateral parts of the dorsal column nuclei and spinal trigeminal complex. Cervical enlargement injections resulted in labeled cells located more laterally in the ipsilateral NRV, while no labeled cells were seen in the nucleus supraspinalis. Only a few labeled cells were present in the contralateral nucleus retroambiguus. After thoracic and lumbar injections retrogradely labeled cells were found primarily in the lateralmost NRV with extensive labeling of cells in the contralateral nucleus retroambiguus. These results indicate that the feline supraspinal projections from the caudal medulla originate from distinct cell groups, similar to that of the primate. Furthermore, the projections from the nucleus supraspinalis and adjacent reticular formation appear to form a somatotopically organized projection system to the different spinal cord levels.

Supported by Grant NS 12481.

914 OPERANT REINFORCEMENT OF FTG UNITS. S. M. Breedlove*, D. J. McGinty, and J. M. Siegel. Department of Psychology and School of Medicine, University of California, Los Angeles, CA 90024 and V.A. Hospital, Sepulveda, CA 91343.

Previous studies have found that cells in the gigantocellular tegmental field (FTG) of the pontine reticular formation discharge during both waking movements and REM sleep. It was hypothesized that FTG discharge related to the motor activation common to these states (Siegel and McGinty. Science 196:676-680, 1977). In this study FTG single unit discharge in cats was increased by operant conditioning using lateral hypothalamic (LH) stimulation as a reinforcement. Behavioral changes accompanying increased FTG discharge could then be observed. This procedure provides an objective means for studying the behavioral correlates of FTG discharge.

Of 22 FTG cells studied, 16 had significantly higher mean 10 second rates during reinforcement sessions than they did in baseline sessions ($p<.05$, two-tailed, t-test). During the reinforcement of 12 experimental cells, a second, control cell was recorded simultaneously. Comparison of unit discharge change in experimental and control cells demonstrates the specificity of the conditioning procedure. Both the experimental cells and the control cells increased discharge during the early reinforcement periods, but during the final 10 minutes of reinforcement the experimental cells maintained or increased firing while the discharge of control cells was reduced. If the discharge of the entire reinforcement session was averaged, the percentage increase over baseline was not significantly different in experimental and control cells. However, the percentage increase in discharge during the final ten minutes of reinforcement was significantly greater in experimental cells than in control cells ($p<.05$, two-tailed, Wilcoxon sign-rank, matched pairs test). For all 22 reinforced FTG cells, increased discharge was accompanied by an increase in motor behavior. For most of the cells, specific, stereotyped movements accompanied the increase in unit discharge during the later portion of the reinforcement session. Typical movements included rotation of the head to the right or to the left, or lifting the head. These stereotyped movements corresponded to the behavioral correlate of discharge determined prior to reinforcement.

In summary, unit firing in most FTG cells can be readily increased by operant conditioning techniques. These operantly-conditioned increases in FTG discharge were accompanied by increased movement, often of a specific nature unique to the cell. The increased FTG firing was not accompanied by sudden REM onset in waking or by cataplexy.

915 BILATERAL CHANGES IN LIMB MOVEMENTS FOLLOWING UNILATERAL CEREBELLAR LESIONS IN HUMANS. John D. Brown and J. D. Cooke. Depts. of Clinical Neurological Sciences and Physiology, Univ. Western Ontario, London, Ontario.

Simple step-tracking movements made by patients with unilateral cerebellar dysfunction were studied. One patient was followed for one year after the development of a unilateral left hemispheric cerebellar hematoma. The second patient, with a focal left hemispheric cerebellitis, was followed for approximately 6 months. During studies the subjects were seated comfortably with their forearm supported horizontally. They grasped a handle at one end of a manipulandum which was pivoted at the other end above their elbow. The subjects were asked to perform alternating flexion/extension movements about the elbow while target and handle positions were displayed to them on an oscilloscope. No restrictions were placed on movement velocities or reaction times. When amplitudes of target movements were varied curves of peak velocity of movement vs movement amplitude showed that the larger amplitude movements made by the affected arm progressively decreased in velocity over a period of months. Smaller amplitude movements were less affected. Following this period and corresponding to the period of clinical recovery, the relation between velocity and movement amplitude moved towards the relation seen in normal subjects. Movements made with and without visual feedback of arm position had virtually identical curves of movement velocity vs amplitude. In both subjects over the course of the study movements made by the clinically "unaffected" arm had velocity/amplitude relations identical to those of the affected arm. It is suggested that, over time, the velocities in the affected limb decrease in order to help overcome the hypermetria consequent to the cerebellar lesion. Further, it is suggested that velocities in the "non-affected" limb are likewise decreased in order to maintain inter-limb co-ordination.

(Supported by PG-1 from the Medical Research Council of Canada)

916 ELECTROMYOGRAPHIC RESPONSES TO SUDDEN ANKLE DISPLACEMENT IN NORMAL AND PARKINSONIAN SUBJECTS. C.W.Y. Chan, R.E. Kearney, G. Melvill Jones. Aviation Medical Research Unit, McGill University, Montreal, Quebec, Canada.

It is well known that in Parkinsonian subjects with akinesia reaction times are increased but reflex latencies remain normal. We have attempted to use this knowledge to distinguish between "reflex" and "voluntary" components of the response to ankle displacement. The electromyographic (EMG) response of tibialis anterior to three different instructions were examined in 9 Parkinsonian patients and 9 age-matched normal humans. The instructions were: as soon as possible (1) dorsiflex the ankle in response to a visual cue, (2) oppose suddenly applied and servo-controlled ankle plantarflexions, (3) relax and allow the ankle to be plantarflexed.

Two principal findings emerged: (1) 6 out of 8 Parkinsonian patients had a significantly longer visual response latency than normal subjects. In the same patients, the 'late' EMG response evoked by opposing ankle plantarflexion (termed the Functional Stretch Response, FSR) was similarly delayed. (2) The intermediate component of the response (termed the Polysynaptic Stretch Response, PSR) was significantly larger in Parkinsonian than normal subjects although its mean latency was the same. Furthermore, its amplitude increased with increases in both displacement amplitude and velocity.

The delay of the FSR in akinesic patients argued against its being a stereotyped reflex. In contrast, the findings that the PSR latency remained unchanged in akinesic patients and that its output is proportional to input characteristics support the view that the PSR is reflex in nature. The increase in PSR in Parkinsonian subjects appears to correspond to the enlarged M2 component in upper limb muscles which has been attributed to an increased gain in long-loop reflexes in Parkinsonian patients[1].

1. Tatton, W.G. and Lee, R.G. Brain Res. 100:671 (1975).

Supported by the Canadian Medical Research Council.

917 INTRACELLULAR AND EXTRACELLULAR EVIDENCE FOR CHANGES IN EXCITABILITY OF MASSETER MOTONEURONS DURING QUIET AND ACTIVE SLEEP. Scott H. Chandler*, Benjamin B. Chang*, and Michael H. Chase. Departments of Physiology and Anatomy, Sch. Med., UCLA, Los Angeles, CA 90024

The brainstem masseteric monosynaptic reflex is depressed during active sleep as compared to quiet sleep (Chase et al., Experientia 24: 47, 1968). Recent studies from our laboratory employing the technique of intracellular recording in chronic cats have demonstrated that the reflex suppression is accompanied by tonic membrane hyperpolarization of masseter motoneurons during active sleep (Nakamura et al., Science, 199: 204-220, 1978). In the present study we have examined the properties of the extracellular field and intracellular spike potential of antidromically activated masseter motoneurons during quiet and active sleep.

In five cats the antidromic field potential was induced by stimulation of motoneuron fibers in the masseter muscle and recorded in the trigeminal motor nucleus with 2 M NaCl glass micropipettes (tip resistance of 1-3 MΩ). During active sleep, the antidromic field potential was reduced to 50-75% of its amplitude during quiet sleep.

The excitability of individual masseter motoneurons was examined by utilizing intracellular recording techniques. Glass micropipettes filled with 2 M K citrate (tip resistance of 8-15 MΩ) were used. During quiet sleep, the intensity of the stimulus delivered to masseter motoneuron fibers was set at a level to produce consistent antidromic invasion of the neuron soma. The induced antidromic spike potential was blocked during the transition from quiet to active sleep. This conduction block was maintained throughout active sleep and was coincident with membrane hyperpolarization of 2 to 10 mV. The cessation of antidromic invasion also paralleled the decrease in neck EMG and distal limb EMG activity that is characteristic of active sleep.

The reduction in amplitude of the antidromic field potential, the blockade of the antidromic spike, and the tonic membrane hyperpolarization provide direct cellular correlates at the level of the final common pathway for the atonia of active sleep.

Supported by USPHS NS 09999.

918 FUNCTIONAL PROPERTIES OF PRIMATE CORTICOMOTONEURONAL CELLS. P.D. Cheney and E.E. Fetz, Dept. of Physiology and Biophysics and Reg. Prim. Res. Ctr., Univ. of Washington, Seattle, WA 98195.

In monkeys making controlled wrist movements, corticomotoneuronal (CM) cells were identified by characteristic post-spike facilitation (PSF) of forelimb muscle activity detected by spike-triggered averaging. Activity of these cells, which has a directly proportional effect on motoneuronal excitability, was quantified during graded dynamic and static wrist responses. Monkeys were trained to alternately flex and extend the wrist between electronically detected hold zones against spring-like loads; thus, wrist displacement from a center zero position required proportional active torques. The same monkeys also performed isometric ramp-and-hold torque responses, alternating between flexion and extension torque zones of variable magnitude. For each cell, response averages of cell and muscle activity, wrist position and torque were compiled for different load levels. On the basis of their dynamic and static responses during the ramp-and-hold movements, all CM cells (n=135) could be classified into one of four basic types: phasic-tonic (59%), tonic (28%), phasic-ramp (8%) and ramp (5%). All CM cells fired during the static hold period, either at constant rates (tonic types) or with gradually increasing rates (ramp types). In addition, some cells of each group exhibited larger phasic responses at the onset of the torque response, with or without any associated wrist displacement. (In contrast, other precentral cells which fired only phasically at movement onset consistently failed to exhibit PSF.) The tonic activity of all CM cells adequately documented (25) was a linear function of static torque over much of the range studied. However, the load sensitivity--i.e., the increment in firing rate per increment in static torque--was consistently greater for extension related CM cells than for flexion related cells (means: 5.2 and 2.5 imp/sec/dyne-cm). Since the mechanics of wrist movements would not seem to fully explain these differences, they may reflect basic differences in the degree of cortical control of flexor and extensor muscles.

To test the participation of CM cells in postulated transcortical reflexes subserving load compensation, activity of 12 CM cells was recorded during transient loading and unloading perturbations of the wrist applied during the hold phase of both flexion and extension. All CM cells exhibited at least those responses consistent with the load compensation hypothesis--i.e., excitation by perturbations which stretched the muscles facilitated by the cell (mean latency: 24 ms). The second EMG response (M2) had a mean onset latency of 30.8 ms; the difference of 6.8 ms agrees well with the mean PSF latency of 7.2 ms for these same cell-muscle pairs. However, half of the CM cells also showed additional responses to load perturbations inappropriate for load compensation.

919 RECRUITMENT ORDER IN THREE DIFFERENT REFLEX RESPONSES ELICITED IN A HINDLIMB FLEXOR MUSCLE IN THE CAT. H.P. Clamann and A.C. Ngai*. Dept. of Physiol., Med. Coll. of Virginia, Richmond, Va. 23298

Several recent reports have suggested that cutaneous afferent inputs may be selectively directed to subsets of the motoneurons of a pool, rather than to the entire pool as predicted by the Size Principle. The organization of three distinct reflex responses of cat tibialis anterior (TA) was studied in cats anesthetized with α-chloralose and paralyzed with Flaxedil (R). TA muscle nerve was cut from the muscle, freed from surrounding tissue, and placed on bipolar electrodes for stimulation and/or recording. Stimulating electrodes were placed either on the proximal stumps of the cut L6 and L7 dorsal roots, or, when these were left intact, on the cutaneous branch of the superficial peroneal nerve, or on the sural nerve. A single stimulus applied to a dorsal root evoked a short (~3.4 msec) latency first response, presumably monosynaptic, a short latency (~4.4 msec) second response mediated by at least one interneuron, and a longer latency (~18.5 msec) third response which could be abolished by spinalization, dorsal root section, or decerebration. All three responses were evoked by homonymous or synergistic flexor muscle nerve stimulation; only the second and third responses were evoked by stimulation of a cutaneous nerve. The activity of single, antidromically identified TA motoneurons was recorded by intra-axonal recording in L7 ventral root. In all three reflex responses recruitment order was generally in order of increasing conduction velocity of axons tested. When the recruitment order of individual motoneurons was tested in the second and third responses simultaneously, a few changes in rank-order of individual cells occurred. These changes appeared to be unrelated to motoneuron size and did not significantly alter the general pattern of recruitment in order of cell size. These results confirm previous reports from this laboratory that changes in spinal afferent input can alter recruitment order of individual motor units in a manner unrelated to their sizes. However, these changes in the recruitment order of individual motoneurons did not alter the over-all positive correlation between critical firing level and conduction velocity. Thus, in response to proprioceptive and cutaneous afferent stimulation, motoneurons of TA appear to be recruited generally in order of their sizes in both spinal and supraspinal reflexes.

Supported by Grant # NS 11677 from NINCDS.

920 A COMPARISON OF VOLITIONALLY CONTROLLED MOTOR UNITS IN THE GASTROCNEMIUS AND SOLEUS MUSCLES OF HUMAN SUBJECTS. M.A. Clendenin and Susan P. Clarke.* Dept. of Anatomical Sciences, Eastern Virginia Medical School, Norfolk, Virginia 23501

In this investigation electromyography was used to record single motor unit activity during different types of volitionally activated movements. Single motor units were sampled from the medial and lateral gastrocnemius and the palpable portion of the underlying soleus muscle. With such extensive sampling of motor units it was anticipated that the distribution of tonic and phasic motor units would reflect the distribution of motor unit types as demonstrated by histochemical studies.

Single motor unit activity was recorded using a sterile coaxial needle electrode in fifty volunteer subjects ranging in age from twenty-one to thirty-seven years. After an initial training period using audio and visual feedback, subjects who were able to isolate and volitionally activate single motor units upon verbal command were asked to alter the firing frequencies of the unit as well as maintain motor unit activation during remote muscle activation.

The results obtained from thirty-five subjects represent motor unit activity recorded from all areas of the lateral gastrocnemius, medial gastrocnemius, and soleus muscles accessible by palpation. Twelve recordings were obtained from the medial gastrocnemius, eleven from the lateral gastrocnemius, and twelve from the soleus muscle.

The data suggests that the motor units sampled be classified as tonic units. Moreover, there appeared to be no significant difference in the properties of the motor units of the medical gastrocnemius, lateral gastrocnemius and soleus muscles. All of the motor units sampled demonstrated a low firing frequency (mean rate of 5.0 to 10.0 impulses/sec), a long interspike inverval (> 100 msec) and a similar response to remote muscle activation. When subjects performed the remote muscle activity of raising the head and shoulders, an inhibition in the firing pattern was observed in 72% of the subjects. There was no significant differences in the characteristics of the inhibition when comparing motor units from these different muscles.

These results indicate that during volitionally activated movements lower threshold tonic motor units are primarily activated. Recordings do not reflect the mix of motor unit types as reflected by histochemical studies, but rather provide an effective means of studying low threshold units.

921 ROLE OF LIMB MECHANICAL PROPERTIES IN SIMPLE HUMAN ARM MOVEMENTS. J. D. Cooke. Dept. of Physiology, Univ. of Western Ontario, London, Ontario.

Studies have been made of phase-plane trajectories (velocity vs position) of simple step-tracking movements made by normal humans. Subjects, seated comfortably with their forearm supported, grasped a manipulandum handle and performed alternate flexion/extension movements about the elbow. Target and handle positions were displayed to the subject on an oscilloscope. Subjects were left free to choose their own strategy of movement; no restrictions were placed on movement times, velocities, etc. The movements were invariably made with velocities well below the maximum possible for each subject. Phase planes of these movements were qualitatively similar to those derived from an analog model in which the arm was simulated as a simple damped spring with mass. In the model, movements were assumed to be made by a step change in the spring constant.

In random trials with the human subjects brief force pulses opposing movement were applied. Both the magnitude of the force and the point in the limb trajectory at which they were applied were varied. Over a range of force inputs the limb trajectory following the force closely resembled the trajectory of unperturbed movements. With greater forces, the trajectory following the perturbation significantly differed from control trajectories, limb velocities being consistently lower than in the control movements. Following forces which actually displaced the limb beyond its initial position, velocities remained higher than control levels over most of the rest of the movement. Application of forces in the model yielded phase planes which were again qualitatively similar to those seen in the humans. Differences between the model and the human could be accounted for by addition, in the model, of a force which mimiced a stretch reflex in response to the perturbing force. The results are interpreted as indicating that a major part of the limb trajectory during performance of some simple movements may be determined by the limb's mechanical properties.

(Supported by PG-1 from the Medical Research Council of Canada)

922 THE VALUE OF UNITARY RECRUITMENT IN REFLEX REGULATION OF MECHANICAL PROPERTIES OF LENGTHENING MUSCLE. P.J. Cordo* and W.Z. Rymer, (SPON: M.M. Mozell). S.U.N.Y. Upstate Medical Center, Syracuse, New York 13210.

When the activated, areflexive m. gastrocnemius (m.g.) and soleus muscles of the cat are stretched at physiologically appropriate velocities, their force responses are grossly non-linear. This non-linearity, termed muscle 'yield' (Nichols and Houk,1976, J. Neurophysiol. 39, 119-142) or 'give'(Flintney and Hirst,1978, J. Physiol. 276. 449-465), consists largely of an abrupt decline in muscle stiffness that intervenes after the muscle has been stretched more than a fraction of a millimeter. When these muscles are subjected to similar stretches in decerebrate cats with intact stretch reflexes, the yield is no longer evident. The neural mechanisms by which the CNS is able to achieve this 'linearization' of muscle force trajectory were investigated in this study.

Examination of the motor output in the stretch reflex of the m.g. revealed several possible mechanisms for the regulation of dynamic mechanical properties. Recruitment of fresh motor units occurred throughout the observed force range (0-2000 grams) although the number of new units declined rapidly at high forces; the distribution of motor unit force thresholds was approximated by a decaying exponential curve. Initial firing rates of motor units recruited during stretch were found to be significantly greater than the rates recorded during isometric activation (using the crossed-extensor reflex). Newly recruited motor units were then observed to increase their firing rates at an average of 2.0 imp/sec/100 grams muscle force increase. Finally, recruitment during stretch was often accompanied by an uncharacteristically short inter-spike interval (< 20 msec) or 'doublet'(Burke, Rudomin and Zajac, 1976, Brain Res., 109, 515-529.

The effects of these patterns of motor unit activation on force production during stretch were then examined using groups of motor units in soleus and single, type-identified motor units in the m.g.. Preliminary observations suggest that motor units recruited during muscle stretch appear to yield only slightly or not at all. The yield is replaced by a more gradual decline in slope of the force trajectory after several millimeters of stretch. When several groups of soleus motor units are sequentially activated during muscle stretch, the trajectory of the force response is strikingly similar to that of the stretch reflex in the decerebrate preparation. The force trajectory resulting from activation of groups of motor units during stretch is noticeably steeper when a doublet is introduced at the beginning of the spike train. The mechanisms of increased initial firing rate and rate modulation appear to have much less influence than unitary recruitment on the dynamic force trajectory of lengthening muscle.

923 CLOSED LOOP CONTROL OF ELECTRICALLY STIMULATED MUSCLE FOR ORTHOTIC PURPOSES. Patrick E. Crago, Applied Neural Control Laboratory, Case Western Reserve University, Cleveland, Ohio 44106

Intramuscular electrical stimulation of paralyzed finger flexors and extensors is successfully used to provide powered grasp in the hands of C-5 quadriplegic patients*. The patient's control would be improved if the non-linear and time-varying properties of the response to electrical excitation could be regulated. This report presents open loop force modulation techniques and closed loop force feedback techniques that have been found to compensate for both non-linearities and time-dependence under isometric conditions. The experiments were carried out in soleus muscles of cats.

A single command signal controls the stimulus parameters. (In an orthosis, this signal would be derived from a non-paralyzed portion of the patient's body.) The signal must control both recruitment (at low forces) and temporal summation (at high forces). Recruitment is varied by stimulus pulse width (PW) modulation at a fixed amplitude and at the maximal interpulse interval (IPI) that allows an adequately fused contraction. However, recruitment (force versus PW) is non-linear, ie. regions of high slope are separated by regions of low slope. This is a significant problem since approximately 1/2 to 2/3 of the total force range is controlled by recruitment. For larger forces the degree of temporal summation is increased by decreasing the IPI. The command signal linearly modulates IPI instead of frequency, since force is a much more linear function of IPI. Complete linearity of force modulation has not been achieved by open loop techniques.

Closed loop force feedback systems have been studied with modulation of recruitment (PW) or temporal summation (IPI), and with modulation that switches from recruitment to temporal summation at an intermediate point. Simple proportional controllers with loop gains of about 10 cause noisy, unstable operation when modulating recruitment; this appears to be due to the discontinuous nature of recruitment. Controllers with low proportional loop gain (about 1), but with the addition of an integrator, have perfect steady state linearity, good compensation for time dependencies, and do not show the noise seen with high loop gain proportional controllers. Ratios of integral to proportional gain of about 10 yield the fastest response time with no compromise to stability. This type of controller also works well with modulation of temporal summation and at the transition between modulation of recruitment and temporal summation.

(Funded by NIH,NINCDS Contract Number NOS-NS-2-2314)

*Peckham, P.H. and Mortimer, J.T. in Functional Electrical Stimulation: Applications in Neural Prostheses, ed. F.T. Hambrecht and J.B. Reswick, Marcel Dekker, New York, 1977.

924 REFLEX MODULATION DURING ONGOING MOTOR TASKS. J. R. Dufresne* and J. F. Soechting. Laboratory of Neurophysiology, University of Minnesota, Minneapolis, Minnesota 55455.

Control systems with proprioceptive feedback are apparently utilized during a variety of motor tasks, but the manner of their engagement and regulation by central mechanisms is unresolved. This question was addressed by experiments on human subjects in which brief torque-pulse disturbances were introduced at several phases of ongoing, sinusoidal forearm tracking tasks. "Pulse responses" of biceps/triceps EMG activity were obtained by subtraction of perturbed and unperturbed task records. Integration of these responses over an interval of 50-100 msec following the pulse gave a quantitative measure of reflex EMG activity, and these data were fit by a least-squares procedure to a sinusoid at the tracking frequency.

The results of this study indicate that reflex activity leads the ongoing EMG activity of the same muscle by approximately 100° during both position- and isometric force-tracking tasks. Moreover, large phase shifts of the ongoing EMG with respect to forearm tracking velocity, obtained by using large elastic loads at different tracking frequencies (0.7 to 1.8 Hz), failed to significantly alter this phase lead of reflex activity. The results are in agreement with tendon-tap and H-reflex studies demonstrating a large increase in reflex activity prior to the initiation of movements. They suggest: (1) that changes in dynamic behavior of the spindle which depend on absolute length and velocity are not the main reason for the observed behavior, and (2) that activities resulting from the intentionally generated motion are not involved in the observed behavior. Although the mechanism responsible for the constant phase relation between changes in reflex amplitude and EMG activity are still unexplained, a tentative interpretation of its functional significance can be offered. This is the following: that the reflex gain is increased prior to the intentional activation of the muscle in order to provide for load compensation and that the constant phase lead described above will compensate for the delay introduced by the low-pass filter characteristics of the muscle.

(Supported by NIH grant #02567 and a grant from the American Parkinson Disease Association.)

925 ANALYSIS OF THE MOTOR UNIT POPULATION IN CAT FLEXOR DIGITORUM LONGUS (FDL) MUSCLE. R. P. Dum*, R. E. Burke and J. A. Hodgson*. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20014.

As part of a larger study, we have examined the motor unit population in the FDL muscle of normal cats. The number and location of FDL motoneurons was studied using retrograde transport of horseradish peroxidase. Labeled cells were found in a narrow column about 10 mm in length along the dorsolateral margin of the ventral horn in the caudal half of L6 and rostral third of L7. A total of about 165 cells were labeled, of which 38% fell into the gamma size range (see Burke et al., J. Neurophysiol. 40:667,1977). We used intracellular recording and stimulation methods in pentobarbital anesthetized cats to study motoneuron and muscle unit properties (initial tension for mechanical recording - 20 gm) and to record peak amplitudes of FDL and FHL group Ia EPSPs (Burke et al. J. Physiol. 234:723,1973; J. Neurophysiol. 39:447,1976). Four normal FDL muscles were studied histochemically. Motor unit types and muscle fiber types were identified according to criteria in these references. The data are summarized in the table below.

UNIT TYPES	FF	FR	S
Number and percent	25 (28.7%)	49 (56.3%)	10 (11.5%)
Axon Cond. Vel. (m/s)	99.8±7.1 (25)	98.3±6.9 (49)	84.1±8.7 (10)
FDL Ia EPSP (mV)	3.1±1.1 (14)	3.7±1.0 (26)	3.6±1.4 (7)
FHL Ia EPSP (mV)	0.8±0.3 (14)	2.5±1.0 (26)	2.2±0.8 (7)
Tw. Time to Peak (ms)	33.8±5.9 (25)	35.1±4.9 (49)	56.2±13.8(10)
Tetanus Tension (gm)	31.7±14.8(25)	5.3±3.3 (49)	1.1±1.0 (10)
(data given as means ± SD; number of observ. in brackets)			
Histochemical fiber proportions in FDL	59.1%	30.1%	10.8%

The organization of the FDL motor unit pool is fundamentally the same as found for the cat medial gastrocnemius (MG) but there are some interesting differences. The correlation between the strength of Ia excitatory input and motor unit type is much less strong than in MG. The twitch contraction times of FDL type S units are somewhat shorter than in MG. Most striking, however, is the large proportion of type FR units in the FDL pool (56%; as compared to 23% in MG), while only 30% of FDL muscle fibers have the histochemical profile characteristic of FR units. Considering the mean tetanic tensions of FDL type FF and FR units in relation to the relative frequencies of these motor unit and muscle fiber types in the FDL nucleus and muscle, respectively, we can conclude that an average FDL type FF unit must contain about 3 times as many muscle fibers as an average type FR unit.

926 DOPAMINERGIC MODULATION OF A BURSTING PACEMAKER OSCILLATION: POTENTIATION BY THEOPHYLLINE
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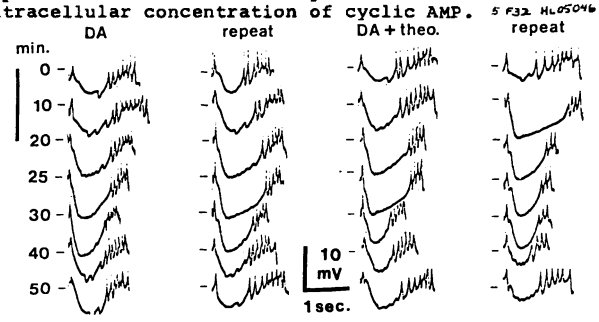
Dopamine (DA) is localized in the stomatogastric nervous system of the spiny lobster along the input nerves to the stomatogastric ganglion (SGG) from the commissural ganglia (Kushner & Maynard, Brain Res. 129 13,'77; Barker, Kushner, & Hooper, Brain Res.in press). Electrical stimulation of this pathway increases the frequency of the pyloric rhythm generated in the SGG. Bath-applied DA mimics this effect with a slower time course (Anderson & Barker, Ns.Ab.III:522) by increasing the amplitude (A) and frequency (F) of the endogenous membrane potential oscillations of the pacemaker cells (PD & AB). Columns 1 & 2 below are individual oscillations from a PD cell at the indicated intervals during and following two consecutive 20 min exposures to 40 μM DA. Note that increases in A are due to increases in the post-burst hyperpolarization (pbh). Increases in F are due to increases in the rate of depolarization since the duration of the pbh is relatively constant. Simultaneous exposure to inhibitors of cyclic nucleotide phosphodiesterase would be expected to enhance those effects of DA which are mediated by a DA-activated adenyl cyclase. Exposure to 40 μM DA plus 1 mM theophylline (cols. 3 & 4) enhances the magnitude of the F response and decreases the time to maximal A response. Potentiation of the F response is due to an increased rate of depolarization (compare cols. 1 & 2 with cols. 3 & 4 at 30 min). It is thus proposed that the DA-induced increase in the rate of depolarization is mediated by an increase in the intracellular concentration of cyclic AMP.



927 CENTRAL PROGRAMS CONTROLLING RAPID LIMB MOVEMENT IN THE CAT.
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In a previous study it was shown that under isometric conditions, rapid force adjustments could be described by a pulse-step model. The initial peak force was determined by the amplitude of a pulsatile command to agonist muscles of approximately constant duration and corresponding to the rising phase of dF/dt. The terminal force was controlled by a later steady output. The present study was undertaken to determine if position adjustments could also be considered to reflect a similar pulse-step control policy.

Cats were trained to position a lever with their forearm and to match a target level which was stepped at random times. The difference between the target level and the angular position of the lever determined the horizontal position of a compensatory display. Because of inertia and friction in the device, step perturbations took 200 msec to fully shift the display, but the derivatives of its motion were proportional to the size of the step. The cats responded to the perturbation by rapidly adjusting the position of the lever. The direction and extent of the initial lever displacement correlated with those of randomly varied perturbations. The peak velocity achieved was characteristically a linear function of the displacement over a large range of perturbation sizes and under different loading conditions. Increasing velocities were achieved by corresponding increases in the peak acceleration. While the duration of movement often increased as its amplitude became larger, the times from onset to peak velocity and acceleration did not.

An unexpected increase in spring load opposing movement resulted in undershoot of the first position change even when the required change in terminal force was as small as 20 gm. An increase in friction resulted in slowing of the response but a correct final position. After a few trials, the displacement, velocity and acceleration returned to control values in both conditions. These compensatory adjustments were accomplished by increases in the force output by the animal. In the case of the spring load both initial and terminal forces were increased; with friction, only the initial forces were increased.

These observations show that, in a tracking situation, position adjustments are determined by central programs which specify an intended force output in relation to derivatives of target motion. This intended output is dependent on the magnitude and configuration of anticipated loads. The different adjustments in motor output in the presence of spring and frictional loads indicates that phasic and tonic (pulse and step) components of the motor program can be controlled separately.

Supported by Grant NS 12730.

928 WHEEL RUNNING ACTIVITY OF RATS AFTER "FAR-LATERAL" HYPOTHALAMIC LESIONS.
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In addition to producing a temporary aphagia and adipsia, destruction of the tuberal region of the hypothalamus lateral and ventral to the fornix ("mid-lateral hypothalamus") produces a permanent decrease in the wheel running activity of rats to 10% of preoperative levels. Destruction of a region lying more laterally that includes the extreme lateral portion of the hypothalamus and the medial portion of the subthalamus ("far-lateral hypothalamus") also produces aphagia and adipsia which may be more permanent than that produced by "mid-lateral" hypothalamic damage and may be caused by the interruption of fiber tracts in this region. Experiments were performed to determine whether "far-lateral" hypothalamic lesions also produce a permanent decrease in wheel running activity.

Adult male and female Sprague-Dawley rats were housed individually in activity cages consisting of a small living compartment, in which food and water were kept, and an activity wheel with a Veeder counter. These cages were kept in a room in which the temperature was maintained at 21° ± 1°C and the room lights were on a 12 hour on-off cycle. The female rats were given food ad libitum, but the male rats were kept on a restricted feeding regimen to maintain body weight constant at 280 ± 5 gms. All rats were given water ad libitum. After a control period of 8 weeks during which time wheel running activity was measured, symmetrical electrolytic lesions were placed either in the "far-lateral hypothalamus" or in adjacent regions of the brainstem of 20 rats under hexobarbital anesthesia (20 mg/100 gm). The rats were returned to their activity cages immediately after recovery from anesthesia and were force-fed until they began to eat and drink spontaneously. Their wheel running activity was measured for at least 8 weeks after placement of the lesions. At the conclusion of the experiment, the location of each lesion was verified histologically.

None of the "far-lateral" hypothalamic or subthalamic lesions produced a permanent decrease in wheel running activity comparable to that observed after "mid-lateral" hypothalamic lesions, even though each of these lesions damaged one or more of the structures and fiber tracts within the subthalamus. These data are consistent with the hypothesis that the change in wheel running activity observed after the placement of "mid-lateral" hypothalamic lesions is due to the destruction of neurons located in this area rather than to the destruction of nerve fibers coursing through this region.

929 MYOTATIC RESPONSES IN HUMAN ANKLE FLEXORS AND EXTENSORS EVOKED BY FORCED ANKLE FLEXION.
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Torques were applied to dorsiflex or plantarflex the ankles of seated human subjects who were performing various motor tasks. The resulting ankle rotation evoked responses which were visible in the EMGs of the triceps surae and anterior tibial muscles.

Stretching the ankle extensors evokes brief and highly synchronized myotatic reflex in the triceps surae at a latency of about 45 msec. The magnitude of this response is highly and linearly correlated with the rate of stretch under all conditions studied. The slope of the linear regression curve characterizes part of the reflex loop "gain". We find the tonic voluntary isometric contraction of the extensors increases the extensor loop gain while contraction of the flexors decreases the extensor loop gain. Both effects are proportional to the degree of contraction.

Slow voluntary rotations of the ankle act in an analogous manner but fast plantar rotations involving strong phasic extensor contraction is associated with an often profound reduction in reflex gain.

These relationships are also characteristic of flexor responses to stretch with one important difference. The latency of the myotatic reflex in the relaxed tibial muscle is about 85 msec and less synchronized than in the extensors. Tonic voluntary isometric contraction not only increases the reflex gain but also shortens the reflex latency. A moderate contraction of 0.5 KgM. can reduce the latency to that in the extensors.

We conclude that segmental reflexes subserve a short latency, low threshold mechanism during posture and slow movement. This may provide some servolike properties to the joint. The brevity of the myotatic response and the fact that supraspinal response of only slightly longer latency will supervene in a normal, conscious subject makes the usefulness of a servo characterization for segmental load compensation very uncertain.

(Supported by NIH grants NS-00196, NS-12877 and NSF grant ENG-7608754)

930 CINEMATOGRAPHIC AND ELECTROMYOGRAPHIC ANALYSIS OF STEPPING AND SWIMMING IN RATS. J.A. Gruner* and J.A. Altman, (SPON: L.F. Jaffe). Dept. Biol., Purdue Univ., W. Lafayette, IN 47907.

Although locomotion has been extensively studied in cats and dogs, and to some degree in rats, the emphasis has been primarily on stepping. Only briefly has swimming in mammalian quadrupeds has been mentioned in the literature, and no joint angle or electromyographic (EMG) data have, to the authors' knowledge, been presented. Yet a detailed study of swimming is important because many mammals do swim of their own accord, and a comparison of swimming and stepping might provide information as to the neural organization of locomotor movements. A synchronized cinematographic and EMG analysis of stepping and swimming in normal rats was therefore performed.

Limb movements and muscle activities for stepping were much the same as previously reported for cats (Engberg and Lundberg, Acta physiol. scand., 86: 92, 1969) and dogs (Tokuriki, Jap. J. Vet. Sci., 35: 433, 1973) except that (1) in cats both the knee and ankle maxima occurred at foot liftoff, whereas in rats the knee maximum occurred at foot contact; and (2) there was no evidence of a double bursting pattern in rat flexormuscles.

When swimming and stepping were compared in rats, the yield phase of stepping (flexion in knee and ankle joints following foot contact) disappeared and there were differences in the pattern of limb movement during the flexion or returnstroke phase of swimming. The altered returnstroke movements were associated with simultaneous bursts in the flexors at the end of the extension or powerstroke phase which were unique to swimming. The limits of flexion and extension of the femur were nearly identical in both cases, as were the relative onsets of extensors and flexors except for the added flexor bursts at the end of powerstroke. (Supported by USPHS Grant NS-10267 and ERDA #E (11-1)-2000).

931 THE SYNAPTIC ORGANIZATION OF THE TRIGEMINAL MOTOR NUCLEUS IN THE OPOSSUM. James E. Hamos* and James S. King, Department of Anatomy, The Ohio State University, Columbus, Ohio, 43210.

The synaptic organization of the opossum trigeminal motor nucleus was examined by utilizing Nissl preparations, Golgi impregnations, 1μm thick sections, and ultrathin sections. The cell bodies of trigeminal neurons have areas that measure from 150-2700μm^2 with a peak range from 700-1750μm^2. Sessile spines are frequently found on neuronal somata. Each neuron has 2-6 primary dendrites with a diameter of 6-10μm that extend in all planes from the cell body. Within 300μm from the soma, the primary dendrites divide into secondary dendrites and these, in turn, may bifurcate into thinner tertiary branches. Dendritic outgrowths are often present and may be sessile spines, thin-necked thorns or branched appendages. The overall diameter of the dendritic tree often extends as much as 1mm with a rare branch leaving the confines of the nucleus to enter the neighboring reticular formation.

The perikarya and initial dendritic trunks of trigeminal neurons are characterized by many synaptic contacts which cover as much as two-thirds of the cell membrane. Synaptic endings may be classified into three general categories. Small terminals, 1-3μm in diameter, which contain round synaptic vesicles account for the majority of somatic endings. Somatic spines are typically post-synaptic to this variety of bouton. Other small terminals, 1-3μm in diameter, contain pleomorphic synaptic vesicles and comprise the second most common category. Finally, there are large boutons, with round vesicles, that are 2-6μm in diameter and are always related to a subsynaptic cistern. These terminals are the least frequently encountered on the soma and proximal dendrites and very often interdigitate with adjacent synaptic endings.

Boutons en passant are typical features of the neuropil surrounding trigeminal neurons and are often seen to arise from myelinated axons. Boutons similar to the two smaller endings found on the cell bodies also typify those seen on small diameter dendrites. These data provide the basis for experimental studies designed to identify the origins of the pre-synaptic profiles in the trigeminal motor nucleus.
(Supported by N.I.H. Grant No. NS-08798.)

932 IMPROVED HEAD CONTROL IN CEREBRAL PALSY PATIENTS FOLLOWING TORQUE STEP PERTURBATIONS OF HEAD POSITION. F.A. Harris, Univ. of Wash. Sch. of Med., Seattle, WA 98195.

Torque steps were applied about the axes for head tilt in pitch and roll, using a pneumatically powered apparatus capable of influencing position of the head relative to the shoulders, in cerebral palsy patients. The patients' own head movements triggered application of torque steps opposing such movement. Application of a torque step was initiated when the head moved outside of an adjustable-width deadzone centered on the setpoint selected by the system operator (usually the gravitationally neutral head position) and terminated when the head returned to the setpoint. The apparatus delivered a maximum of 70 in-lb torque about either axis. The subjects were instructed either to yield to or resist forces thereby applied to the head, according to which of two circumstances prevailed.

Passive stabilization of the head, which facilitated self-feeding and diagnostic, hygienic and restorative dental care, was accomplished by instructing the subjects to yield to applied forces. In an attempt to strengthen the neck muscles through resistive exercise (i.e., contraction against constant or increasing load), with the goal of thereby improving voluntary head control, subjects were instructed to tilt the head in a particular direction until the deadzone limit was exceeded and then endeavor to maintain that deviated position despite the continuing application of force tending to return the head to the setpoint. This procedure was carried out for all four directions of tilt, with the number of repetitions dictated by the subject's endurance.

Stripchart and FM tape recordings of pitch and roll signals from head tilt transducers mounted on a separate headband worn during pre- and postexercise periods, and simultaneous cinematography, were carried out for comparison of voluntary head control before and after the period of "automated exercise." Those signals recorded on FM tape were digitalized; computer-plotted "scattergrams" thereby derived, representing the domain within which the head was held and the amount of time it was held at each point within that domain, were used to study alterations in head control resulting from the exercise procedure. It was determined that the range of "involuntary" head motion was narrowed, and subjects were able to hold the head at or near the setpoint for increasingly longer periods of time, following individual exercise periods as brief as ten minutes' duration. Cumulative improvement and functional carry-over were obtained with repetition at weekly intervals. Therefore, procedures used in investigation of neural mechanisms involved in postural stabilization and control of voluntary movement are applicable as well to rehabilitation of individuals with neurological disorders such as (but not limited to) cerebral palsy. (Supported by DE01479 from Nat. Inst. Dental Res.)

933 THEORETICAL FORMULATION RELATING INTRAFUSAL MECHANICS TO THE NONLINEAR DYNAMIC RESPONSIVENESS OF MUSCLE SPINDLE PRIMARY ENDINGS. Z. Hasan*. (SPON: R.G. Durkovic). Neurosurgery Lab., Upstate Medical Center, Syracuse, New York 13210.

For large ramp stretches, the dynamic responsiveness of primary endings varies substantially depending upon the absence or presence of γ_D or γ_S activity. However, for small sinusoidal stretches the differences in dynamic responsiveness are marginal and of unexpected direction (ref. 1,2). In order to resolve the apparent inconsistency a formulation is developed based on the following assumptions: 1.The extra tension related to movement in the polar zones of intrafusal fibers depends upon a fractional power (1/3) of the speed of movement of the polar zones. This assumption is a generalization of a property reported for extrafusal muscle (ref. 3). 2.The equatorial zone behaves as an elastic element. 3.The applied stretch is distributed between the polar and equatorial zones, and the nerve ending responds to the length of the equatorial zone and to its time-derivative.

From this formulation it is seen that large dynamic responsiveness for large stretches goes hand in hand with small dynamic responsiveness for small stretches. The result is contrary to expectations based on linear systems analysis but in the context of the nonlinear formulation there is no contradiction. Using suitably chosen sets of parameters corresponding to the primary ending when deefferented and when under the influence of γ_D or γ_S activation, the differences in dynamic properties for large stretches are explained. In particular, the slower adaptation seen in the presence of γ_D activity (ref. 4) is predicted. The 'dynamic index' varies as approximately the square root of velocity and depends upon fusimotor state. Response to a continuing stretch at constant velocity is predicted, in the steady state, to be a linear function of instantaneous length and a nonlinear function of velocity. Using the same sets of parameters the responses to small sinusoidal stretches show large phase leads at low frequencies (ref. 5), and the manner in which the predicted gain at a fixed frequency depends upon stretch amplitude in different fusimotor states is in accord with observations (ref. 2).

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2.Hulliger, Matthews & Noth.J.Physiol.267:811(1977).
3.Hill.J.Physiol.199:637(1968).
4.Emonet-Dénand, Laporte, Matthews & Petit.J.Physiol.268:827(1977)
5.Hasan & Houk.J.Neurophysiol.38:663(1975).

934 PHENYTOIN REDUCTION OF EXTENSOR TONE AND GAMMA MOTONEURON ACTIVITY IN THE DECEREBRATE CAT. N. Hershkowitz*, T. M. Mahany*, L. Baizer *, and A. Raines. Dept. Pharmacol., Sch. Med. & Dent., Georgetown University, Washington, DC 20007.

Previous work has demonstrated that phenytoin (DPH) directly suppresses deefferented muscle spindle activity (Anderson and Raines, JPET 191: 290, 1974). Because increased muscle tone in the decerebrate animal results from enhanced gamma motoneuron activity (Eldred et al., J. Physiol. 122: 498, 1953) and thus depends upon the integrity of segmental afferent input to the spinal cord (Sherrington, J. Physiol. 22: 319, 1898), we were interested in determining whether DPH would diminish decerebrate extensor tone. A midcollicular transection was performed on cats under methoxyflurane anesthesia. Anesthesia was discontinued and the extensor tone quantified by determining the force necessary to produce total flexion around ankle and knee joints when each limb was pushed against a platform affixed to an isometric force transducer. A cumulative dose-response relationship was estimated by measuring the force every ten min during an intravenous infusion of 1 mg/kg/min of DPH. DPH produced a dose dependent reduction in the extensor tone with 25% reduction occurring at 10 mg/kg and 50% reduction at about 40 mg/kg.

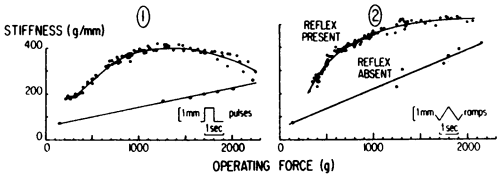
Because the suppression of gamma motoneuron activity may also bring about a reduction in tone, the influence of DPH on this activity was determined in decerebrate cats. A laminectomy was performed on 11 cats and the dorsal roots S_3-L_3 were bilaterally divided. Spontaneous activity of single gamma motoneurons isolated from ventral roots L_6 or L_7 was monitored. Cumulative dose-response relationships were estimated as above. DPH produced a dose dependent depression of the gamma motoneuron activity with 35% depression occurring at 10 mg/kg and 50% depression at about 20 mg/kg. To confirm the gamma nature of the muscle rigidity in our preparations a unilateral dorsal ganglionectomy (S_1-L_5) was performed on 4 untreated decerebrate cats. As expected, muscle tone was eliminated in the hindlimb of the operated side.

Thus, the capacity of DPH to suppress muscle tone in the decerebrate cat appears to be the result of the depression of muscle spindle function. This depression is produced by both a direct action on the spindle as well as a reduction of gamma motoneuron activity. It is felt that the mode of drug administration, which fails to allow time for drug effect to fully develop, underestimated DPH's potency. The observations reported here may provide a rational basis for the use of DPH in the treatment of muscle hypertonicity characterized by high gamma motoneuron tone.

Supported by NINCDS grants 10667 and 12566.

935 FACTORS AFFECTING THE GAIN OF THE STRETCH REFLEX AND SOLEUS MUSCLE STIFFNESS IN PREMAMMILLARY CATS. J.A. Hoffer and S. Andreassen*. Physiol. Dept., Univ. of Alberta, Edmonton T6G 2H7, Canada.

The role of the stretch reflex during movement has been the subject of several interpretations. Recently Nichols & Houk (J.Neurophysiol.39:119,1976) presented evidence to suggest that the stretch reflex regulates muscle stiffness rather than length. We applied incremental length changes to soleus, recorded the resulting changes in force, and used $\Delta F/\Delta \ell$= stiffness as index of gain, to investigate 1) the variability in the gain of the stretch reflex in the high decerebrate, which shows a wider behavioral répertoire than the intercollicular preparation; 2) changes in gain caused by stimulation of several peripheral and brainstem structures, and 3) the role of tendon organ pathways in the regulation of stiffness.



The reflex-mediated response to 1mm, 500ms rectangular pulse stretches was moderately dependent on the operating force (Fig.1), while the muscle response alone was markedly dependent (cut soleus nerve was stimulated at 10-50Hz to generate operating forces). Reflex responses were similar in the range ℓ_{max}-16mm to ℓ_{max}-1mm. In response to 1mm, 500ms ramps, muscle stiffness doubled, while the net reflex contribution was unchanged (Fig. 2) .

The gain of the stretch reflex was remarkably constant for each operating point (note absolute deviation of data points). The gain along spinal pathways could not be modified notably by stimulating selected peripheral and brainstem structures, although transient decreases were sometimes observed.

When the muscle was active near peak levels of contraction for $\geq$ 5 sec, stiffness values began to rise, both with reflex absent and present. Since little or no reflex-mediated compensation for muscle stiffness potentiation was observed, we concluded that the tendon organ feedback gain was low in this preparation.

Our results indicate that under these conditions the stretch reflex contribution to total stiffness is constant for each operating point, independently of type of perturbation or of changes in muscle stiffness. However, it is likely that in intact systems the stretch reflex can compensate more adequately for increased muscle stiffness during sustained high levels of contraction.
(Supported by MDA of Canada and Tech. Research Council of Denmark)

936 RESPONSES OF MONKEY PRECENTRAL CORTICAL CELLS DURING A CONTROLLED JAW BITE TASK. Donna S. Hoffman* and Erich S. Luschei* (SPON: M.A. Ruda). Dept. Physiology and Biophysics and Regional Primate Ctr., Univ. of Washington, Seattle, Washington 98195.

Single unit discharges were recorded in the precentral face area of monkeys while they exerted graded isometric biting forces across a pair of metal plates. One hundred out of 204 recorded units showed increases in discharge rate that were temporally related to, and often occurred prior to, the onset of isometric jaw force. About 25% of these task-related neurons showed a consistent relationship between their rate of maintained firing and static biting force, as force was varied between 0.1 and 7.4 kg. Such a result has been previously reported for cells recorded in the precentral arm and hand areas and is consistent with the idea that precentral cortex participates in controlling muscle tension.

The sensitivity of precentral cells to changes in jaw position and to changes in the force applied to the incisors was tested by sinusoidally moving the lower plate of the bite bar system while monkeys were applying force across the two plates. Most task-related cells examined during this procedure exhibited a sensitivity to the derivatives of positional and force stimuli applied to the jaw.

Single pulse, low intensity (<100μA) electrical stimuli applied at the end of most microelectrode penetrations produced widespread bilateral inhibition of jaw closing muscle activity at short latencies (about 7 ms). Stimuli applied in a few penetrations adjacent to the central sulcus produced short latency excitation of contralateral masseter muscle activity in addition to inhibition of activity in other jaw closing muscles bilaterally. Task-related cells were recorded only within the cortical area which could be stimulated with low currents to produce effects on jaw muscle activity.

The results of this study, together with prior studies, provide compelling evidence for a precentral role in control of jaw muscle activity. In addition, the similarity of unit firing patterns recorded in both precentral jaw and forearm areas suggests that the role of the precentral cortex with respect to movement may be common to different muscular systems.
(Supported by grants RR-00166 and NS-08596 from NIH and GM-00666 from USPHS).

937 ORIGINS OF CORTICAL AND CEREBELLAR PROJECTIONS TO THE RED NUCLEUS IN THE MONKEY. D.R. Humphrey, R. Gold* and D.J. Reed*. Lab. of Neurophysiology, Emory Univ. Sch. Med., Atlanta, GA 30322.

As a prelude to a study of their movement-related behavior in the conscious animal, retrograde cell labeling techniques were used to define the distribution of cortico- and cerebellorubral cells in rhesus and cynomolgous monkeys. Horseradish peroxidase (Sigma VI, 30-50 % solution) was injected (0.05-0.2 μL) into the red nucleus (RN) bilaterally in 10 animals. Survival times ranged from 48-72 hr, and after removal the brain stem, cerebellum and cerebral cortices were sectioned at 50 μ and reacted with diaminobenzidine. A cobalt chloride modification was also used to enhance visualization of the reaction product (Adams, J.D., Neurosci., 2: 141, 1977). All sections were examined under both bright and darkfield illumination.

Our major results may be summarized as follows. (1)Labeled corticorubral (CR) cells were found in the supplementary motor area and in areas 8, 6, 4, 5 and 7 on the convexity of the hemisphere. Areas 8 and 6 projected most heavily to the rostral portion of the parvocellular division of the nucleus, and areas 6 and 4 to its mid and caudal portions. Dense labeling was found in area 4 only in the region of the precentral arm area. Labeled cells were less numerous in areas 5 and 7, and very sparse in areas 3, 1 and 2. All labeled cells were small (modal transverse dia. = 13 μ), pyramidally shaped, and situated within layer V just dorsal to the row of large pyramidal cells. Maximum observed packing density was comparable to that of corticospinal tract cells (1 cell/(0.1 mm)^3 average in layer V). (2) Only a few labeled CR cells were found following injections confined to the mid and caudal regions of the magnocellular division of the RN, which is the source of rubrospinal efferents. (3) Cerebellar projections to the parvocellular division of the RN were derived principally from the dentate nuc. (ratio of 2:1 when compared to interpositorubral efferents), whereas those to the magnocellular division came principally from nuc. interpositus (ratio = 2:1).

Our findings with respect to the CR projection thus agree well with the degeneration studies of Kuypers and Lawrence (Brain Res., 4: 151, 1967), with the exception that we find little evidence for a significant direct cortical input to the magnocellular division of the RN. Our results suggest instead that the major cortical projection is to the parvocellular division. Since this division also receives a significant input from the dentate nuc., and projects back toward the cerebellum by way of the olive, it may be involved in both cortico-cerebellar and cerebello-brain stem-cerebellar loops which play some higher order role in the programming of voluntary movement. (Supported by NIH Grant NS 10183).

938 WIDESPREAD REFLEX RESPONSES TO CUTANEOUS STIMULATION IN MAN REVEALED BY EVOKED EMG. R.E. Kearney, C.W.Y. Chan, A.P. Arrott*. Aviation Medical Research Unit, McGill University, Montreal, Canada.

Animal experiments have demonstrated that cutaneous stimuli applied at a single site can elicit complex and widespread reflex responses. We have used an evoked EMG technique to study these responses in normal humans.

Stimuli consisted of electrical pulses applied to the median arch of the subject's foot at a level of about three times sensory threshold. Surface EMG's were measured from a tonically contracting muscle, full wave rectified and then band pass filtered. Stimulus related responses were extracted from noise by ensemble averaging and Wiener filtering. To date, we have succeeded in demonstrating repeatable responses in a variety of limb flexors and extensors as well as in a number of back extensors.

Limb flexor responses consisted predominantly of an early excitation followed by inhibition. Responses in limb extensors were somewhat more complex but were in general reciprocal to the flexor responses. In individual subjects, a characteristic pattern of response has been observed in the back extensors at a variety of spinal levels. An early inhibitory wave could be identified whose latency increased as progressively higher spinal levels were studied. It thus appears to be due to inter-segmental spinal reflexes. In some subjects, a later wave could be identified whose latency was shorter at higher than lower levels. This suggests a contribution from a long loop reflex such as the spino-bulbo-spinal reflex.

We conclude that, in normal man, a single cutaneous stimulus can elicit a complex, widespread reflex response which is organized reciprocally between flexors and extensors. Furthermore, some of the complexities of the response may arise from the interaction of spinal and long loop reflexes.

Supported by a grant from the Canadian Medical Research Council.

939 LOCALIZATION OF BRAIN STEM TARGETS IN THE FASCICULARIS MONKEY BY A HISTOLOGICAL X-RAY MATCHING METHOD. P. R. Kennedy, H.-G. Ross, V. B. Brooks. Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1.

Targets in the monkey's brain stem are difficult to localize mostly because they are not stationary during head movements. The short supply of Rhesus monkeys (Macaca Mulatta) makes the Fascicularis monkey (Macaca Irus) an attractive alternative. A brain atlas is available for this species in the standard prone position (1), and corrections for the sitting position can be approximated from (2). Surprisingly, however, even that cannot provide accurate stereotaxic guidance for deep targets, presumably because of the variable shape of the external auditory meati of the Fascicularis.

We have solved this problem by (a) matching sagittal brain stem sections with an outline of the brain stem visualized by contrast X-rays in 3 monkeys, and (b) locating in experimental animals the desired target in relation to bony landmarks that are also visible on X-rays taken during implantation. This method is independent of conventional stereotaxic coordinates, and is used with the monkey sitting in a modified stereotaxic frame. This is important since it is also the position during experimentation. Skull X-rays can be taken by elevating above the metal frame, holders for ears, eyes, and mouth. Ear bars are made of acrylic.

Outlines of the brain stem in 3 Fascicularis monkeys (2-2.7 kgs) were made with contrast X-ray studies using Conray (Na-Iothalamate, Mallinckrodt Canada, Ltd.) or Metrisimide (Amipaque, Nyegaard, Oslo, Norway) or air. These brain stem outlines were matched with sagittal histological brain stem sections from 1 monkey, using as matching points the interpeduncular fossa, pons, ponto-medullary junction, medulla and fourth ventricle. Then the desired brain stem target was related to bony landmarks in the composite picture. At implantation, a plain lateral skull X-ray was taken to reveal bony landmarks as well as metal markers whose projections intersected at stereotaxic zero. Using these landmarks, the desired target position was transferred to this X-ray from the composite picture to obtain stereotaxic coordinates for immediate implantation.

Accurate implantation has been confirmed with AP and lateral X-rays in one chronically prepared Fascicularis monkey to date.

Refs. (1) Shantha, T. R. et al. Karger, Basel, 1968.
(2) Smith, O. A. et al. J. Comp. Neurol. 145: 1-24, 1972.

(Supported by MRC of Canada (PG-1). H.-G.R. is a Fellow of Die Deutsche Forschungsgemeinschaft)

940 RECRUITMENT AND DISCHARGE PROPERTIES OF HUMAN MOTOR UNITS IN LOW TO HIGH FORCE ISOMETRIC CONTRACTIONS. C.G. Kukulka* and H.P. Clamann (SPON: A.J. Szumski). Dept. of Physiol., Med. Coll. of Va., Richmond, Va. 23298

The activities of single motor units (n=46) from human biceps brachii muscle were monitored at various forces of isometric contractions (0-88% maximum contraction). Identification of single motor units at high forces was made possible through the use of an analog circuit which took the time derivative of the waveform, thereby selecting units according to the rise or fall times of their action potentials. Recruitment of motor units was observed up to 84% maximum voluntary contraction (MVC), with 36 of 46 units recruited below 50% MVC and 10 of 46 units recruited above 50% MVC. Units recruited at higher forces, on the average, had a tendency to fire at rates higher than those recruited at lower forces. Mean firing rates for grouped data, when plotted against contractile force, and analyzed by linear regression, revealed a correlation coefficient of r = 0.5 and a slope of 1 impulse $sec.^{-1}$ 3.6 kg^{-1} change in force. The maximum firing rates did not exceed 30 impulses $sec.^{-1}$, even at forces as high as 88% MVC. It appears that in brachial biceps, during isometric contraction, the two mechanisms for grading the contraction, recruitment and rate coding, have complementary roles. At low forces (below 50% MVC), a large number of motor units whose firing rates are relatively low may be recruited. As the force increases towards maximum, the limitation in firing rates of units already active necessitates the recruitment of additional units. Our findings reveal that recruitment occurs throughout most of the range of isometric forces, with the number of units recruited declining as the force of contraction increases. Previous investigations have reported that units recruited at the high forces produce greater twitch tensions than units recruited at low forces. Therefore, although fewer units are recruited at the higher forces (above 50% MVC), their larger twitch tensions would contribute a greater proportion to the total force output than the twitches of units recruited at lower forces (below 50% MVC).

941 ULTRASTRUCTURE OF CEREBELLAR AFFERENTS IN THE VENTRAL MEDIAL THALAMIC NUCLEUS IN THE CAT. K. Kultas-Ilinsky, I. Ilinsky, P.A. Young, and K.R. Smith. Depts. Anat. and Surg., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

The ventral medial nucleus (VM) of the thalamus receives cerebellar projections particularly from the fastigial nucleus (Angaut & Bowsher, Brain Res., 24:49, 1970; Faull & Carman, J. Comp. Neur., 178:495, 1978). The purpose of the present study was to identify cerebellar fibers and their terminations in the VM and to compare their ultrastructural characteristics with those of previously described nigral afferents in this nucleus (Ilinsky et al., Anat. Rec., 187:611, 1977; Kultas-Ilinsky et al., Neurosci. Abstr., 3:40, 1977).

Bilateral electrolytic lesions were placed in the brachium conjunctivum by means of a precise stereotaxic technique described earlier (Ilinsky et al., Neurosci. Abstr., 3:396, 1977). The animals were allowed to survive for 3, 4 or 6 days. Numerous very large synaptic boutons (some up to 8 μm in length) and large to medium size myelinated fibers were found degenerating in the VM. At earlier stages the degeneration appeared as aggregations of synaptic vesicles into several clusters with swelling of some vesicles in the synaptic bouton. Later the cytoplasm around the clusters became filled with neurofilaments and glycogen particles. The mitochondria showed a clearing of the matrix and reduction in the number of cristae. Eventually the electron density of the boutons increased until no fine structural details could be resolved, at which stage two types of glia (an astroglia and a dark type presumably microglia) participated in the phagocytosis of the degenerating boutons. In general the pattern of degeneration may be classified as one with filamentous hyperplasia. The degenerating boutons were engaged in asymmetrical contacts with two types of dendrites: a common type and a vesicle-containing type. The majority of degenerating boutons were located in glomeruli where the same bouton contacted both types of dendrites.

It is concluded that cerebellar and nigral projections possess different types of synaptic contacts in the VM, the cerebellar being asymmetrical and the nigral symmetrical. They also differ in size and in their types of degeneration. However, the synaptic sites of both types of boutons are identical, although the cerebellar terminals seem to outnumber nigral terminals. Preliminary data on the diameters of the synaptic vesicles indicate that those in nigral terminals tend to be more elongated although both populations can be classified as pleomorphic.

(Supported in part by USPHS grant FR 05388.)

942 LOAD COMPENSATING RESPONSES OF HUMAN ABDOMINAL MUSCLES. Robert Lansing and Loren Meyerink*. Department of Psychology, University of Arizona, Tucson, AZ 85721.

Positive pressure loads (6-12 cm H_2O, 2 sec duration) were applied unexpectedly at the external airway as subjects made a constant expiratory effort against steady pressure. The latency and form of internal oblique, external oblique, and rectus abdominis EMG responses were obtained from surface recordings; data reported here were recorded over the lower anterior internal oblique covered only by the aponeurosis of external oblique. Pressure and volume changes measured at the mouth reflected the combined action of many respiratory muscles. We examined the effects of the response task, practice, and stimulus expectancy in 8 subjects. Response tasks were defined by instruction and training with a cathode ray feedback display.

When subjects did not try to oppose the load no mechanical or EMG responses were recorded in spite of rapid lung inflation. Given the task of maintaining their pre-load position in spite of the load displacement, subjects corrected to within 50-100 ml of their original volume and there was a 2-phase EMG response: an initial burst, phase I (latency 45-140 ms, duration 100-300 ms, amplitude 50-500 uV), followed by tonic activity of varying amplitude, phase II, continuing to load offset. We believe the phase I burst is a simple ballistic response of the kind conventionally recorded in simple reaction time RT tasks, serving here to oppose the initial change in pressure. The ensuing phase II potentials may represent the subject's proprioceptively guided efforts to achieve positional correction. Intercostal responses to transient loading of voluntary expiratory efforts, similar in latency and duration to our phase I burst, have been reported (Newsom Davis, J. & Sears, T.A., J. Physiol. 209: 711-738, 1970) but important differences in method make direct comparisons premature.

Our interpretation of the phase I burst is based on results obtained when subjects made a single brief response to the loads in a traditional RT task. The latency and form of these RT EMG responses were similar to those of the phase I potentials for any given subject. Practice and increased expectancy produced identical reductions in the phase I and RT EMG latencies. These practiced load RTs averaged 65 ms (range 45-120), showing that latencies of 45-70 ms do not rule out conventional RT processes. We obtained biceps RTs as short as 50 ms for 2 subjects in response to the respiratory loads, so rapid RTs in this latency range cannot be considered unique to load compensatory action in the respiratory system. The finding (Hufschmidt, H., Kilimov, N., & Linke, D., EEG & Clin. Neurophysiol. 43: 622, 1977) of voluntary biceps RTs to contralateral elbow movement of 40-70 ms supports this conclusion.

943 DIRECT SOMATOSENSORY INPUTS FROM THE THALAMUS TO THE MOTOR CORTEX. K. D. Larsen, H. Yumiya*, and H. Asanuma. Rockefeller Univ., New York, NY 10021.

Neurons in the motor cortex have somatotopically organized, somatosensory receptive fields. However, some studies suggest that not all of the somatosensory inputs to the motor cortex arrive by way of the sensory cortex. Horseradish peroxidase (HRP) and electrophysiological techniques were used to determine if some somatosensory information is transferred to the motor cortex directly from the thalamus. In HRP experiments, 0.15 µl of a 50% solution of the enzyme was injected into the distal forelimb region of the motor cortex, identified as the focus of the evoked potential elicited with radial nerve stimulation. Many HRP-containing cells were found in the ventral lateral nucleus (VL), but the rostral portion of the ventral posterior lateral nucleus (VPL) also had labeled cells. Cells in the rostral VPL were studied in electrophysiological experiments. An array of eight electrodes, implanted at a depth of one mm in the motor cortex, was used to antidromically activate cells with intracortical microstimulation. The receptive fields of the antidromically identified cells and their neighbors were examined, and the locations of projection cells were marked with electrolytic lesions. Forty cells in the VL-VPL border area were antidromically activated, each from only one cortical electrode, with latencies of 0.7-1.7 msec ($\bar{x}$ = 1.3). Thirty-two of these could be driven with natural stimulation, 12 of which had receptive fields on the skin and 20 of which were driven from deep structures. In eight cases, the cell in thalamus was recorded simultaneously with cells in the cortex using the electrode which previously activated the thalamic cell antidromically. In six of these simultaneous recordings, four of them involving cells receiving inputs from skin and two from deep structures, the receptive fields were almost identical in the thalamus and the cortical site. These results show that somatosensory inputs traveling in a thalamo-motor cortical path contribute to the formulation of receptive fields in the motor cortex.

944 DECOMPOSITION OF SUPERIMPOSED ACTION POTENTIAL TRAINS. Ronald S. Le Fever* and Carlo J. De Luca. Dept. of Orth. Surg., Children's Hosp. Med. Ctr., Harvard Med. Sch. and M.I.T., Boston, MA

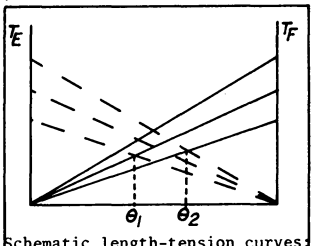
A computerized technique has been developed for the separation of superimposed action potential trains, recorded with indwelling EMG electrodes, into the individual action potential trains of each motor unit contributing to the recording. This technique is also directly applicable to extracellular neural recordings consisting of action potentials from several neurons.

The computer program which performs the decomposition may be employed in automatic or interactive modes, depending on the quality of the data to be decomposed. In the interactive mode the program seeks assistance from the operator only when it has exhausted its schemes for assigning an action potential to the appropriate train. The accuracy of the program in automatic mode is typically 95% for the decomposition of six superimposed action potential trains, but will vary with the quality of the data. Each time the program assigns an action potential to a particular motor unit it averages this new action potential into that motor unit's stored action potential form. These stored forms are compared to each successive action potential in the recorded data. The assignment decision is based on two criteria: the mean squared difference between a particular action potential and each of these stored forms, and the probability that this action potential belongs to each motor unit given the previous occurrences. The mean squared error criterion is algebraically equivalent to techniques using matched filtering, orthogonal decomposition and template matching.

The decomposition program works best when more than one simultaneously-recorded signal of the neuromuscular events is available for analysis. Our motor unit action potential recordings are obtained by using a special electrode consisting of 4 enameled 25 µm wires cemented together in close proximity. The cut ends of the wires act as the recording surfaces. This arrangement permits a very selective recording of 3 linearly independent differential signals. It was found that the quality of the recorded data could be substantially improved by proper recording and filtering techniques. The time duration of the action potentials (hence the amount of superposition) can be decreased by locating FET preamplifiers very near the recording site. This effectively increases the bandwidth of the recorded action potentials. By subsequently filtering out the lower frequencies, action potentials with time durations less than 500 µ sec may be obtained. A combination of analog and digital filtering is employed for this purpose.(Supported in part by NIAMDD grant #AM 19665, and by a joint grant from the United Cerebral Palsy Res. and Ed., the C.A. Dana and the Hearst Foundations.)

945 TRANSITION FROM MOVEMENT TO POSTURE: AN ELECTROMYOGRAPHIC (EMG) STUDY. Francis Lestienne*, Andres Polit* and Emilio Bizzi. Dept. Psychol., M.I.T., Cambridge, MA 02139.

Recent experimental evidence has indicated some characteristics of the programs which subserve limb movements and posture in monkeys (Bizzi et al., J. Neurophysiol., 1976; Polit and Bizzi, Science, 1978). In particular it has been suggested that motor programs specify, through the selection of appropriate length-tension curves in both agonist and antagonist muscles, an equilibrium point that correctly positions the arm in relation to a target (Fig. 1).



Schematic length-tension curves; T_F=Flx.; T_E=Ext.; θ=joint pos.

To further investigate this process we recorded the alpha motoneural input to forearm flexors and extensors (EMG activity) in eight adult subjects. Their forearm was fastened to an apparatus that permitted flexion and extension in the horizontal plane. 17 lights, spaced at 5° intervals, were mounted on a perimeter arc centered around the axis of rotation of the elbow. The subjects were asked to point, without sight of the arm, to whichever light was on and to hold the arm at that position for about two seconds. During the holding time we recorded the rectified and filtered EMG activity from elbow flexors and extensors. Averaged integrated EMG activity of flexors and extensors for each target indicated that (1) the transition from one position to another was attained by modulating the EMG of both agonists and antagonists. The alternative possibility that CNS would implement final arm position by specifying a new level of activity in the agonist muscles while the neural input to the antagonists remains constant was not borne out by these experiments. (2) The final level of EMG activity recorded from either flexors or extensors, while highly variable from trial to trial, was strongly correlated to position and not to such parameters as velocity, direction and amplitude of movement. Taken together these results reveal some aspect of the way in which the CNS implements the shift in equilibrium point between agonists and antagonists. They provide some idea about neural pattern of activity underlying the attainment of an invariant goal such as pointing with the forearm to a target. (Research supported by NIH grant NS09343.)

946 THE UNIT ACTIVITY OF PRIMARY AND SECONDARY AFFERENTS FROM CAT HINDLIMB MUSCLE SPINDLES DURING NORMAL WALKING. Gerald E. Loeb and Jacques Duysens. Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD 20014.

Chronically implanted "floating" microelectrode wires were used to record primary afferent unit activity from the L7 and S1 dorsal root ganglia during unrestrained treadmill locomotion. Using manipulation, electrical stimulation and conduction velocity criteria, we identified 21 primary and 13 secondary spindle endings originating in various hindlimb muscles. Units were held for 1-24 days, during which period it was sometimes possible to implant EMG and/or length gauges in the muscle of origin to facilitate detailed comparison of spindle and muscle activity.

During walking, the activity of a given spindle primary was usually consistent in similar step cycles but was usually poorly correlated with absolute muscle length and was apparently unrelated to velocity of muscle stretch. It could change markedly for similar movements performed under different conditions. Secondaries appeared to be predominantly passive indicators of muscle length during walking, but could demonstrate apparently strong and rapid fusimotor modulation during other motor activities such as postural changes and paw shaking.

Spindle activity modulation not relatable to muscle length changes was assumed to be influenced by fusimotor activity. In certain muscles (particularly those not immediately causing an ongoing movement), this presumption leads to the conclusion that gamma motoneurons may be activated out of phase with homonymous alpha motoneurons as well as by more conventional alpha-gamma motoneuron coactivation. Simultaneous recordings of two spindle primary afferents from extensor digitorum longus indicated that spindles within the same muscle may differ considerably with respect to this presumed gamma motoneuron drive.

On the basis of this preliminary survey, we would propose that the various "servo-control" hypotheses regarding tightly linked pathways for the simultaneous, proportional activation of alpha and gamma motoneurons may require considerable qualification. Our data suggests that even within a given motoneuron pool, voluntary or reflex activity may significantly and independently alter the alpha-gamma relationships for a given movement. Simultaneous activation of alpha and gamma motoneurons could often be inferred under particular circumstances, but the data as a whole indicate the ability to independently control the spindles as sense organs within their parent muscles. Activity in the alpha and gamma systems generally appears correlated during use of muscles as prime movers in a task and uncorrelated when the muscles are used as stabilizers or passive sensors of limb position for a given movement.

947 POSTURAL ANALYSIS IN CORTICAL AREA 5. W.A. MacKay, H.C. Kwan, J.T.Murphy and Y.C.Wong*. Dept. Physiology, Univ. of Toronto, Toronto, Ont., Canada, M5S 1A8.

In order to evaluate the relative importance of peripheral and central inputs to area 5 in a behaving primate, one may directly compare the activity of neurons, sensitive to joint rotation, during similar active and passive movements. Such a study was undertaken in 2 monkeys trained to move a manipulandum into specific positions guided by the visual display of a cursor and a target. By flexing or extending his hand, the monkey maintained the cursor on the target line as the latter was step displaced or as step torques were applied to the hand.

The most common type of neuron found (N=358) responded dynamically to rotation of one or more joints. Only 15 cells were dominated by wrist inputs. Passive and active wrist rotations gave the same response, excitation in one direction and inhibition in the opposite direction. The cells with dominant elbow, shoulder or leg joint inputs also showed directionally selective responses which were similar for both passive and active movements. Another group of 108 neurons responded tonically to maintained joint postures. Virtually all were large cells located in deeper layers of the cortex. Again the majority behaved similarly for passively-imposed postures and active maintenance of the same joint position(s). However, a few neurons were encountered which either phasically or tonically altered their firing rate during the task, but could not be driven by passive manipulation of the arm. In spite of a lack of sensory input, the activity of these cells was often closely related to joint angle or to isometric contractions acting on the joint in the same direction. The modulations of firing rate generally preceded any movement or attainment of targets.

We conclude that proprioceptive input is a major determinant of activity in area 5, being processed in a manner which produces an accurate measure of joint angle in probable output cells. But a central input is also present and appears to monitor the 'motor drive' to specific joint vectors. It is likewise modulated into a postural signal.

Supported by MRC of Canada.

948 PROPRIOCEPTIVE SHAPING OF A CENTRALLY-INITIATED MOTOR PROGRAM DURING MOVEMENT OF THE CRAYFISH CLAW. John D. Marrelli* and James L. Larimer, Dept. Zool., U. of Texas, Austin, TX 78712.

The resistance reflex is generally thought to be inoperative during voluntary movements of the crayfish walking legs.[1] However, there is evidence[2] for a large increase in the resistance reflex sensitivity (2X) during attempted movements of the restrained crayfish claw. Evidence is presented here concerning the interaction of the proprioceptive inputs from the claw with the centrally-initiated motor activity that produces movements about the merus-carpus (MC) joint. In this series of experiments all of the joints of the limb except the MC were fixed. This joint was allowed to flex and lift the limb off a rest position on one stop to a contact with another stop. The lift-off and movement to the second stop was monitored as well as flexor motorneuron activity. Sensory receptors within the limb were selectively destroyed and their effects on flexor motorneuron behavior were observed. In the intact system, the dominant behavior was complex but consistent. There was a pre-lift-off acceleration of motorneuron activity followed by a post-lift-off inhibition. Upon contact with the second stop, the motor activity again accelerated briefly then terminated abruptly. Disruption of sensory receptors distal to the MC joint eliminated the abrupt cessation of the motor program after contact, with cessation becoming variable. Destruction of the myocordotonal organ as well as the distal sensory cells resulted in long-lasting oscillations of the motorneuron activity after contact with the stop. Destruction of the remaining cordotonal organs of the joint (MC1 and MC2) eliminated the inhibition of the post-lift-off motorneuron activity. The resulting waveform of the flexor motorneuron frequency resembled a rectangular pulse with a slight overshoot at the onset. These data indicate a proprioceptive modification of a centrally-generated motor program. In particular, the inhibition of flexor motorneuron activity during flexion suggests strongly an active role of the resistance reflex in the shaping of the motor program to achieve some peripheral matching of load with central control. (1. P. J. Mill, Structure and Function of Proprioceptors in the Invertebrates, 1976; 2. J. D. Marrelli and W. Evoy, 6th Soc. for Neurosciences, 1976.) (Supported by NIH NS-05423 and by NIMH NS-05075).

949 CAN EMG ACTIVITY BE USED TO DISTINGUISH "SLOW" VERSUS "FAST" MOVEMENT? N. Mayer, S. Naylor*, and H. Rosenholtz*. Dept. of Rehab. Med., Temple U. Health Sciences Center, Phila., Pa. 19140.

By considering movement displacement and its associated integrated EMG (IEMG) as processes which go to 100%, we have derived the "delta parameter" which describes how the EMG distributes itself with respect to displacement as a function of movement velocity. Subjects were asked to move a pivoted rod forward at different self-generated rates and various accuracy controlled amplitudes. Surface EMG from the agonist anterior deltoid was integrated over the course of the movement and displacement was measured by a potentiometer. Each movement was divided into a series of ten percent amplitude bins and the corresponding percentage accumulation of IEMG was identified by computer. The value of the delta parameter was obtained by adding up the differences between percent IEMG and percent displacement occurring at each 10 percent amplitude bin of the movement. Accordingly, if the IEMG process goes to 100% more quickly than the displacement process, the delta parameter will be positive. Conversely, the delta parameter is negative when IEMG rises more slowly than the movement. Results show that the delta parameter for anterior deltoid increases linearly with velocity at each movement amplitude while the slopes of the best fit lines vary inversely with amplitude. The delta parameter changed sign (from negative to positive) at different threshold velocities depending on the movement amplitude. However, when average accelerations were calculated for each movement amplitude, the delta parameter changed sign at similar accelerations independent of differences in amplitude or velocity of movement. These findings indicate that the distribution of IEMG with respect to movement displacement is quantitatively measurable and varies with movement speed and acceleration. Orchestration of central motorneuron drive according to the acceleration requirements of a given movement might be responsible for sign and magnitude changes in the delta parameter. It is suggested that a negative delta parameter for the agonist muscle might be a useful classifier of "slow" movements while positive values would be indicative of "fast" movements.

950 EFFECTS OF LESIONS IN DIFFERENT AREAS OF SENSORIMOTOR CORTEX ON M2 AND M3 STRETCH RESPONSES IN MONKEYS. Alan D. Miller and Vernon B. Brooks. Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1.

Perturbations applied to a monkey's forearm can produce three successive EMG responses in stretched upper limb muscles (M1, M2, M3 (1). M1 peaks at 15-20 ms following perturbation onset, i.e. at the latency of a spinal stretch reflex. M2 and M3 peak at latencies of 40-50 and 65-75 ms and are thought to depend on participation of supraspinal structures that have not been fully defined. Lesions of postcentral arm region (said to have involved areas 1, 2, 3b, most of 3a, and some of 5) abolish M1 (1). Many precentral neurons discharge in response to perturbations at latencies which would permit their participation in the generation of M2 and M3 (2). We made a series of lesions through the sensorimotor cortex to determine which areas contribute to M2 and M3.

Cebus monkeys were tranquilized with Atravet, and their forearms were firmly strapped to a manipulandum handle which could be displaced along a fixed and repeatable trajectory using a torque motor (3). Biceps EMG responses, obtained with fine-wire intramuscular electrodes, were filtered, full-wave rectified, and digitized online at a sampling rate of 1000 Hz using a PDP computer; 25 trials were averaged in 5 ms bins. Unilateral suction lesions were made sequentially in 4 monkeys in the arm area of pre- or postcentral cortex, which was first mapped using surface anodal stimulation. The extent of the lesions, which were histologically confirmed, involved some underlying white matter.

Lesions down the rostral bank of the central sulcus, which included all of area 3a, the overlying part of area 4, and the bottom part of area 3b, greatly reduced M2 and M3 in the contralateral arm in 2 cases studied. In contrast, lesions of the precentral cortex sparing that part overlying area 3a had little, if any, effect on M2 and M3 (relative to M1) in 3 animals. A lesion of the postcentral cortex sparing most of the caudal bank of the central sulcus also left M2 and M3 unchanged in relation to M1.

The results suggest that area 3a and/or the immediately overlying part of area 4 is essential for the normal occurrence of M2 and M3. This region may be part of a transcortical loop which mediates M2 and M3 and/or may provide tonic facilitation for subcortical structures which in turn mediate M2 and M3.

(1) Tatton et al. Brain Res. 1975, 96: 108-113,
(2) Conrad et al. Brain Res. 1975, 94: 219-236, Evarts, Science 1973, 179: 501-503,
(3) Cooke & Eastman, Exp. Brain Res. 1977, 27: 491-500.

Supported by MRC of Canada (PG-1). A.D.M. was the recipient of an Ontario Graduate Scholarship Program predoctoral fellowship.

951 PROJECTION OF THE NUCLEUS CUNEIFORMIS TO PONTINE RETICULAR FORMATION IN THE RAT. Cheryl A. Miller* and Harry M. Sinnamon. Lab. of Neuropsychology, Wesleyan Univ., Middletown, CT 06457.

The influences of the midbrain reticular formation (MRF) on behavior could be mediated by connections to the reticulospinal system. In the cat these projections have been described in anatomical and electrophysiological studies. The present study describes this pathway in the rat. Single units were recorded extracellularly in DA Agouti rats (N=16) anesthetized with halothane (0.5-0.75%). Constant current monophasic pulses (0.1-0.3 msec) were delivered through stationary, twisted pair, stainless steel electrodes. In the principle experiment the recording electrode was advanced slowly through the pons while the MRF (primarily n. cuneiformis) was stimulated at 1 Hz, 500μA. Responses and latencies were determined for various regions of the pons. Since stimulation of the n. cuneiformis could activate passing fibers originating in other structures such as the tectum, a complimentary experiment was performed. In it the range of antidromic response latencies was determined for cells in n. cuneiformis that responded to stimulation in the various pontine regions. The limits of this range, adjusted for synaptic delay, provided the criterion for classifying a response in the pons to n. cuneiformis stimulation as monosynaptic.

Cells in the ipsilateral n. cuneiformis were antidromically activated by stimulation in reticularis gigantocellularis (RG) with a latency range of 0.75 to 1.3 msec. Monosynaptic excitatory projections from n. cuneiformis to RG were seen in 4 of 23 cells ($1.45 < \text{latency} < 2.0$ msec). Multisynaptic (latency > 3.8 msec) and non-cuneiformis (latency < 1.0 msec) projections were found in 5 other RG cells. The reticularis pontis caudalis (RPC) also showed an ipsilateral projection with 4 of 27 cells having latencies compatible with the antidromic latency range (1.4 to 2.4 msec). While the reticularis paragigantocellularis (RPG) apparently receives a projection from the n. cuneiformis (antidromic latency 0.8 to 2.2 msec, 5 cells), no monosynaptic excitations have been found in 9 RPG cells tested to date. RG and RPC appear to receive a monosynaptic excitatory projection from the contralateral n. cuneiformis which is as yet unconfirmed by antidromic studies. The reticularis parvocellularis shows no indication of ipsilateral projection in 16 cells tested. In summary these results obtained from the rat suggest a major slow conducting (1 to 4 m/sec) excitatory projection to the RG and RPG from the n. cuneiformis.

952 EVALUATION OF SPONTANEOUS FUSIMOTOR EFFECTS FROM AVERAGED DORSAL ROOT NEUROGRAMS TRIGGERED BY THE ACTION POTENTIALS IN A GAMMA EFFERENT. K.S.K. MURTHY, Y. YOON*, B.M. RIGOR*, P.L. GILDENBERG, and K.G. CHATMAJIAN*. Departments of Surgery (Neurosurgery) and Anesthesiology, University of Texas Medical School, Houston, Texas, 77030.

It is possible to record the spontaneous activity of single gamma efferents, preserving the integrity of their peripheral connections. Using such a technique, a simultaneous recording of multiunit afferent activity in dorsal roots has been undertaken in this study to permit identification of the type of gamma efferent being recorded. Adult cats were studied under halothane anesthesia after induction with ketamine hydrochloride. Surgical procedures included left hind limb dissection to expose the muscle nerves for stimulation and a laminectomy (L_4 to S_1). Blood pressure was monitored through a cannula in the left common carotid artery and an intravenous drip of lactated Ringers' solution maintained through the experiment. No peripheral nerves were sectioned. No roots were severed. While recording from the ventral rootlets (L_7), the filaments were teased in continuity until a single spontaneously discharging gamma efferent was isolated. Conduction velocity and muscle of destination were established by antidromic stimulation of various muscle nerves. The dorsal root (L_7) filaments were then searched for spindle afferents from the same muscle innervated by the efferent under study.

An envelope of the multiunit afferent discharge was obtained with a ratemeter (time constant 40 msec) and averaged, with the sweep being triggered by the spontaneously occurring action potentials in the γ efferent. Two types of responses correlating with fast or slow intrafusal contractions can be obtained. The faster response presumably results from contraction of nuclear chain intrafusal fibres and causes a peak in afferent activity within 15 msec after the occurrence of the γ spike. Multiple peaks in afferent response are observed at low rates of spontaneous activity in the γ efferent, probably due to oscillations in the nuclear chain fibres (Boyd, 1976). The slower response shows maximum afferent activity about 50 msec after the occurrence of the γ spike and may correlate with contractions in nuclear bag intrafusal fibres. It remains to identify the types of γ efferents contributing to the fast and slow responses observed here.

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953 ORGANIZATION OF POSTURAL ADJUSTMENT CONTROL SYSTEMS IN HUMANS. Lewis M. Nashner and Marjorie H. Woollacott. Neurological Sciences Institute, Portland, Oregon 97209.

The EMG activity of four leg muscles (gastrocnemius, tibialis anterior, hamstrings and quadriceps) was measured while freely standing humans were subjected to unexpected movements of a platform capable of six independent degrees of motion (horizontal, vertical and rotational displacements of each foot). Earlier studies have shown that a neurally programmed fixed pattern of activity occurs in the four leg muscles in response to ankle joint rotation caused by anterior-posterior sway (G-H coupling for anterior body sway and T-Q coupling for posterior body sway). This fixed pattern has been termed the sway synergy. The present data show that vertical body movements causing predominantly knee joint rotation elicit a different fixed pattern of activity in the leg muscles (G-Q coupling for downward movement and T-H coupling for upward movement), which has been termed the suspensory synergy. This fixed pattern of EMG activity among proximal and distal leg muscles also appears to be programmed neurally (and not caused independently by stretching proximal and distal muscles), since the response pattern is not altered when one of the platform bases is rotated during a vertical change in height so that the ankle motion is unexpectedly dissociated from that of the knees. The sway synergy appears to be elicited whenever movements occur largely about the ankle joints, whereas the suspensory synergy is elicited by movements about the knee joints. Two subclasses of the suspensory synergy are observed, depending on whether synchronous or reciprocal vertical motions are used. During synchronous leg motions (described above) the G-Q and T-H pairs resist the movements, whereas during reciprocal motions they follow the platform movements.

Reorganization of activity from the sway into the suspensory synergy is evident in the first trial following unexpected changes from sway to vertical perturbations. This is in marked contrast to changes in the gain of the muscle responses which, as shown in earlier studies, takes three to five trials to adapt when the relation between proprioceptive inputs and postural sway motions is unexpectedly changed.

The following hypothesis is consistent with these experimental observations: the synergic arrangement of the muscles is organized at a lower level in the nervous system, based on the pattern of proprioceptive inputs. The adaptive changes, which require several trials, involve the integrative functions of the CNS and utilize more complex combinations of somatosensory, vestibular, and visual inputs.

954 PEDALING AND THE MONKEY MOTOR CORTEX. Edward J. Neafsey, Dennis Shaw*, and Arthur A. Ward, Jr. Dept. Neurol. Surg., Univ. Wash., Seattle, WA. 98195

Single units were recorded from the leg region of one monkey's precentral motor cortex during performance of a pedaling movement. The pedaling involved reciprocal movements of the legs, and within each leg there was a cyclic activation of flexors (TA) and then extensors (G-S) as determined by EMG recordings. The monkey's arms and head were restrained. Over 80% of the 38 units recorded thus far showed some modulation of their activity related to the movement. Thirty-seven percent were activated during the flexion phase of the contralateral leg (+F), 39% during the extension phase (+E), and 5% paused during execution of the flexion-extension sequence. The remaining 19% showed no clear modulation during the movement. The modulation of some units could be explained on the basis of their observed receptive fields determined during passive movements of the leg. For example, one unit was activated during contralateral knee extension during pedaling and also responded to passive extension of the same knee. However, this pattern was not always found and many cells had no obvious receptive fields. The +F and +E cells were also studied during arm and head movements. Individual cells displayed either a consistent increase, a consistent decrease, or no change in activity during these other movements. All three types of changes were found among the +F cells, while only decreases or no change were found among the +E cells. The 25 electrode penetrations were spaced 1 mm apart and formed a 5 x 5 grid. The location of +F and +F cells were plotted on this grid. Surprisingly, the general pattern observed was that of large adjoining zones of either +F or +E cells with little overlap between zones.

(This project was supported by NIH research grant number NS-04053 awarded by the National Institute of Neurological and Communicative Disorders and Stroke, PHS/DHEW.) Dr. Neafsey is also an affiliate of the CDMRC at the University of Washington.

955 PYRAMIDAL TRACT AND RED NUCLEAR PROJECTIONS FROM THE PRIMATE SUPPLEMENTARY MOTOR AREA. C. Palmer*, E.M. Schmidt and J.S. McIntosh. Lab of Neural Control, NINCDS, NIH, Bethesda, MD 20014

There have been several investigations as to whether the supplementary motor area (SMA) has a pyramidal tract projection. Coulter et al (Brain Res. 103:366, 1976) failed to find a pyramidal projection from SMA in cats from cervical and lumbar HRP injections though Murray and Coulter (Neurosci. Abs. 3, 1977) with the same method found the projection in primates. In this study we sought neurons in the SMA projecting to the pyramidal tract (PT) and red nucleus (RN) in four rhesus monkeys using electrophysiological techniques. Neurons were found with antidromic invasion from stimulation of the pyramids or red nucleus and recorded with glass coated PT/IR microelectrodes, their impedances ranging from 1 to 2.5 Meg at 1 KHz. The criteria for antidromic invasion were a constant threshold and latency, the latency also being constant with high frequency stimulation; these ranged from 200 Hz to 500 Hz. When the cells had spontaneous activity, antidromic invasion was tested with the spike collision method. The pyramidal tract was stimulated with a bipolar coaxial stainless steel electrode 0.5 mm in diameter. The average threshold for PT cells was 365 μA; for RN neurons 401 μA (the threshold ranged from 50 μA to 650 μA for PT neurons and 80 μA to 800 μA for RN neurons, with pulse duration of 0.1 msec). RN neurons were stimulated via a similar bipolar electrode 0.2 mm in diameter or via an array of two monopolar stainless steel electrodes, 0.2 mm in diameter separated by 1 mm, with current balancing resistors in series. Stimulation occurred via an anode in the scalp. Their location was established from histological reconstruction of the electrode tracks. Fifty-eight projection neurons to the pyramidal tract were recorded and their latencies varied from 0.5 to 13 msec, 53.6% having latencies between 0.5 and 3 msec. Twenty-nine projection neurons to the red nucleus were recorded and their latencies varied from 1.2 to 6 msec. They were more evenly distributed in their range than PT neurons. Antidromically invaded neurons from the same region in the midbrain tended to be clustered together and often units with different antidromic latencies could be recorded at one electrode site. However, PT and RN neurons were rarely intermingled in this way. More RN neurons than PT neurons were recorded in laminae III and the superficial parts of laminae V. The PT neurons were generally found in the deeper parts of laminae V and laminae VI. No evidence was found of collaterals from the pyramidal tract to the red nucleus. Humphrey and Reitz (Brain Res. 110: 162, 1976) investigated PT and RN projections from the hand area of the primate motor cortex and found an average of 7 PT and 2.8 RN neurons per track. We found an average of 3.1 PT and 1.1 RN neurons per tract in the SMA.

956 CONTROLLED LATERAL PINCH AND RELEASE IN THE C6 QUADRIPLEGIC INDUCED BY ELECTRICAL EXCITATION. P. Hunter Peckham* (SPON: F.T. Hambrecht) Case Western Reserve University, Cleveland, Ohio 44106

Electrical excitation of paralyzed muscle provides a technique to elicit controlled contractions in paralyzed limbs. In this study, this approach was applied to the C6 spinal cord injury patient to provide control of lateral pinch (key grip) and release. The C6 quadriplegic retains voluntary motor control of shoulder motion, elbow flexion, forearm supination and pronation, and wrist extension, but is paralyzed otherwise. As the patient extends his wrist (without stimulation), the passive force exerted by stretching of the thumb flexor closes the thumb against the index finger (tenodesis grasp). The force is sufficient to enable him to pick up and hold large, lightweight objects.

Muscle stimulation augments voluntary tenodesis function. Chronically indwelling percutaneous coiled wire electrodes are implanted into thenar muscles (adductor pollicus and/or opponens pollicus), finger flexors, and thumb extensor. The electrodes pass through the skin on the volar and dorsal aspects of the forearm just proximal to the wrist. The force in each muscle is controlled through a recruitment and temporal summation scheme, by modulation of the stimulus pulse width and frequency respectively. A single command signal modulates the stimulus parameters according to a predetermined algorithm. Finger flexor stimulation always precedes stimulation of the intrinsic thumb muscles to provide a stable platform for lateral pinch; release is provided by stimulation of the thumb extensor. The command source for muscle activation is a two level processed myoelectric signal (MES) from a muscle which retains voluntary function. A low level MES increases or decreases the stimuli, a zero level MES maintains the stimulus, and high level MES either reverses the direction of stimulus change or deactivates the stimulation, depending upon the length of time the level is held.

Five subjects have utilized this system and have found its function beneficial in performing tonic tasks, such as eating and writing.

Sponsored by NIH-NINCDS Contract No. NO1-NS-2-2314.

957 ON THE RELATIONSHIPS BETWEEN SELECTED PARAMETERS OF MOTONEURON - MOTOR UNIT "TYPE" AND THE ABSOLUTE VOLTAGE THRESHOLD OF THE MOTONEURON. M.J. Pinter*, R.L. Curtis, M.J. Hosko. Depts. of Pharm. and Anat., Medical College of Wisconsin, Milw. WI 53233

Among the indices of motoneuron-motor unit "type", an inverse relationship has been established between motoneuron size and cell membrane resistance (RES). Positive correlations have been established between membrane resistance and duration of afterhyperpolarization (AHP), between membrane resistance and twitch contraction time (TWT) of the functionally isolated motor unit and between duration of AHP and TWT. The amplitude of the maximum homonymous EPSP is positively correlated with motoneuron resistance. It has been assumed that the absolute voltage threshold (TH) and depolarization (DEP) necessary to elicit an orthodromic response in a motoneuron is independent of the above variables. This study was designed to test the validity of this assumption. We examined functionally isolated motor units of the triceps surae motoneuron pool in the anesthetized cat. Blood pressure, deep rectal, spinal and muscle oil pool temperatures were monitored. Units to be studied were identified by antidromic stimulation of lateral gastrocnemius (LG) - Soleus (Sol) and medial gastrocnemius (MG) nerves. Upon impalement of a motoneuron, the following parameters were measured: AHP duration, cell membrane RES (spike height method), DEP necessary to elicit an orthodromic spike (transected dorsal root stimulation) and resting membrane potential (RMP). Any cell whose RMP was less than 50 mV was excluded from analysis. Positive identification of the motoneuron was made by attaching the appropriate muscle tendon to a strain gauge myograph; TWT, twitch tension and tetanic tension of the motor unit were measured. 122 cells were studied. Full or partial results were obtain from 25 Sol and 97 MG-LG motoneurons.

As reported by others, we found the following pairs displayed significant positive correlations: AHP and RES, AHP and TWT and RES and TWT. We also demonstrated weak correlations among the following pairs: AHP and DEP, ($r = -.22$, $P < .01$), AHP and TH ($r = .21$, $P < .01$) and RES and TH ($r = .35$, $P < .01$). The correlations between TH and RMP ($r = .87$) and between DEP and RMP ($r = .46$) were significant ($P < .001$). Correlations between TWT and TH, TWT and DEP, RES and DEP, AHP and RMP and TWT and RMP were insignificant These data indicate that the TH and DEP of the motoneuron are tightly related to the RMP and minimally related to other indices of motoneuron-motor unit "type". Supported in Part by NIH GRANTS DA00124 and NS07680, by The Dept. of Neurology, MCW and by The Evan and Marion Helfaer Foundation.

958 RELATION BETWEEN EXTRAFUSAL AND INTRAFUSAL ACTIVITY IN THE DECEREBRATE CAT MODEL: A ROLE FOR BETA FIBERS. E. Post*, W.Z. Rymer and Z. Hasan*. (SPON: C.J. Hodge). Neurosurgery Laboratory, S.U.N.Y. Upstate Medical Center, Syracuse, New York 13210.

The relation between extrafusal and intrafusal activation is presently unclear. In some circumstances (such as isometric contraction of human finger flexors (1)) the activation appears tightly coupled whereas in others (such as cat locomotion (2)) the relation is claimed to be less rigid. Furthermore, the physiological role of beta axons, whose presence is now widely confirmed in mammalian muscles, remains largely unexplored.

We have examined the relation between extrafusal and intrafusal activation in the triceps surae muscles of the decerebrate cat. The state of intrafusal activity was deduced from the discharge of identified primary and secondary endings, isolated from small dorsal root filaments. Spindle receptor properties were examined under isometric conditions, during ramp muscle stretch and during isotonic muscle shortening. The state of extrafusal and intrafusal excitation was varied using a range of stimuli, most often the crossed extensor reflex.

In most primary endings (35/42) crossed extensor activation induced a progressive increase in receptor discharge, commencing well before the onset of extrafusal force or emg activity. There was usually no further increase in discharge once extrafusal threshold was reached : in fact, at high forces receptor discharge rates sometimes fell. This sequence of activation suggests that fusimotor neurons are recruited first and saturate before or about a level of excitability equivalent to extrafusal threshold.

A smaller number of primary endings, and a significant fraction of secondary endings accelerated their discharge as force increased. Although it is possible that this acceleration of discharge was mediated via a different class of gamma fibers to that cited above, it is equally likely that beta motoneuronal discharge may have been responsible, since beta axons have been demonstrated in these muscles.

The role of beta fibers may be to increase intrafusal contraction in circumstances where gamma neuronal action is effectively saturated.

(1) Vallbo (1974). Acta physiol. Scand. 90: 319-336.
(2) Prochazka, Westerman, & Ziccone (1977). J. Physiol. 268: 423-448.

959 LARGE DISPLACEMENT ACTION TREMOR OF THE ANKLE IN NORMAL SUBJECTS. R. Pozos, D. Deetz*, and P. Iaizzo* (SPON: Alex Fedinec). Dept. of Physiol., Univ. of MN-Duluth, Sch. of Med. and Nat Polinsky Rehab. Center, Duluth, MN.

Studies concerning the mechanisms of pathological tremors which are characterized by a large displacement and a low frequency infer that the frequency and amplitude of these tremors are unique to the disease state. However, recent reports indicate that large displacement, low frequency oscillations which have been called "physiologic clonus" are induced by fatiguing the muscles. Our studies indicate the presence of an action tremor (5-6 Hz) with a large displacement (2-3 cm) in normal subjects who have not been fatigued.

Subjects were seated and an accelerometer (Gulton AVR 250) was taped to the knee. Electromyographic signals from the tibialis anterior and the soleus were amplified and recorded simultaneously with the motion. Subjects were instructed to raise their heels as high as possible and to slowly lower it.

As the heel was raised a frequency of 7-8 Hz was observed with displacement values of 20-35 micra. As the heel was lowered a frequency of 5-6 Hz was seen with a displacement value of 2-3 cm. This large displacement low frequency oscillation was position dependent in that it only occurred at a certain distance off the floor as the subject was lowering his heel and not during the full range of motion. This action tremor (tremor occurring during motion) was seen every time the subject lowered his heel. Subjects who had been fatigued by running a number of miles also demonstrated this action tremor. In some cases the action tremor in the fatigued subjects progressed into a self sustained oscillation which had the same large displacement and low frequency values as the action tremor. Similar studies were conducted on a number of Parkinsonian patients who had an overt ankle tremor. Their displacement and frequency values were similar to the action tremor seen in control subjects.

Since this study shows that the action tremor has the same displacement and frequency values as the pathological tremor, it would suggest that the mechanism for abnormal tremors is inherent in control subjects. Further since this action tremor is found in non-fatigued individuals, this finding would indicate that alteration of the properties of muscles through fatigue is not a major component of the mechanisms for these oscillations.

This research was supported in part by a grant from the Miller Dwan Research Foundation.

960 ELECTRON MICROSCOPIC CHARACTERISTICS OF FINE GRAIN DEGENERATION IN THE RAT. P.M. Ritschel, K. Kultas-Ilinsky, P.A. Young, L.C. Massopust, and R.A. Owler*. Dept. Anat. St. Louis Univ. Sch. Med., St. Louis, MO 63104.

The purpose of this investigation was to characterize, at the electron microscopic (EM) level, the fine grain degeneration seen in the ventrolateral-ventroposterior (VL-VP) and intralaminar (IL) complexes after lesions in the motor cortex of the albino rat. The Fink-Heimer technique was used to localize the areas of fine grain degeneration with light microscopy. Cores for EM were taken from areas of densest concentration of fine grain degeneration. In the VL-VP complex, a dense area first appeared at 2 days postoperatively, became heaviest at 4 days, and disappeared after 8 days. In the IL the fine grains were observed in the lateral central nucleus at 11 days and in the lateral central and paracentral nuclei at 14 days. At 21 days no fine grains were observed in these nuclei. Injections of tritiated leucine into the motor cortex were used to confirm corticothalamic terminations in these areas.

Ultrastructural studies of the VL-VP area revealed small unmyelinated preterminal axons and synaptic boutons degenerating 2 days postoperatively. The degenerating structures had a dense matrix and occasionally swollen vesicles could be seen. The degenerating boutons appeared in clusters surrounding medium sized dendrites. The number of degenerating structures increased three fold in 4 day survival animals. Only a few degenerating boutons were seen at 8 days; none were observed at day 11. No changes in neurons were observed at the survival periods studied. The same types of degenerating axons and boutons were found within the IL complex at 11 days. At day 14 larger boutons, in addition to the small ones, were seen degenerating. Fragments of large myelinated axons were also observed. There appeared to be dilatations of rough endoplasmic reticulum and a decrease in ribosomal rosettes in neurons in the IL nuclei at day 14 and later. At day 21 very few degenerating boutons were observed.

The fine grain deposits within the VL-VP are the result of small degenerating unmyelinated preterminal axons and boutons. The lack of neuronal changes indicated that alterations in thalamocortical fiber collaterals were not present. The fine grain deposits in the IL appeared to be the result of degenerating terminal axons and boutons of various sizes. However, the changes seen in the neurons here indicate the possibility of some retrograde changes in this area at the longer survival times. (Supported in part by USPHS grant FR 05388.)

961 CUTANEOUS INPUT TO NECK MUSCLE MOTONEURONS OF THE CAT. P.K. ROSE and N. SPROTT*. Department of Physiology, Queen's University, Kingston, Ontario, Canada. K7L 3N6.

Several studies have indicated a close anatomical and functional relationship between the trigeminal system and the upper cervical spinal cord. The aim of the present work was to examine the responses of identified neck muscle motoneurons to stimulation of cutaneous afferents innervating the face. Experiments were performed on adult cats anaesthetized with chloralose (70 mg kg^{-1}). Intracellular recordings were obtained from motoneurons innervating deep dorsal neck muscles (biventer cervicis and complexus, BC-C) more superficial neck muscles (splenius, S), and lateral neck muscles (trapezius, T). Stimulation of the infraorbital (IO) and supraorbital (SO) nerves consistently led to the appearance of EPSPs in ipsilateral S and BC-C motoneurons. In contrast ipsilateral T motoneurons were usually inhibited by SO stimulation and the effects following IO stimulation were mixed. Stimulation of the contralateral IO led to EPSPs in all 3 motoneuron groups although a significant proportion of T motoneurons were inhibited. The response to contralateral SO stimulation was excitatory in T motoneurons, inhibitory in BC-C motoneurons, and mixed in S motoneurons.

Additional experiments using graded electrical stimulation of the IO nerve revealed that the response of S and BC-C motoneurons usually occurred at low threshold (1-2 T) stimulation. Increasing the stimulus strength to 10 T or greater did not alter the nature of the response and led to EPSPs with latencies of 2.0 to 7.0 msec. Gentle tapping of the nose and surrounding vibrissae led to EPSPs and action potentials in S and BC-C motoneurons.

The nature and pattern of the responses of neck muscle motoneurons following stimulation of the IO nerve suggests that cutaneous input originating from the nose will result in lowering of the head to avoid the stimulus. Moreover this behaviour can be triggered by innocuous mechanical stimuli. In contrast responses to SO stimulation would suggest that a more complex movement would result, perhaps related to orientation.

(Supported by the Canadian Medical Research Council).

962 PATTERNS OF CONTRALATERAL LIMB RESPONSES TO NOCICEPTIVE STIMULI DURING LOCOMOTION. S. Rossignol and L. Gauthier*. Centre de recherche en sciences neurologiques, Université de Montréal, Québec, Canada H3C 3T8.

When a nociceptive skin stimulus is applied on a skin nerve of a hindlimb in acute spinal cats treated with Clonidine the contralateral hindlimb extends when it is placed initially in a flexed position and conversely flexes when placed in extension (Grillner and Rossignol, Brain Res., 144: 411-414, 1978). The present study also reports on a reversal of contralateral effects but during locomotion in high decerebrate cats walking on a treadmill The stimuli (100 ms trains, 100 Hz, 1 ms pulses at or above 40X threshold for the largest fibers) were randomly delivered to the superficial peroneal nerve on one side (ipsilateral limb) at different periods of the walking cycle. The EMG activity of selected extensors and flexors was recorded bilaterally. On the ipsilateral side, the stimulus invariably evoked a flexion response irrespective of the step cycle phase. In the contralateral limb, however, increases in amplitude of either the extensor or the flexor EMG bursts were observed with stimuli occurring some time prior to or during the respective bursts and modified the ongoing limb movement. Two general patterns can thus be described. First, when the stimulus is applied during the ipsilateral swing phase, the pattern is an increase both in ipsilateral flexion and contralateral extension. Second, when the stimulus occurs during the ipsilateral extension phase, the ipsilateral extensor is inhibited and replaced by a flexion response while the contralateral flexion is enhanced. In the first pattern, the contralateral limb preserves a stable alternate gait despite the large increase of the extensor burst amplitude. In the second pattern, the alternate gait is suddenly changed to a gallop with both limbs at different degrees of flexion. The contralateral limb flexor burst is then either increased in amplitude which accelerates the swing phase or lengthened which delays the onset of extension or even resets the limb step cycle in order to rephase it properly in alternation with the other limb. This work thus emphasizes that with a nociceptive stimulus the classical pattern of crossed extension is seen only in a certain period of the locomotor cycle when its function then is to sustain the weight of the animal. In other parts of the cycle, this response is not only abolished but replaced by a facilitation of flexor muscles enabling the animal to reestablish an alternate gait pattern within one or two walking cycles.

(Supported by the MRC)

963 SERVO-REGULATION OF MUSCLE CONTRACTION IN MAN: A REEVALUATION. W.Z. Rymer, Z. Hasan* and B.C. Corser*. Neurosurgery Lab., S.U.N.Y. Upstate Medical Center, Syracuse, New York 13210.

The early electromyogram response to abrupt muscle stretch does not serve to reestablish muscle length; rather it appears to contribute to the regulation of net muscle stiffness (1). Previously proposed mechanisms for the neural regulation of muscle have emphasized feedback servo control, using length and force signals originating in muscle to provide the required error signals. Here we address several potential inadequacies of this approach.

Forcible, constant amplitude extensions of the contracting human long thumb flexor, applied over a range of different velocities, produce virtually constant peak forces in spite of widely differing emg responses. In the extreme case, extensions completed before the onset of significant emg action (at 40 msec) produce forces similar to those observed during slower stretches, even though emg action could have made no contribution to the dynamic force trajectory. The observations suggest that intrinsic mechanical properties of muscle are responsible for much of the dynamic force trajectory.

When the mechanical properties of an adjacent, similar synergist were studied, the latter expectation was confirmed. We used percutaneous stimulation of a relaxed index flexor (FDS) to evaluate muscle behavior under 'open-loop' conditions. The deviations between electrically and reflexively activated force for this muscle were small, of late onset, and strongly dependent upon initial force. Furthermore, the most marked deviations occurred at movement cessation where electrically stimulated muscle showed significant force adaptation.

Since the length afferent signal is essentially controlled in our experimental paradigm, the only available correctional feedback signal capable of compensating for the inadequacies of intrinsic muscle force arises from tendon organs. However, tendon organ information appears to act simply as a proportional feedback signal with little dynamic component, hence such information could not be used (alone) to prevent undesirable deviations in force trajectory. This is consistent with the conclusion that the dynamic force trajectory in our subjects may rely largely upon intrinsic muscle properties. The emg response may be issued in response to a qualitative prediction of impending decline in intrinsic muscle stiffness, rather than as a correction activated by measured error.

(1) Nichols & Houk (1976). J. Neurophysiol. 39: 119-142.

964 REFLEXOGENIC AND PSYCHOGENIC REFLEX MODULATION: DIFFERENTIAL EFFECTS ACCORDING TO POLYSYNAPTIC AND OLIGOSYNAPTIC PATHWAYS IN HUMANS. Jerome N. Sanes*, James R. Ison, and Alice A. Adelson*. Dept. Psychology, Univ. of Rochester, Rochester, NY 14627.

Electromyographic activity recorded from orbicularis oculi in humans, was elicited by percutaneous electrical stimulation of the supraorbital branch of the trigeminal nerve. The reflex consists of an early brief ipsilateral response (R1) with a latency of about 10 msec and a more prolonged later consensual response (R2) with a latency of 30-40 msec. An oligosynaptic trigemino-facial path mediates R1, a polysynaptic path through the pontomedullary reticular formation mediates R2. The plasticity of R1 and R2 was investigated as a function of sensory stimulation preceding reflex elicitation, voluntary delivery of the shock and repetition of the shock.

In Experiment 1 brief tones (S1), at 70 dB SPL, were presented from 5 to 800 msec before the eliciting stimulus (S2). In Experiment 2 tones of intensities varying from 30 to 70 dB SPL preceded S2 at a fixed lead time of 100 msec. In each experiment S1 facilitated R1 and inhibited R2. Facilitation of R1 developed more rapidly than did inhibition of R2. The time course of facilitation had two peaks, at 50 and at 800 msec lead times, whereas inhibition had a single peak at 200 msec. Both effects increased linearly with S1 intensity. Contrary to earlier reports the reflex threshold for R1 was greater than that of R2 (11.0 mA vs. 2.3 mA). In Experiment 3 self-presentation of S2 provided reflex thresholds of R1 and R2 which were roughly equivalent. Increased S2 intensity increased both R1 and R2, but self-delivery inhibited R2 whilst markedly facilitating R1. In Experiment 4 repetition of S2 (rates of .2, .5, and 1/sec) resulted in habituation of R2 and sensitization of R1, both effects directly related to presentation rate. We note that prior experiments which have reported equivalent reflex thresholds use either self-presentation or repetitive delivery; these conditions are favorable for R1, unfavorable for R2.

We believe these collective findings reveal two processes, one reflexogenic and the second psychogenic, involved in the modification of R1 and R2. The reflexogenic process manifests itself in the time course of R2 inhibition, in the early facilitation of R1 following the tone, and in the habituation of R2. Psychogenic processes, having to do with awareness of stimulus imminence, seem responsible for facilitation of R1 at the 800 msec S1-S2 interval, for the potentiation and inhibition of R1 and R2 by self-delivery, and for the facilitation of R1 by repetitive shocks: these latter effects suggest the presence of a central reflex modulatory mechanism which is activated by warning stimuli as well as by volitional events.

(Supported by PHS grant NS 12443.)

965 SYNCHRONY OF VIBRISSA MOVEMENT WITH THETA AND ALPHA RHYTHMS IN RATS: NEUROANATOMICAL SUBSTRATES. K. Semba and B. R. Komisaruk. Inst. Anim. Behav., Rutgers Univ., Newark, N.J. 07102

Normal rats show two types of rhythmical vibrissa movement, one often synchronized with theta waves of the limbic system (Komisaruk, JCPP, 1970, 70, 482), and the other synchronized with what are probably alpha waves of the thalamocortical system (Semba, Szechtman, & Komisaruk, Soc. Neurosci. Absts., Vol. III, 1977, No. 889). The theta-synchronized movements occur at a dominant frequency of about 7/sec, appear during exploratory sniffing behavior, and are of large amplitude. The alpha-synchronized movements occur at a dominant frequency of about 9/sec, appear while the rat stands still, and are of small amplitude (a fine tremor). Thus the facial nucleus, which is the final common motor pathway, apparently receives input convergently from these two systems. In the present study, we have also observed that: (1) The two types of movement rarely, if ever, appear together and the same is true of the two brain wave patterns. Topographically, the predominant appearance of the alpha waves is in the anterior half of the cerebral cortex, whereas that of the theta waves is in the posterior half. (2) Bilateral ablation of either the entire neocortex or just the sensorimotor area eliminated the vibrissal alpha-tremor movement while leaving vibrissal theta-exploratory movement normal. The tremor movements showed a slight recovery (short irregular bouts of very small amplitude) 7 weeks after surgery in a few rats. Unilateral neo-decortication eliminated normal contralateral tremor movement for 3-6 weeks in some rats, whereas in some rats, no detrimental effect of this operation on tremor was observed. (3) Sensory feedback from the tremor movement to the CNS does not seem to play a critical role in generation or maintenance of the tremor, for cutting the maxillary division of the trigeminal nerve did not abolish the tremor. The transection did not affect the exploratory vibrissa movement, either. (4) Depletion of monoamines with tetrabenazine (10 mg/kg, i.p.) induced the alpha-tremor and the associated synchronized EEG activity, but not theta-exploratory vibrissa movements or theta activity. We are currently investigating the effect on the alpha-tremor activity of septal lesions, which disrupt exploratory vibrissa movement and theta waves. Preliminary results with cerebellar ablation indicate that it selectively abolishes the alpha-tremor of the vibrissae, but not the theta-exploratory movement of the vibrissae.

966 PERIPHERAL, SPINAL AND SUPRASPINAL MECHANISMS OF SUSTAINED CLONUS. Arthur M. Sherwood* and Milan R. Dimitrijevic. Dept. Clin. Neurophysiol., Tex. Inst. for Rehab. and Res., Houston, TX. 77030.

Sustained ankle clonus can be described as a repetitive stretch reflex, elicited by activation of stretch receptors during the relaxation period of the cyclically stretched muscle(Hagbarth et al, J. Neurol., Neurosurg., Psych., 38: 636-641, 1975). The persistence of the clonus rate in spite of changing loads led Walsh to conclude that a central pacemaker played a key role in controlling the clonus rate(Walsh, J. Neurol., Neurosurg., Psych., 39: 266-274, 1976).

Testing which of the two mechanisms, peripheral or spinal, is the predominant factor in controlling the rate of clonus in spinal cord injury patients, we used EMG, position and force measurements of the effects of additional afferent volleys applied at various phases of the clonus cycle. The afferent volleys, whether from electrical stimuli(H-reflexes), phasic stretch(T-wave) or tonic stretch(tonic vibratory reflex), produced an output only if their arrival coincided with the stretch afferent volleys producing the next clonus cycle. We have found that the frequency of clonus is constant within $\pm$ 1 Hz, and that this frequency cannot be altered appreciably by modifying the peripheral or central experimental conditions. This constant frequency is not determined by peripheral factors, but rather by the centrally determined period of unresponsiveness to additional proprioceptive volleys between two successive contractions.

We have also found that the contribution of supraspinal facilitation of segmental reflex mechanisms is essential to sustained clonus. This facilitation maintains the state of segmental excitability, which in turn governs the incoming stretch receptor volleys. Examining 27 incomplete and 39 paralyzed spinal cord injury patients for the presence of clonus in the quadriceps and triceps surae muscles bilaterally, 14% of the incomplete but only 4% of the paralyzed patients showed sustained clonus, although both groups exhibited clonus(50% of incomplete and 57% of paralyzed).

967 DYNAMIC PROPERTIES OF CAT TENUISSIMUS MUSCLE. J. F. Soechting and Sofia Sanchez Robles* (SPON: C. K. Knox). Laboratory of Neurophysiology, University of Minnesota, Minneapolis, Minnesota 55455.

The dynamic characteristics of the tension generated by an isolated tenuissimus muscle-nerve preparation were evaluated under several experimental conditions with the aim of providing a general model description for this muscle incorporating some of the non-linear features of its behavior. Average muscle twitch was calculated by randomly stimulating the muscle nerve at different mean frequencies under isometric conditions. Mechanical stiffness of the muscle was determined by imposing sinusoidal changes in length while stimulating the muscle at a constant frequency. The interaction between mechanisms responsible for generating active tension and the mechanical stiffness was evaluated by simultaneously imposing random changes in muscle length and random electrical stimulation. In agreement with the 3-element model for muscle, the average twitch response under this condition was identical to that obtained under isometric conditions. The effect of immediate past history of muscle activation on the active tension was ascertained from 2nd order Wiener kernels characterizing the incremental active tension due to two stimuli separated by various times. A model for this muscle incorporating some of these non-linear features will be presented. (Supported by NIH Grant NS-02567.)

968 MUSCLE RECEPTOR RESPONSES TO CONTRACTIONS OF SMALL GROUPS OF CONCURRENTLY AND INDEPENDENTLY ACTIVE MOTOR UNITS. E. K. Stauffer, R. A. Auriemma* and G. P. Moore. Univ. Minn.-Duluth Sch. Med., Duluth, MN 55812 and Dept. Biomed. Engr., USC, Los Angeles, CA 90007.

Responses of single muscle receptor afferents to contractions of motor units in the cat tibialis posterior were studied in order to derive how the response of a receptor to any given motor unit was modified as additional independently stimulated motor units were activated.

Single motor units were functionally isolated from ventral rootlets at the L_6-L_7 level. Single spindle group Ia, group II and tendon organ afferents were identified and isolated from corresponding dorsal root filaments.

Responses of each afferent were recorded during periodic (1/sec) stimulation of every motor unit individually, and PST histograms were constructed to show the average behavior of the afferent spike train to the motor unit contraction. Average motor unit contraction profiles were also calculated using force measurements from the tendon. Following this, the same afferent was tested as two or more motor units were stimulated at mean rates of 5-10 pulses/second by concurrent and independent Poisson pulse trains obtained from Geiger counter-triggered stimulators. In every instance the form of the PST histogram describing the average behavior of the afferent response during the motor unit contraction changed qualitatively and quantitatively as additional motor units were activated, usually revealing a striking relationship between the time course of the afferent PST histograms and each averaged motor unit tension record.

A new two-dimensional cross-correlation technique was used in addition to determine whether responses to summed motor unit contractions could be predicted from the response to single contractions, or whether non-linear interactive effects occur between successive contractions of a single motor unit or between motor units known to effect a common receptor.
(Supported by funds from Univ. Minn. Grad. Sch., Minn. Med. Found. and NIH Grants GM 27732 and NS11298.)

969 CHANGES IN THE RECRUITMENT THRESHOLD OF MOTOR UNITS PRODUCED BY SKIN STIMULATION DURING SLOWLY INCREASING VOLUNTARY MUSCLE CONTRACTIONS IN MAN. J.A. Stephens* and R. Garnett* (SPON: D.G. Stuart). Sherrington Sch. of Physiol., St Thomas's Hosp. Med. Sch. London ENGLAND

The force of abduction of the index finger produced by contraction of first dorsal interosseous muscle (1DI was recorded using a strain gauge placed against the lateral side of the proximal interphalangeal joint. Motor unit action potentials were recorded using a mono polar concentric needle electrode inserted into 1DI.

Once a single motor unit action potential had been isolated, the subject was required to make repeated isometric contractions following a target on an oscilloscope screen. The task was so arranged that for each contraction the subject's force rose linearly with time until it reached approximately twice the level at which the motor unit first started to fire. The subject then relaxed. Each ramp contraction lasted approximately 10 sec and was repeated every 18 sec.

After at least 6 control contractions, the index finger was stimulated via ring electrodes placed on either side of the proximal interphalangeal joint (50 pps, pulse width 0.1 ms). Stimulus strength was set at 4x threshold for perception. Such stimulation is not painful but elicits a sensation similar to having the finger firmly gripped. With one exception, the recruitment threshold of units gradually changed during stimulation reaching a new level after some 2-4 min. Low threshold units (control recruitment threshold < 150 g or approximately 6% maximum;n=17) had their recruitment threshold raised by skin stimulation. Increases in threshold ranged from 21 to 188 g or 0.31 to 72 times control values. High threshold units (control threshold > 150 g; n=7) had their recruitment threshold lowered. Reductions in threshold ranged from 13 to 418 g or 0.28 to 0.95 times control values. For the majority of units recruitment threshold returned to control within 1-2 min after the end of stimulation.

We conclude from these results that cutaneous stimulation alters the recruitment order of motor units during normal voluntary muscle contractions in man. This finding coupled with previous observations in the decerebrate cat (Kanda et al, 1977) lends further support to the view that the recruitment order of motor units is not fixed but depends in part on the nature and distribution of input to the motoneurone pool.

Kanda, Burke & Walmsley. Exp Brain Res 29 57-74 1977

970 STABLE OSCILLATIONS OF A SKELETAL MOTOR CONTROL SYSTEM. Robert N. Stiles, Dept. of Physiology and Biophysics, Univ. Tenn. Cntr. Hlth. Sci., Memphis, TN 38163.

Postural tremors of the hand are suggestive of an instability of the neuromuscular system controlling that structure. However, these continuous oscillations may also reflect the transient responses of a stable system to continuous internal disturbances. According to R.W. Jones (Principles of Biological Regulation, Academic Press, NY, 1973, pp 240-244), the possible operating points of a physical system are of two kinds, either stable or unstable. If a system is unstable, any disturbance will cause the system to move away from a steady-state level and never return. Therefore, any kind of disturbance can be used to "test" the system for stability. In the present study, a pulse of force (an external disturbance) was repetitively (about one/sec) imposed upon the outstretched hand of a normal human subject. This was done over different 16-sec periods during a 40-60 min period that the subject continuously maintained the hand extended against gravity. During this 40-60 min period, postural hand tremor occurred having root-mean-square (rms) displacement amplitudes ranging from 30 to 4500 micra. Hand motion was detected with an AVR-240 accelerometer mounted 16 cm from the wrist, and the voltage analog of acceleration was digitized at 64/sec. Bipolar, surface EMG's from a wrist extensor muscle were digitized 1024/sec, rectified and smoothed, resulting in amplitude demodulated EMG records with an equivalent sampling rate of 64/sec. Auto- and cross-spectral analyses were performed on the simultaneously obtained 16-sec records of EMG's and postural hand oscillations. When applied between recordings of tremor of different rms displacement levels, the pulse disturbances consistently resulted in damped (die-away) oscillations of the outstretched hand. These results indicate that the system producing both large- and small-displacement amplitude hand tremors should be considered stable. High coherence values were obtained between the amplitude demodulated EMG and hand tremor for tremors with amplitudes above about 100 micra. Similar coherence values were obtained between the EMG's and damped oscillations of the hand. Therefore, the presence of amplitude modulation of wrist extensor EMG's which is highly coherent with the postural hand tremor is not sufficient for the maintenance of these stable oscillations. (Supported in part by USPHS Grant NS-08692.)

971 MULTIPLE REPRESENTATION IN THE PRIMATE MOTOR CORTEX: A NEW CONCEPT OF INPUT-OUTPUT ORGANIZATION FOR THE REPRESENTATION OF DISTAL FORELIMB. Peter L. Strick and James B. Preston. Depts. of Neurosurgery and Physiology, SUNY, Upstate Medical Center, and V.A. Hospital, Syracuse, New York 13210.

The motor output and somatosensory afferent input of area 4 were mapped in the squirrel monkey using microstimulation and unit recording. The squirrel monkey was chosen because none of its forelimb area of motor cortex is buried within a sulcus. Animals were initially anesthetized with pentobarbital sodium (25 mgm/Kgm). Ketamine HCl (5-10 mgm/Kgm) was given as needed to maintain anesthesia. Glass-coated, platinum-iridium microelectrodes (approx. 1 MΩ) and a closed chamber system were used for microstimulation and unit recording.

The results from microstimulation showed that there are two, spatially separate hand-wrist representations in area 4. Stimulation in area 4 just rostral to area 3a evoked movements of the hand (thumb and fingers). Rostral to this hand region was a zone from which wrist movements were evoked. Moving further rostrally in area 4, there was an abrupt transition to a second zone of hand representation and anterior to this a second zone of wrist representation. The hand and wrist movements evoked and their thresholds were similar in the two motor representations. We have termed the hand and wrist representation adjacent to area 3a the caudal motor representation, and the second, more anterior hand and wrist representation, the rostral motor representation.

The results of single unit recording in area 4 demonstrate that the caudal and rostral motor representations receive different patterns of somatosensory afferent input. Units driven by cutaneous stimulation were concentrated in the caudal representation while units driven by joint and/or muscle stimulation were concentrated in the rostral representation.

On the basis of these observations, we believe that the two motor representations reflect two motor systems in area 4 which are designed to control different aspects of distal forelimb motor behavior. (This study was supported in part by funds from the Veteran's Administration Research Fund and USPHS Grant N502957.)

972 NUCLEUS OF THE CRUS CEREBRI (NCC): A PREVIOUSLY UNDISCOVERED CELL GROUP FORMING A RETICULUM AROUND MOTOR SYSTEM FIBERS DERIVED FROM THE CEREBRAL CORTEX AND NEOSTRIATUM. Konrad Talbot (1) and Larry L. Butcher (1,2). Dept. of Psychology (1) and Brain Research Inst. (2), U.C.L.A., Los Angeles, California 90024.

The cellular group we term NCC, a neuronal cluster embedded in the rat and cat pes pedunculi between the caudomedial tip of the subthalamic nucleus (Sub N) and the rostrolateral tip of the substantia nigra, pars compacta (SN,pc), receives no mention in the neuroanatomical literature. It cannot, however, be grouped with neighboring structures; for example, the NCC is not continuous with the Sub N or the SN,pc, and its neurons are readily distinquished in Nissl preparations from nerve cells in those structures. In this context, we summarize our studies on the rat NCC.

Compared to most brain nuclei, the NCC is certainly small: its approximately 700-1000 neurons are located within A-P, M-L, and D-V dimensions of 0.545, 0.884, and 0.545mm, respectively. Nissl material and especially pharmaco-histochemical preparations for acetylcholinesterase (AChE) reveal that the neuronal somata (1) range in maximum diameter from 8-37um (85% are 15-26um), (2) exhibit a wide variety of shapes most of which are ovoid or triangular, and (3) possess 1-4, but usually 2-3, enzyme-reactive processes. Most NCC neurons demonstrate appreciable but not intense AChE activity (unlike Sub N and SN,pc), many display appreciable NADH-diaphorase activity (again unlike Sub N and SN,pc), and none fluoresce for catecholamines (unlike SN,pc) even after nialamide (100mg/kg) treatment.

Characteristic of the NCC is the aggregation of its somata and processes, especially at caudal levels, to form a reticulum through which course bundles of peduncular fibers. This is particularly interesting since silver degeneration studies (e.g., Rinvik, 1968, Exp. Br. Res., 5, 129) and our own autoradiographic investigations indicate that the NCC in both the rat and cat is so situated that many, if not all, those bundles consist of motor system fibers projecting from the motor cortex to midbrain and thalamic nuclei and from the rostro- and centro-lateral neostriatum to the SN. Although gating mechanisms come to mind, further work is clearly required to elucidate the possible functional significance of the close association between NCC neurons and motor system fibers.
(This study supported by USPHS grant NS 10928 to L.L.B.)

973 EVIDENCE FOR A MULTISYNAPTIC STRETCH REFLEX OF THE JAW ELEVATOR MUSCLES IN THE CAT. Anthony Taylor*, Michael J. O'Donovan and Kwabena Appenteng*. (SPON: Mary Wetzel). Dept. Physiol., St. Thomas's Hosp. Med. Sch., London SE1 7EH, England.

The monosynaptic connections of jaw elevator muscle spindles to elevator motoneurones are sparse and weak. Thus by spike-triggered averaging from spindles, individual monosynaptic EPSPs were found in only 15% of motoneurones and their mean amplitude was 18μV. In spite of the weakness of this projection, powerful stretch reflexes of the jaw elevators can be obtained under certain conditions. This may be explained by the existence of multisynaptic components, for which we have some evidence. First, there are interneurones close to the motor nucleus specifically excited by spindle input, and since there is no Ia antagonist inhibition of jaw muscles, these interneurones could be excitatory to the motoneurones. Secondly, ramp and hold jaw opening generates synaptic noise in elevator motoneurones which is seen to continue to increase into the hold phase, when spindle output is declining rapidly. This could be due to progressive build-up of activity in an interneurone pool. Thirdly, naturally evoked spindle afferent volleys can generate late excitation of motoneurones which is abolished by small doses of barbiturate. In addition it is noted that IC records from jaw elevator motoneurones commonly show ongoing individual EPSPs related to jaw stretch with amplitudes up to 10 times the average monosynaptic individual EPSP. These seem likely to be due to interneurones driven by spindles and suggest a potentially powerful pathway. A weak stretch reflex of the elevators may indeed by greatly enhanced by other intra-oral stimulation which could be explained by convergence on to excitatory interneurones.

974 FORELIMB FUNCTION AFTER DORSAL COLUMN NUCLEI LESION OR DORSAL RHIZOTOMY. D. E. Teodoru*, T. Tran* and A. J. Berman. Dept Neurosurg., VA Hosp., Bronx, N.Y. 10468

Four monkeys were subjected to unilateral lesion of dorsal column nuclei (DCN) and four to dorsal rhizotomy C2-T3 (DR). Postoperatively, spontaneous behavior was systematically observed and forelimb movements compared on two tasks. One task required limb movement forward into a cylinder. Latency from presentation of the container until hand entry was a measure of coarse reach; time from entry to grasp and withdrawal with food was a measure of coarse grasp. The second task required reaching and grasping a food pellet from one of three positions atop a narrow cylinder. This task yielded measures of fine reach and grasp as well as allowing detailed evaluations of the movements involved.

DCN lesion was followed by ataxia in the immediate postoperative period. At the time of testing, however, 6 mos after surgery, reach latency was unaffected but an impairment of grasp was evident. The form of coarse grasp was similar to that of the intact limb, consisting of a prehensile movement with all the fingers opened and closed together. Fine grasp was initially unsuccessful, apparently due to incoordination of thumb-index opposition. When grasp became successful, it consisted of scooping movements involving all fingers or independent scooping by thumb or index. When vision was occluded with the precision test, reach time of the lesioned limb continued to be comparable to that of the intact limb, but grasp was never successful. DR monkeys failed to contact the target with their operated limbs in the fine test. On the coarse task, however, one monkey was successful. In this case, reach time was considerably longer than for the contralateral intact limb. Grasp time was greater than for the intact limb, but, despite severe injury to two fingers on the denervated hand, was far less than for animals with DCN lesions.

The proximal to distal pattern of recovery observed after DCN lesion is in contrast to the distal to proximal recovery seen after DR in this and previous experiments. After DCN lesions there is chronic deficit in coordinated finger movements with only transient involvement of proximal limb musculature. After DR, fine finger movements recover before movements involving the shoulder. We suggest that DCN lesion interrupts afferent input to motor cortex modules mediating finger coordination. In addition, non-pyramidal inputs which can interfere with finger coordination are released from the pyramidal inhibition normally supported by afferent input ascending via DCN. Thus a distal deficit predominates. DR interrupts gamma loops through which non-pyramidal influences operate, resulting in a proximal deficit, with the pyramidal system free to recover its control over distal musculature. Supported by NIH Grant #2 RO1 NS12330 to A.J.B.

975 EFFECTS OF FOCAL INJURY IN AREA 6 ON THE ACTIVATION OF NEURONS IN THE AREA 4 MOTOR CORTEX. Floyd J. Thompson and Joseph J. Warner* Dept. of Neuroscience, College of Medicine and College of Veterinary Medicine, University of Florida, Gainesville, Fla.

The activation patterns of motor cortex neurons were investigated to determine the nature of physiological changes which occur subsequent to focal injury in area 6. The fundamental question investigated in these experiments was: Do injury related changes in sensory transmission to the motor cortex involve mechanisms intrinsic to the cortex or to subcortical systems which contribute to cortical neuron excitability?

The motor cortices of cats were exposed and covered with a closed chamber. Bipolar concentric electrodes were inserted into the contralateral dentate nucleus, in the ipsilateral medullary pyramidal tract, and into the ipsilateral entopeduncular nucleus. A bipolar concentric electrode was also placed in the posterior intermediate sulcus in the upper cervical spinal cord to activate the contralateral gracile and cuneate tracts. These electrode placements provided activation of cortical neurons by four functionally different but powerful sources. Cortical neuron activation patterns were studied by analysis of laminar and single unit evoked activity. Focal injuries were made in area 6 using a silver ball electrode to deliver radiofrequency to the cortical surface. Histological examination of the studied neural tissue revealed exact locations of cortical lesions and electrode tracts.

These studies have shown that evoked activity of motor cortex neurons can be enhanced, diminished, or abolished for several hours in a spatially widespread manner, following a focal lesion in area 6. However, within the same animal, these changes in the evoked activity were different depending on the input tested. For example, in one animal, the evoked activity in the lateral postcruciate gyrus elicited by dentate stimulation was reduced by 50-60% for 3 hours following the production of the lesion. However, in the same region, the evoked activity elicited by entopeduncular nucleus stimulation was increased by 20-50% for five hours following the injury. Changes of this nature have been observed to persist for up to]2 hours, then return to control values. In a different experiment, single unit activity was evoked by stimulation of the gracile and cuneate tracts following focal area 6 injury. In the same recording region, however, dentate stimulation evoked no activity during that time.

The experiments to date strongly suggest that even focal injury to the cortex may have profound effects on subcortical systems which contribute to the excitability of the motor cortex. (Supported by The American Heart Association, Suncoast Chapter, 7/77 AG 613)

976 EFFERENT PROJECTIONS OF THE DEEP MESENCEPHALIC NUCLEUS OF THE RAT. Randolph B. Veazey*, (SPON: C.M. Severin). Department of Anatomy, University of Texas Medical Branch, Galveston, Texas 77550.

Efferent projections from the deep mesencephalic nucleus were examined using autoradiographic and horseradish peroxidase techniques. In one series of rats unilateral injections of tritiated leucine were made and confined to either the medial or lateral part of the deep mesencephalic nucleus (DMN). Following injections of tritiated leucine into the lateral part of the DMN two groups of fibers were observed. One group coursed ventrally above the substantia nigra to enter the ipsilateral zona incerta. Some of these fibers entered the subthalamic nucleus where mesencephalic terminations were observed in its ventromedial part. Other fibers within the zona incerta coursed anteriorly to enter and pass through the internal capsule into the entopeduncular nucleus where mesencephalic terminations were observed. The remaining fibers passing through the internal capsule entered and terminated in the medial portion of the globus pallidus. The second group of fibers arising from the lateral part of the DMN coursed dorsally and rostrally to enter and terminate in the dorsomedial nucleus of the thalamus. Following injections of tritiated leucine into the medial part of the DMN two groups of fibers were observed. One group coursed ventrally and posteriorly to enter the ventral tegmentum of the pons. Some of these fibers terminated in the caudal part of the pontine reticular nucleus. Other fibers in the ventral tegmentum of the pons passed posteriorly through the ventral portion of the medullary reticular formation to enter the spinal cord. Some of these fibers terminated in the gigantocellular nucleus of the medullary reticular formation. The second group of fibers arising from the medial part of the DMN coursed dorsally and posteriorly through the dorsal portion of the pons and medulla to enter the spinal cord. To verify the findings of the autoradiographic study, another series of rats received injections of 33% horseradish peroxidase confined to the terminal areas demonstrated by autoradiography. The DMN was then examined for the presence of HRP-positive labelled cells. Based on the autoradiographic and HRP studies, the lateral part of the deep mesencephalic nucleus projects to the ventromedial portion of the subthalamic nucleus, the entopeduncular nucleus, medial portion of the globus pallidus, and dorsomedial nucleus, whereas the medial part of the deep mesencephalic nucleus projects to the caudal part of the pontine reticular nucleus, the gigantocellular nucleus of the medullary reticular formation, and the spinal cord.

977 CORRECTION OF ONGOING MOTOR OUTPUT IN THE CAT. D. Vicario, T. Blunk* and C. Ghez. Rockefeller Univ., New York, NY 10021.

We have previously shown that rapid isometric responses made by cats tracking sudden target perturbations, using a compensatory display of their force error, are controlled by a pulsatile neural signal of constant duration and variable amplitude (Ghez and Vicario, Soc. Neurosci. Abstr., 3: 271, 1977). The peak force achieved under these conditions is a linear function of the peaks of its first and second derivatives. Thus, the responses were scaled from their onset and the peak of the force derivative (dF/dt) was predictive of the intended final force. In the present study, we have investigated the cat's ability to make corrections in the force it applies following a second target perturbation presented at varying interstimulus intervals (ISIs) after a first.

As previously described, cats were trained to apply force isometrically to a strain gauge with their forearms to match a target force level which was stepped at random times. On random trials a second target step followed the first at controlled intervals. The motor effects of changes in the neural output could be detected rapidly under isometric conditions, where mechanical delays and reflex effects are minimized. Latencies in this task are very short; mean daily latencies measured from the perturbation to the onset of dF/dt were 60-80 msec. The peak dF/dt is reached after another 30-55 msec.

When the second perturbation preceded the peak dF/dt (of the response to the first) by more than a reaction time, and required a reduction in force applied, the peak dF/dt decreased progressively with shorter ISIs. Peak forces were proportionally reduced. The cat could abort its response entirely with the shortest ISIs (10-20 msec), though in such cases EMG activation of remote synergistic muscles was still occasionally present. Accompanying these reductions in amplitude were reductions in the time from onset to peak dF/dt, indicating that the output pulse had been shortened in its duration. These data, obtained under isometric conditions, are not consistent with intermittent sampling models which imply central refractoriness. The timing of the observed corrections in ongoing motor output suggests that responses may be updated by newly acquired sensory information with no detectable increase in reaction time.

Supported by Grant NS 12730.

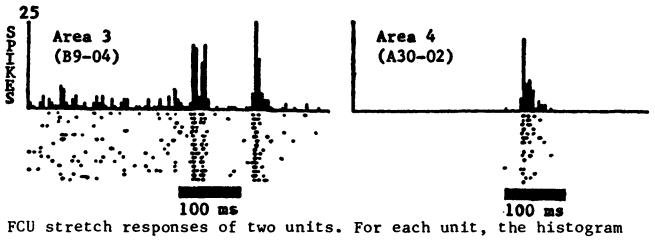
978 UNIT RESPONSES TO ELECTROMAGNETIC MUSCLE STRETCH IN PRIMATE SENSORIMOTOR CORTEX DURING SKILLED ACTIVITY. Jonathan R. Wolpaw. Laboratory of Neurophysiology, NIMH, Bethesda, Md. 20014.

Motor cortex units respond to limb displacements delivered during limb movements or maintained postures. Such displacements activate skin, joint, and muscle receptors. The present study assessed response to relatively pure muscle receptor activation.

Monkeys working for liquid reward grasped a torque motor handle and held it in mid-position, wrist neither flexed nor extended, against a range of steady-state torques. Stimuli given at pseudo-random intervals were of two kinds: (1) 50 ms DC torque pulses(TP), flexor or extensor, superimposed on the steady state torque and producing a hand displacement;(2) 100 ms 70 gm DC stretch of the muscle flexor carpi ulnaris(FCU), achieved by applying force via an external electromagnet to a 2 gm coated iron slug chronically imbedded in the distal musculotendinous junction (Wolpaw and Colburn, Br Res 141 (1978) 193-196). The latter stimulus was presumed to produce relatively selective activation of muscle stretch receptors for it caused no detectable change in handle position and monkeys appeared to ignore it.

In two monkeys (Macaca mulatta) 307 units in areas 4, 3(a & b), and 1 responded to flexor and/or extensor TP, the primary search stimuli. Of 259 units which responded within 60 ms of TP onset, 45% of those in area 4 and 63% of those in areas 1 and 3 also responded to FCU stretch. In all three areas FCU stretch responses were predominantly phasic ("on" or "on-off"), and units with short response latencies (10-20 ms) occurred.

Studies in anesthetized primates show that, while unit responses to muscle afferent stimulation are strongest and earliest in area 3a, responses also occur in area 4. The present study indicates that in awake behaving primates there is widespread short latency sensitivity to muscle stretch in area 4, as well as in areas 3 and 1, even when the ongoing performance is not disturbed.



FCU stretch responses of two units. For each unit, the histogram gives the sum of the 16 individual trials shown in the raster. The bar represents 100 ms FCU stretch.

979 COMPARISON OF LOAD COMPENSATION BY NORMAL AND DEAFFERENTED MONKEYS. Richard M. Wylie and C.F. Tyner. Dept. Med. Neurosci. Walter Reed Army Inst. Res. Washington, D.C. 20012.

Three normal and two operated (unilateral forelimb dorsal rhizotomy) monkeys were trained to perform a weight compensation task. The task required flexion of the forearm (held in a restraint which limited gross movement to rotation at the elbow in the vertical plane) from a horizontal rest position through a criterion arc of about 40 degrees and return to the rest position. No external cue signaled crossing the criterion level: reinforcements were delivered only after return of the arm to the rest position. After initial training with no external load, external loads were suspended from the arm restraint. Flexion through the criterion arc lifted the load against gravity about 16.5 cm. When the load was held constant over many one and one half hour sessions, the average height reached was independent of load. The loads were then varied between short blocks of responses within a daily session. On the first lift of each trial, both normal and deafferented monkeys generated load dependent lifts: acceleration and velocity measured during the rising phase of the lift and peak height achieved were all inversely related to the load. After the first few responses, the normal animals generated lifts in which acceleration, velocity and peak height were all independent of load. Although on successive lifts within a trial, the deafferented animals reduced the effect of load on position, the effect remained sufficient to appear in trial averages.

Comparison of the waveforms of acceleration generated by the two groups of animals reveals a striking difference between groups. On all lifts, including the first of each trial, the normal animals generated acceleration curves which rose monotonically to a maximum early in a lift and then fell smoothly to the maximum deceleration near the top of the lift. In contrast, deafferented animals generated acceleration curves with multiple relative maxima which indicate discrete increments of force during the rising phase of the lift. The increments of force are assumed to be corrections the animal makes in the trajectory of his arm. The corrections appear at latencies of more than 100 msec. from the start of movement and at heavier loads are seen on many lifts through the trial. The corrections are not a generalized ataxia because they were not seen when the animal was run on the same load in repeated sessions. The latencies of the corrections indicate that deafferented monkeys lack servo loops with rapid feedback. The contrast in the smoothness of regulation between the two groups suggests that servo loops with short loop delays contribute, in the normal monkeys, to the performance of this task.

980 THE CELLS OF ORIGIN OF INTERHEMISPHERIC CONNECTIONS IN THE PRIMATE MOTOR CORTEX. Julius D. Zant* and Peter L. Strick. (SPON: E.G. Keating). Depts. Neurosurg. and Physiol., S.U.N.Y., Upstate Med. Center and Veterans Administration Hospital, Syracuse, N.Y. 13210.

Multiple injections of 30-50% HRP (Sigma, type VI or Boehringer-Mannheim I, in saline) were made unilaterally into the motor cortex of 3 rhesus and 2 cynamolgus monkeys. In some animals the primary somatic sensory area was also injected for comparison. After a 1-3 day survival, the animals were sacrificed, perfused with an aldehyde-phosphate fixative and their brains processed for retrograde HRP transport using benzidene as a substrate (Mesulam, J. Histochem. Cytochem. 24: 1273, 1976).

There was a clear relation between the intensity of HRP retrograde labeling and the size of labeled neurons in the motor cortex on the side opposite the HRP injections. This was particularly evident for neurons in layer III, where large callosal neurons were more heavily labeled than small ones.

There are a number of marked differences in the laminar distribution and topographical organization of callosal neurons of the motor and somatic sensory cortices. First, callosal neurons are found in all layers of the motor cortex, except layer I. These neurons are most concentrated, however, in layers III, V and superficial layer VI, forming a distinct bilaminar pattern. In contrast, most callosal neurons in the somatic sensory cortex are found in layer III and only occasional callosal neurons are seen in layer V. Second, the mediolateral strips of labeled neurons which are a prominent feature of the somatic sensory cortex (jones, et. al. Science 190: 572, 1975) sre not as distinct in motor cortex. Third, many more callosal neurons are found in the arm and leg areas in the motor cortex than in the arm and leg areas of the somatic sensory cortex.

Finally, while the representation of the arm in area 4 lacks distinct strips of callosal neurons it does contain two "holes" in which callosal neurons are not found. On "hole" is buried in the central sulcus and the other is located on the cortical surface near the central sulcus. Current concepts indicate that callosal "holes" are found in regions of sensory cortex containing the representations of distal musculature. Recently, Strick and Preston (Brain Res. in the press) have demonstrated that there is a double representation of the hand in area 4 of the squirrel monkey. Therefore, the callosal "holes" in the arm area of area 4 suggest that a double representation of the hand also may exist in the motor cortex of the rhesus monkey.

(Supported in part by funds from the Veterans Administration Medical Research Service and Department of Neurosurgery, Upstate Medical Center).

981 DO INSECT MUSCLE CELLS SERVE AS SENSORY-MOTOR INTEGRATORS? Sasha N. Zill* and David T. Moran* (SPON. W.E. Hahn). Dept. Anat., Sch. Med., Univ. of Colorado, Denver, Colo. 80262

Insect locomotor patterns are widely assumed to be generated by the central nervous system and modified by peripheral feedback. Our studies of cockroach leg reflexes suggest that one level of integration of the effects of sensory feedback with centrally generated patterns occurs at muscle cells that, in insects, are multiply innervated and non-spiking.

The posterior trochanteral flexor (coxal levator) muscle lifts the leg in walking. It is innervated by both inhibitory (axon #3) and slow excitatory (axons #4,5,6) motoneurons. Pearson and Bergman (J. Exp. Biol. 50:445, 1969) studied this innervation and found that the inhibitory axon, a branch of the widespread common inhibitory neuron, was most effective in reducing tension produced by axon #4 - the smallest excitatory axon - but had little effect on tensions produced by the larger excitatory axons (#5,6).

Pearson and Fourtner (J. Neurophysiol. 38:33, 1975) have characterized an interneuron that depolarizes during rhythmic leg movements and is believed part of a central flexor burst generator in locomotion. It excites a group of flexor motoneurons - specifically axons #3,5,6 but not axon #4.

We have studied the effects of stimulation of a variety of leg sense organs upon trochanteral flexor motoneurons. Campaniform sensilla are sense organs that monitor cuticular strain caused by external load and muscular contraction. Chordotonal organs monitor joint angles. Stimulation of distal tibial campaniform sensilla and coxal chordotonal organs excites the trochanteral flexor. In both cases activity in axon #4 is increased with little effect on the other flexor motoneurons (#3, 5,6).

These results suggest that trochanteral flexor motoneurons may be divided into two groups, those driven predominantly by central interneurons (axons #3,5,6) and those driven by proprioceptive feedback (axon #4). Since the inhibitory axon (#3) most effectively eliminates tension produced by axon #4, the interaction between these axons on the muscle cell membrane would effectively integrate centrally generated patterns and proprioceptive feedback. We are presently testing this hypothesis by simultaneously recording sensory and motor activity in walking animals. (Supported by NSF Grant BMS-73-06766 and NIH Anatomy Training Grant GM-01981.)

NEUROCHEMISTRY

982 BIOCHEMICAL CHARACTERIZATION AND IN VITRO MAINTENANCE OF NEURONS FROM ADULT RAT BRAIN ISOLATED BY THE PERFUSION METHOD. H.H. Althaus*, W.B. Huttner*, P.J. Gebicke*, V. Neuhoff* and J.D. Lane. Max-Planck-Institut für exp. Medizin, Forschungsstelle Neurochemie, 3400 Göttingen, FRG

Recently a method has been developed to isolate neurons from adult rats which retain a reasonable part of their processes (Althaus, et al., Hoppe-Seyler's Z. Physiol. Chem. 358, 1155, 1977). These neurons have a preserved plasma membrane to which synaptic complexes are attached (Huttner, et al. in preparation; abstract, this meeting). Scanning and transmission electron microscopy of the preparations will be presented. In these isolated neurons, the composition of lipids, amino acids and several enzyme activities have been evaluated, as well as the uptake and incorporation of (^{3}H)-uridine and (^{14}C)-leucine into nuclear and protein fractions. The protein composition of cytoplasmic and particulate fractions of the neurons at different ages have been studied by SDS microgel-electrophoresis and pathological conditions such as experimental hyperphenylalaninemia result in changes in membrane-bound proteins. Moreover these neurons can be maintained in culture and are capable of regenerating their fibers. (supported in part by SFB 33).

983 EFFECTS OF CHRONIC ANTIPSYCHOTIC DRUG TREATMENT ON DOPAMINE METABOLITES IN PRIMATE BRAIN. N. G. Bacopoulos*, D. E. Redmond, J. Baulu* and R. H. Roth. Departments of Pharmacology and Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06510

Haloperidol, 0.5 mg/kg, i.m., (chronic maintenance dose) was given daily for 19 days to adult male and female feral vervet monkeys (Cercopithecus aethiops) trapped and maintained in group outdoor cages on the island of St. Kitts, West Indies. On the 20th day chronically treated and untreated (control) animals were injected with 1.0 mg/kg haloperidol i.m. (acute challenge dose) or saline. Four hours later brains were removed under nembutal anesthesia and dissected into regions that were frozen in liquid nitrogen and kept in a -80° C freezer. Dopamine metabolites (3,4-dihydroxyphenylacetic acid-DOPAC- and homovanillic acid-HVA) were measured by mass fragmentography. The acute challenge dose of haloperidol increased DOPAC and HVA in basal ganglia of previously untreated animals but had lesser effects in chronically pretreated animals. Conversely, chronic pretreatment with haloperidol enhanced the HVA-elevating effects of the acute challenge dose in dorsal frontal, orbital and cingulate cortex. In the temporal, parietal and occipital cortex, the acute haloperidol challenge dose had the same effects on HVA concentration in chronically pretreated and previously untreated control animals. HVA was quantitatively the principal metabolite of dopamine in cortical regions, with DOPAC constituting less than 5% of total metabolite. No significant amount of the conjugated forms of these metabolites were found in any of the brain regions analyzed.

These observations demonstrate that following chronic haloperidol treatment the responsiveness of dopamine neurons in the basal ganglia to the HVA-elevating effects of haloperidol is diminished, whereas the responsiveness of frontal cortical and cingulate neurons is enhanced. These regional differences in the response of central dopamine neurons to chronic haloperidol treatment may be relevant to the clinical action of this drug. Clinical studies have indicated that tolerance develops to the sedative and extrapyramidal effects of haloperidol while the therapeutic antipsychotic effects of this drug require one or more weeks of continued treatment to be manifested. (Supported in part by USPHS grant MH-14092, the Guggenheim Foundation and postdoctoral fellowship USPHS 1-F32-MH07146-02 to NGB.)

984 EFFECTS OF SPINAL CORD TRAUMA ON MYELIN (NEUROSCIENCE ABST.). Naren L. Banik*, James M. Powers* and Edward L. Hogan. Depts. Neurology, Biochemistry and Neuropathology, MUSC, Charleston, SC 29403

The essential role of descending fiber tracts in the mediation of motor paresis following spinal cord trauma warrants study of the biochemical events occurring in these myelinated axons. In this study the effects of trauma on the myelin sheath have been examined after experimental spinal cord trauma was produced in rats by dropping a 10 g weight 30 cm upon surgically exposed and dura-invested spinal cord. Specimens of normal and traumatized spinal cord were removed at 1, 2, 3, 4, 8, 12, 24 and 72 hrs. after injury and myelin was prepared. Hemorrhages, necrosis and edematous swelling in the lesion area were evident on light microscopy, while electron microscopic study revealed that dissociation of the myelin lamellae with the formation of vesicular structures and damaged axons were most prominent between 4 and 12 hrs. Granular degeneration of axons preceded vesicular damage to myelin which occurred first in the innermost myelin lamellae. After 12 hrs., aggregates of vesicular myelin debris were scattered throughout the lesion and by 72 hrs. the vesicular distortion of myelin was diminished but still present. The predominant morphologic lesions at the latter stage were axonal degeneration including mineralization and clusters of lipid-laden macrophages. The yield of myelin was reduced by 15 and 30% at 4 and 8 hrs. respectively following trauma. At 72 hrs. the yield was decreased to 50% of the normal value. No significant change in the level of adenosine 2', 3' cyclic phosphohydrolase (CNP) activity was found in myelin at 4 hrs. after trauma. At 8 and 72 hrs. the CNP activity was decreased by 15 and 20% respectively compared to control. Myelin proteins were separated by SDS-PAGE; striking reductions in all myelin proteins were observed in samples from the lesion. The main loss was in myelin basic protein with reductions of about 65% large basic and 45% small basic proteins while the losses were 15% for the proteolipid and 10% for the Wolfgram proteins from myelin (at 4 hrs.) compared to control. Greater decreases of these proteins were found at 24 and 72 hrs. following trauma. For example, at 24 hrs. there was a loss of 85% large B.P., 70% small B.P., 30% PLP and 35% WP. The relevance of these results to the pathophysiology and ultimate functional disability of spinal cord trauma will be discussed.

This study was supported by NIH-NINCDS Grant No. NS11066.

985 EFFECT OF WALLERIAN DEGENERATION ON MYELIN PROTEIN SYNTHESIS IN SCIATIC NERVE OF RAT. Margaret E. Bell and Richard C. Wiggins. Dept. Neurobiol. and Anat., UTMS, Houston, Texas 77025.

We used a double isotope procedure to compare the in vitro incorporation of glycine into myelin protein synthesis in control sciatic nerves and in nerves undergoing Wallerian degeneration. Wallerian degeneration was induced in rats by surgically performing a unilateral left neurotomy at the level of the sciatic notch. Each rat whose left sciatic nerve was cut also received a sham operation on the right side. At 1,3, and 5 days after surgery, we removed the distal stumps of the sectioned nerves and the contralateral sham operated nerves. In addition to the contralateral control, for each experimental rat there was a control rat (no surgery) whose intact sciatic nerves were removed at the same time as the experimental nerves. All nerves were transferred into screw-top test tubes containing 0.3 ml Krebs-Ringer-bicarbonate buffer, pH 7.4, 3 mg/ml glucose, radioactive glycine, and an atmosphere of 95% O_2 and 5% CO_2. Nerve sections undergoing Wallerian degeneration and the contralateral control nerves were placed in buffer containing 5 μCi of 3H glycine. Sciatic nerves of control rats were placed in buffer containing 10 μCi of 14C glycine. At the end of a 3 hr incubation period at 37°C with continuous shaking, the tissues were rinsed 3 X in cold buffer. Each 3H-labelled nerve was combined with a 14C-labelled nerve from a control rat, and the two were homogenized together in 0.29M sucrose. Purified myelin was prepared according to Wiggins et al. (Brain Res. 89:99, 1975). The myelin pellets were delipidated 3 times with ether-ethanol (3:2, v/v), solubilized in Protosol, and prepared for liquid scintillation counting. The sciatic nerve 3H/14C ratios (degenerating/control and sham operated/control) at 1,3, and 5 days are shown in Table 1 (sample size in parentheses):

Table 1	1 day	3 day	5 day
3H/14C = degenerating / control	1.15±0.18(9)	1.35±0.12(8)	0.75±0.10(5)
3H/14C = sham operated / control	1.15±0.19(7)	1.06±0.09(6)	1.32±0.47(3)* 1.12±0.16(2)*

As can be seen from the data, no significant increase or decrease in myelin synthesis appears at 1 day following initiation of degeneration. By 3 days post transection, however, myelin synthesis appears to be increased by about 30% in response to injury. At 5 days after surgery, this transient stimulation of myelin synthesis in the cut nerves has attenuated as a result of nerve degeneration, and myelin synthesis has decreased by 45%. (The high value at 5 days for the sham control results from a single sample; by omitting this number one gets a mean of 1.12.)*

986 EXTRACELLULAR PROTEINS OF THE VERTEBRATE BRAIN. Larry I. Benowitz and Victor E. Shashoua, Department of Biological Chemistry, Harvard Medical School. McLean Hospital, Belmont, MA 02178.

Several types of nervous system cells are known to depend upon extrinsic proteins for their development and function (e.g., Levi-Montalcini and Angeletti, Physiol. Rev. 48:534, 1968; Lim *et al.*, Science 195:195, 1977). Assuming that analogous factors play a role in the functional development of many other CNS elements, we have begun to survey proteins of the brain's extracellular and cerebrospinal fluid (ECF). In the fish, chick, and mouse, the ECF, obtained from perfused brains either by sucrose extraction or by directly drawing off the cerebrospinal fluid, was found to contain proteins markedly different from those appearing in serum or in the cytoplasmic fraction of whole brain. Polyacrylamide-SDS gels of the various protein fractions were compared in terms of staining patterns (Coomassie blue and PAS glycoprotein stains) and isotope incorporation profiles. Our primary evidence for the selective secretion of proteins was obtained by a double-labeling method. ECF proteins labeled with ^{3}H-valine from one animal were combined with ^{14}C-labeled cytoplasmic or serum proteins from another animal. The mixture was separated electrophoretically on gels, which were then cut and counted for ^{3}H and ^{14}C. High ^{3}H:^{14}C ratios, indicative of a relative enrichment in the ECF, were found predominantly for low molecular weight proteins. In the goldfish, the ECF was found to be particularly enriched in β and γ (see Abstract: Shashoua, this volume), proteins whose metabolism is altered by training (Shashoua, Science 193:1264, 1976). The β protein derives from a family of astrocyte-like cells of the brain's matrix zone (Benowitz and Shashoua, Brain Res. 136:227, 1977). In the chick, nearly 1/3 of all the precursor incorporated into ECF proteins after 1 hr of labeling appears in a 17,000 dalton molecular weight product. Tremendous animal-to-animal variations were noted in the labeling only of the 17,000 dalton secreted protein. The mouse brain was also found to have proteins which are rapidly labeled and secreted into ECF. We are now attempting to localize the origin of various secreted brain proteins and to study their relationship to brain function. (Supported by grants from The McKnight Foundation, The Benevolent Foundation of Scottish Rite Freemasonry, Northern Jurisdiction, U.S.A. and The Medical Foundation of Boston.)

987 IN VIVO PHOSPHORYLATION OF POSTSYNAPTIC DENSITY PROTEINS. Robert F. Berman*, William J. Kinnier*, John P. Hullihan* and John E. Wilson. Department of Biochemistry, University of North Carolina, Chapel Hill, North Carolina 27514.

Protein phosphorylation, a possible mechanism modulating synaptic transmission, was examined in postsynaptic density-enriched fractions isolated from rat brain. In vivo phosphorylation was carried out by injecting rats intraventricularly with 1 mCi of ^{32}P-orthophosphate in 20 µl saline. After a 30 minute isotope incorporation period, the rats were decapitated, and postsynaptic densities (PSD) were isolated from pooled cerebral corticies as described by Cotman, Banker, Churchill & Taylor (J. Cell Biol., 1974, 63, 441-455). In vitro phosphorylation was attempted by incubating unlabeled PSD's, isolated from non-injected rats, with 5 µM [$\gamma^{32}P$]ATP as described previously (Hullihan, Wilson & Williams, Biochim. Biophys. Acta, 1977, 499, 139-149). In vivo and in vitro treated PSD's were then fractionated by SDS-polyacrylamide slab gel electrophoresis, stained with Coomassie blue, and scanned for absorbance at 600 nm. The Coomassie blue protein banding pattern was typical for PSD's, and identical for in vivo and in vitro treated PSD's. The principal polypeptide component occurred in a single band at an apparent molecular weight of 51,000, and did not co-migrate with purified tubulin or actin. The gels were dried and placed in contact with X-ray film. The resultant autoradiograph showed a major peak of radioactivity associated with the 51,000 M.W. component for the in vivo labeled PSD fraction. Additional minor peaks of radioactivity were also observed. In contrast, no ^{32}P incorporation into PSD proteins was observed following the in vitro ^{32}P-labeling procedure. These results indicate that proteins associated with the postsynaptic density, although difficult to phosphorylate under in vitro conditions, readily incorporate phosphate in vivo and may represent a major class of phosphoproteins. They also open the possibility that the phosphorylation state of these proteins may play a role in their suggested function as modulators of synaptic transmission (Blomberg, Cohen & Siekevitz, J. Cell Biol., 1977, 74, 204-225). (Supported by NIH Grant NS-07457).

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989 CYCLIC GMP LEVELS IN THE CEREBROSPINAL FLUID AND CEREBELLUM OF THE MONKEY: EFFECTS OF MORPHINE. G. N. Catravas, S. J. Wright, Jr.* and J. B. Katz*. Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

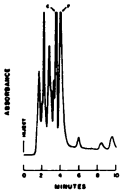
Silastic Pudenz catheters were chronically implanted in the fourth ventricle of monkeys and were connected to compressible polyethylene Ommaya reservoirs placed subcutaneously over the occiput for aspiration of cerebrospinal fluid (CSF). Administration of morphine (20 mg/kg IM) to the awake animal significantly elevated the levels of cGMP in the CSF. Following hemicraniectomy biopsies of cerebral and cerebellar cortex were taken from monkeys under anesthesia, given 20 mg/kg (IM) morphine sulfate. Only cerebellar cGMP levels were found to change significantly showing a more than 30% decrease compared to anesthetized controls. Naloxone (0.3 mg/kg IM) blocked the changes observed in both CSF and cerebellar cGMP levels. Although the controlling factors of brain and CSF cGMP levels are not well understood, our results indicate that, under some conditions, a reciprocal relationship may exist between cGMP levels in certain brain regions and in CSF.

990 KINETIC AND MECHANISTIC STUDIES OF CHOLINE ACETYLTRANSFERASE FROM BOVINE BRAIN. L. P. Chao* and L. L. Hsu* (SPON: D. Leelavathi). TRIMS, Houston, Texas 77030; Dept. Neurol. Sch. Med., UCLA, Los Angeles, Ca. 90024

Choline acetylthransferase (acetyl-coenzyme A: choline O-acetyltransferase, EC 2.3.1.6., CAT) from candate nuclei of bovine brains was purified to homogeneity according to the procedure previously reported by Chao and Wolfgram (J. Neurochem. 20, 1075-1081, 1973). The CAT activity was determined by measuring the radioactive acetylcholine produced from acetyl-coA and choline (Brandon and Wu, personal communication; Fonnum, J. Jeurochem 24 407-409, 1975). Kinetic studies of the purified brain CAT at varying acetyl-CoA concentrations and fixed excess choline concentration or vice versa resulted in linear double reciprocal plots. The Km values obtained from Lineweaver-Burk plots are 30uM and 1mM for acetyl-CoA and choline chloride respectively. Our kinetic data also indicate that product inhibition by acetylcholine is competitive with respect to choline and noncompetitive with respect to acetyl-CoA while product inhibition by CoA is competitive with acetyl-CoA and noncompetively with choline. Initial studies of effects of neurotransmitters,neuroleptics and psychotomimetics on the CAT activity showed that r-aminobutyric acid, dopamine, bufotenine, ipronia zid and 1, 2, 3, 4 - tetrahydro-B-carboline slightly inhibited the CAT activity at 0.1mM concentrations whereas norepinephrine, serotonin, cAMP, haloperidol, chozapine and mescaline did not affect the CAT activity to a significant degree. The possible kinetic mechanism(s) of CAT and the drug reaction mechanism(s) will be discussed.

991 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC): NEW INSIGHT INTO CYCLIC AMP METABOLISM. Major L. Cohn, Maria I. Chitas* and Joseph A. Feldman*, Dept. Anes., Magee-Womens Hosp., Univ. Pgh. Sch. Med. & Univ. Duquesne, Pittsburgh, PA 15213.

The metabolic pathways regulating the degradation processes of adenosine 3':5' monophosphate (cAMP) are well established. Cyclic nucleotide phosphodiesterase (PDE) converts cAMP to adenosine 5' monophosphate (5'AMP). Subsequently 5'AMP is rephosphorylated to ATP or further metabolized to adenosine and then adenine. By an alternate pathway, 5'AMP may be deaminated to inosine monophosphate (5'IMP) and converted to inosine by the catalyst 5'-nucleotidase. The removal of ribose converts inosine to hypoxanthine. We are now reporting a new HPLC technique by which we separate, identify and quantify cAMP and its metabolites in rat brain tissue. The retention times for 5'AMP, cAMP, adenine and adenosine were recorded at 2.87; 6.40; 8.46 and 9.60 min. respectively. When beef heart PDE was added to standard cAMP solutions in tris buffer pH 7.4 (0.06 M) three absorbance peaks appeared, the first at 2.87 min. which corresponds to the retention time of 5'AMP; the second and third (labeled C and F) at 3.37 and 3.84 min respectively. Thus, C and F retention times do not correspond to those of adenine and adenosine whose peaks did not appear. When rat brain slices were incubated at 30°C in tris buffer, pH 7.4 (0.06 M) with cAMP, C and F as well as 5'AMP and adenosine appeared at their usual respective retention times. With prolonged incubation cAMP and 5'AMP peaks disappeared while C and F peaks significantly rose. Next, we tested whether C and F peaks appear only under anaerobic conditions by sacrificing groups of rats in a microwave oven set at 5 sec. time exposure. The removed brains were homogenized in perchloric acid (0.4 N), centrifuged and the supernatant neutralized with 3M tris. The ultra-filtered samples were chromatographed. The major peaks of 5'AMP, cAMP, adenine, adenosine, C and F appeared at usual retention times (Fig). Interestingly, C and F peaks have the same retention times as inosine and hypoxanthine. Mass spectral studies are in progress to confirm the identification of all peaks. (NIDA 00605)



992 GANGLIOSIDES ASSOCIATED WITH ISOLATED NEURITES AND SOMATA OF SENSORY AND SYMPATHETIC NEURONS IN VITRO. Carson Cornbrooks, Richard P. Bunge and David Gottlieb. Dept. Anat. and Neurobiol., Wash. U. Sch. of Med., St. Louis, MO 63110.

The superior cervical ganglion (SCG) and the dorsal root ganglion (DRG) of the embryonic or neonatal rat can be cultured as explants which generate a profuse network of fibers (neurites) in vitro. It has been established that these neurons express in culture many of their known in vivo characteristics: 1) When grown with supporting cells the neurites of the larger DRG neurons, but not those of SCG neurons, become myelinated in culture; 2) The SCG neurons receive cholinergic synapses from cocultured spinal cord (Ko et al., 1976a, Brain Res., 117: 437) and provide synapses to cocultured target (fat) cells, and to one another (O'Lague et al., 1974, PNAS, 71: 3602; Ko et al., 1976b, Brain Res., 117:461). These ganglia may be taken for culture after the embryonic period of neuroblast proliferation, but before a large number of supporting cells have been generated. If these cultured explants are then treated with several pulses of antimitotic agents, they may generate a profuse outgrowth of neurites free of all supporting cells (Estridge, 1977, Nature, 268: 60). Because it is thus possible to obtain neurites free of contaminating elements (Schwann cells and fibroblasts) they offer a model system for axonal biochemistry. We have used this system to study the cellular distribution of gangliosides. Established cultures with neuritic halos 3 mm or more in width were incubated with ≃ 5 μCi of ^{3}H-glucosamine for 48 hours. Neurites and somata were separated by dissection, and mixed with approximately 1 gram of fresh rat brain. Gangliosides were extracted by the method of Tettamanti (1973, BBA, 296: 160), followed by chromatography on Sephadex G25-80 to remove traces of labeled precursors. Ninety-five percent of the labeled material was TCA-PTA precipitable. Mild acid hydrolysis released approximately 80% of the label which comigrated with authentic sialic acid when assayed by column chromatography on AG1-X8 resin. Radioactive gangliosides were analyzed by thin layer chromatography. In the case of both SCG and DRG somata and neurites labeled material comigrated with GM1, GD1a, GD1b and GT. A large amount of label migrates in the disialo-region but does not correspond to GD1a or GD1b. Small amounts of label are associated with GO, GM2 and GM3. These results indicate that numerous gangliosides are associated with both the cell somal region and the neurites of two different types of PNS neurons.
Supported by NIH Grants NS 09923, NS 12867 and the Muscular Dystrophy Association.

993 CENTRAL MYELINATION IN VITRO: EFFECT OF REDUCED CALCIUM AND MAGNESIUM. William Craelius, Nicki A. Newby and Frances C. Thomas* Dept.Biol. Sci., Stanford U., Stanford, CA 94305.

It has been suggested that deficiencies in calcium and magnesium experienced during infancy may lead to improper development of myelin (Goldberg,1974;Intern. J. Envir. Stud.,6,121). This hypothesis has been tested in vitro using slices of spinal cord from 10 to 12 day old chick embryos. Control culture media contained approximately 2.0 mM Ca and 1.0 mM Mg, and test media was identical except that the concentrations of Ca and Mg were approximately 0.7 and 0.4 mM. After one to three weeks incubation at 37 C, cultures were fixed and prepared for electron microscopy. Central type myelination, in various stages of growth, was readily observed in both control and test spinal cord slices after 7 days of culture. In the control tissue, at 7 days, many axons were tightly myelinated with 50 or more layers of lamellae. Myelin grown in the reduced Ca and Mg media, however, usually appeared loose and irregular, and was not as well formed as control myelin.

The optimal ionic requirements necessary for the proper growth of myelin have not been explored previously, and these results suggest that there may be certain minimum levels of calcium and/or magnesium that promote adequate myelination. Since only a moderate reduction in calcium and magnesium produced a noticeable deficiency in myelin growth, these results may be physiologically relevant. As suggested by Goldberg, the demyelination experienced by victims of multiple sclerosis may occur as a result of improper early development of myelin, due to shortages of calcium and magnesium. Published lipid analyses of myelin taken from multiple sclerosis victims have found developmental abnormalities, among which are increased unsaturation in fatty acids, particularly in cerebrosides, and decreased fatty acid chain length (Woelk & Borri,1973:Europ.Neurol. 10,250; Fewster et al,1976:J. Neurol. 209,119). Both of these abnormalities would render myelin unstable as predicted by O'Brien (1965: Science,14,1099).

Demyelination in multiple sclerosis may be partially a result of improper myelination; the present results suggest that deficiencies in calcium and/or magnesium could be responsible for the developmental errors.

994 ISOLATION OF MANNOSE-CONTAINING OLIGOSACCHARIDES IN HYPERPLASTIC GINGIVA FROM A CASE OF MANNOSIDOSIS. Peter F. Daniel* and Ira T. Lott* (SPON: Marjorie B. Lees). Eunice Kennedy Shriver Center for Mental Retardation, Inc., 200 Trapelo Road, Waltham, MA 02154.

We have reported an unusual case of mannosidosis (Kistler et al., Arch. Neurol. (1977) 34, 45-51) with massive gingival hyperplasia. The gingiva was infiltrated with histiocytes containing large amounts of storage material staining positively for carbohydrate. Gingival tissue was excised from this patient for dental indications, and 3.4 g were available to us for analysis. The tissue was homogenized in distilled water and sonicated to release any water-soluble material. Proteins were precipitated by addition of trichloroacetic acid (5% final concentration), and the supernatant was fractionated on Sephadex G-50 and Biogel P-2, followed by ion exchange chromatography on Dowex 50 to separate oligosaccharides from glycopeptides. Oligosaccarides represented the major storage material; very little carbohydrate was present as glycopeptides. Six oligosaccharide fractions were obtained by chromatography on an analytical Biogel P-2 column (-400 mesh) at 55° C and were analyzed by TLC on silica gel G with n-propanol/H_2O 8:3 as solvent; sugars were visualized by spraying with orcinol. Fraction 1 did not migrate; fraction 2 contained only minute amounts of orcinol positive material and was not further analyzed; fraction 3 contained at least four slow-migrating components; fractions 4-6 each contained one major component. Fraction 6 co-migrated with standard Man α 1, 3 Man β 1, 4 GlcNAc, which is the major sugar isolated from mannosidosis urine. Carbohydrate analysis of the crude fractions from Biogel P-2 by GC-MS revealed that fraction 1 contained only glucose and was presumably a dextrin. Fractions 3-6 each contained mannose and glucosamine together with small amounts of glucose. The molar ratio of mannose to glucosamine in fractions 4-6 was determined to be 5, 3, and 2 respectively. Control tissue (4.5 g) obtained from hyperplastic gingiva associated with phenytoin administration showed no components corresponding to the mannose containing fractions. The sole orcinol positive peak in the control co-eluted with fraction 1.

In comparison with published compositional data (Strecker et al., Biochimie (1976) 58, 579-586) on mannosidosis urine, the present results from gingival tissue show a striking absence of an oligosaccharide containing 4 moles of mannose.

995 BRAIN NUCLEAR PHOSPHOPROTEIN METABOLISM WITH BEHAVIORAL STIMULATION. S. Diane Davis* and John Eric Wilson (SPON: D.L.McIlwain). Neurobiology Program & Dept. Biochem., UNC, Chapel Hill NC 27514.

The effects of electric footshock and footshock avoidance training upon the incorporation of 32P into rat brain nuclear proteins were studied in 100 day old Wistar albino rats. After 35 min of labeling and 5 min of avoidance training (preceded by 5 min of acclimation to the apparatus) rats were killed by decapitation, the brains homogenized, and the nuclei sedimented through 2.4 M sucrose containing CaCl2 and Triton X-100. Nuclei were further fractionated into nucleoli, heavy chromatin, and light chromatin by sonication and density gradient centrifugation. Avoidance training caused an alteration of the distribution of the label between the two chromatin fractions studied, with a relative increase in the fraction sedimenting through 2 M sucrose compared to the fraction that did not sediment through 2 M sucrose. Electric footshock alone caused increased incorporation into the chromatin proteins of both fractions studied. No significant findings were observed in the nucleoli, the nonchromatin nuclear proteins, or the 10% CCl3COOH soluble proteins of the nucleus. Preliminary work indicated that ACTH administration duplicated the effects of electric footshock.

996 EFFECTS OF SOME MONOAMINE OXIDASE INHIBITORS (MAOI'S) ON THE DEAMINATION AND UPTAKE OF meta-TYRAMINE (m-TA), para-TYRAMINE (p-TA) AND DOPAMINE (DA) BY RAT STRIATUM in vitro. L.E. Dyck* and A.A. Boulton. Psychiatric Research Division, University Hospital, Saskatoon, Sask., Canada S7N 0W8.

During recent investigations on the uptake of m-TA, p-TA and DA into rat striatal slices; nialamide was included in the medium at all times and appeared to inhibit MAO. However, in release studies it became evident that the MAO was active and the deaminated metabolites were being washed into the bathing medium. Deamination occurred in the decreasing order: p-TA>m-TA>DA. In order to determine whether this differential rate of deamination was a consequence of an innate substrate selectivity of MAO, a substrate selective inhibition by nialamide, or a selective protection from MAO by intraneuronal storage; the ability of rat striatal homogenates to deaminate m-TA, p-TA and DA were assessed with and without the addition of MAOI'S. Their effect on uptakes was also evaluated.

MAO activity of striatal homogenates was assayed by measuring the decline in amine substrate concentration and the production of acid metabolites. Preincubation (20 min, 10 μM MAOI) and incubation (37 min, 10 μM MAOI, 10 nM tritiated amine) were performed at 37°C. After the incubation, the amines were extracted into di-2-ethyl-hexyl-phosphoric acid, while the acids were extracted at pH 1.0 into ethyl acetate. In the absence of drugs, homogenates deaminated p-TA and m-TA faster than DA. Approximately 75, 50 and 10% of the p-TA, m-TA and DA were converted to p-hydroxyphenylacetic, m-hydroxyphenylacetic and dihydroxyphenylacetic acids, respectively. The efficacy of the MAOI'S in inhibiting MAO with all three substrates in increasing order was nialamide, iproniazid, pargyline, clorgyline, catron and parnate.

Uptake of 10 nM amine into slices was assessed after a 5 min incubation. MAOI (10 μM) was included during preincubation (20 min). Nialamide, iproniazid, pargyline and clorgyline did not exhibit any significant effect on uptake of the amines; however, catron and parnate, whose chemical structures most clearly resemble the amines themselves, significantly reduced the uptake of m-TA and p-TA. Parnate significantly reduced DA uptake.

It is clear that at this concentration of nialamide and iproniazid no interference with uptake of the three amines occur, but little protection of p-TA and m-TA from MAO occurs. Catron and parnate which are the best inhibitors of MAO for all three amines, unfortunately also inhibited, markedly, their uptakes. Pargyline and clorgyline, which are good inhibitors of MAO for all three amines, did not interfere with their uptakes and they therefore appear to be suitable for use in transport studies.

Supported by the Department of Health, Province of Saskatchewan and the Medical Research Council of Canada.

997 CYCLIC NUCLEOTIDE-MODULATED PHOSPHORYLATION OF PROTEIN THAT IS SPECIFIC TO PHOTORECEPTOR CELLS OF RETINA. Debora B. Farber and Richard N. Lolley. Depts. Ophthalmology and Anatomy, UCLA School of Medicine, Los Angeles, CA 90024, and Devel. Neurology Lab., V.A. Hospital, Sepulveda, CA 91343.

Rod photoreceptor cells of dark-adapted retinas possess high levels of cyclic GMP. Upon bleaching of rhodopsin by light, cyclic GMP-phosphodiesterase is activated, and the concentration of cyclic GMP falls rapidly. Cyclic GMP activates a protein kinase from rod outer segments (ROS) and modulates preferentially the phosphorylation of a soluble, 30,000-dalton (30K) protein. In order to confirm that the 30K protein is localized specifically in photoreceptor cells of rodent retinas, we studied its phosphorylation in vitro, as a function both of visual cell maturation during postnatal life and visual cell degeneration in selected inherited disorders of mice and rats. In dark-adapted retinas of control animals, the incorporation of phosphate into the 30K protein increases at the time during postnatal life when photoreceptor cells are differentiating and developing ROS. Thereafter, the phosphorylation of this protein increases slightly, reaching its highest level by adulthood. From early postnatal life to adulthood, phosphorylation of the 30K protein is enhanced by exogenous cyclic nucleotides. C3H/HeJ mice are affected with an inherited disorder that causes photoreceptor cell degeneration beginning about 10 days postnatally. Prior to 10 days, when C3H visual cells are viable, basal and cyclic nucleotide-dependent phosphorylation of the 30K protein is demonstrable and similar to that of control retinas. However, after 10 days, when rod visual cells are degenerating, phosphorylation of the 30K protein diminishes and is undetectable by 20 postnatal days. A different type of inherited blindness occurs in Royal College of Surgeons (RCS) rats, and visual cells degenerate later in life. In retinas of RCS rats, basal and cyclic nucleotide-dependent phosphorylation of the 30K protein proceeds normally until morphological pathology is demonstrable. As photoreceptor cell degeneration occurs, the phosphorylation of the 30K protein is reduced, and it disappears when visual cells are depleted from the retina. These data indicate that the soluble 30K protein is localized selectively in photoreceptor cells of the retina and perhaps exclusively in ROS. (Supported by NIH Grant EY00395 and the Medical Research Service of the Veterans Administration.)

998 METHODS FOR MEASURING BLOOD GABA CONTENT. J. Ferkany*, L. Smith*, W. Seifert*, R. Caprioli* and S.J. Enna (SPON:I.J. Butler). Depts. Pharmacol., Neurobiology and Anat., and Biochem., Univ. Texas Med. Sch., Houston, Texas 77025.

Several recent studies have indicated that the ability to measure blood levels of putative neurotransmitters may provide useful clinical and basic scientific information. While there is little evidence that GABA serves a transmitter function in peripheral nerves, the ability to detect this agent in blood may provide new insights into the biological role of this amino acid in mammalian systems. While previous attempts to measure blood GABA have been unsuccessful, the present report describes two methods which are capable of accurately determining the content of GABA in mammalian blood. For the study, whole blood was withdrawn from a variety of mammalian species into syringes containing 15% EDTA, and 1-3 ml portions were added to tubes containing 7% perchloric acid, then vortexed and centrifuged. The resultant clear supernatant was neutralized with 4N KOH, then analyzed for GABA content using a previously described radioreceptor assay (J. Neurochem. 28:1121, 1977) or by gas chromatography-mass spectrometry (GC/MS). For the latter assay, the neutralized supernatant was lyophilized, redissolved in 0.1 M ammonium acetate buffer and the GABA eluted from a Sephadex G-15 column. For GC/MS assay, the GABA in the eluate was converted to a N-pentafluoropropionyl, methyl ester derivative and GABA content quantified using d_2-GABA as an internal standard. Analysis of rat blood samples using both procedures indicated that the two assay methods yield virtually identical results, with whole blood GABA levels varying from 500 to 1000 pmoles/ml. Using the radioreceptor assay it was found that blood GABA content is stable for up to 24 hrs at room temperature and that the amino acid is found in both formed elements and plasma. Whole blood GABA concentrations range from 500 to 1300 pmoles/ml in 8 mammalian species with human values averaging about 900 pmoles/ml. In vivo administration of aminooxyacetic acid increases both blood and brain GABA levels to a similar extent. Thus, blood GABA determinations may be a useful tool to correlate clinical response to the biochemical effect of GABA transaminase inhibitors and for investigating the physiological function of this amino acid in peripheral tissues. (Supported in part by the Pharmaceutical Manufacturers Association, the Huntington's Chorea Foundation, Merck Sharp and Dohme, and USPHS grants RCDA NS-00335 (S.J.E.) and MH-29739.)

999 ONTOGENIC DEVELOPMENT OF TRITIATED WATER FORMATION DURING LEUCINE METABOLISM IN RAT BRAIN. G. N. Fuller and R. C. Wiggins, Dept. Neurobiol. & Anat., Univ. of Texas Med. Sch. Houston, TX 77025

Following the injection of tritiated amino acid a large portion of brain soluble radioactivity is in the form of tritiated water (TH_2O), and we show here that the portion increases with postnatal age. Long Evans female rats at various ages were injected intraperitoneally (IP) with L-(4,5-^{3}H(N))-leucine (50uCi/100g body wt.). After 10 min animals were sacrificed and cerebral cortex, liver, blood and sciatic nerve tissue samples were quickly excised and homogenized immediately in a deproteinizing solution of 10% trichoroacetic acid (TCA). The TCA precipitable fraction was then pelleted by centrifugation at 700g for 10 min. Two equal aliquots were taken from the resulting supernatant of each sample. Total soluble radioactivity was determined in one aliquot by adding Biofluor directly to the sample followed by liquid scintillation couting. The other aliquot was dried under nitrogen at 50°C and resolubilized in the original volume of water before determining the evaporative loss of radioactivity (TH_2O). The TH_2O percentage of total radioactivity, that is, the percentage of radioactivity lost through evaporation of the acid soluble fraction was:

	% TH_2O ± SEM			
AGE(d)	CEREBRAL CORTEX	LIVER	BLOOD	SCIATIC NERVE
1	7±0.49	7±0.58	9±1.47	-
8	13±1.54	6±0.48	9±1.28	8±0.82
15	17±0.82	7±0.37	14±1.1	6±0.97
20	22±1.8	9±1.6	18±1.7	11±1.0
25	30±1.4	12±1.3	16±1.3	17±10
30	37±2.1	12±1.7	19±2.3	14±3.3
ADULT	45±1.6	14±0.60	25±1.2	28±3.17

The proportion present as TH_2O is either constant or increases slightly during the first two postnatal weeks. However, during the 3rd and 4th postnatal weeks hydrogen exchange from leucine to water increases markedly, and reaches essentially adult values at this time. A less striking increase is observed in liver and blood. Results for L-(4,5-^{3}H)-valine at 10 and 40 days are comparable to those for leucine. At least for the case with leucine, the percentage of radioactivity present as TH_2O was the same in brain cerebellum, cerebral cortex, and medulla. This work was supported by U.S. Public Health Service Grant NS-14355.

1000 EFFECT OF A CLASS OF NATURALLY-OCCURRING TETRAHYDROISOQUINOLINES ON CATECHOLAMINE METABOLISM IN RAT ADRENAL MEDULLA; IMPORTANCE OF METHYLATION. Matthew P. Galloway*, William J. Burke*, Alex Kosloff*, Deborah L. Lieberman*, John Mitchell*, and Carmine J. Coscia* (SPON: H.B. Sarnat) Departments of Biochemistry and Neurology, St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Two tetrahydroisoquinoline derivatives, 3'-O-methylnorlaudanosolinecarboxylic acid (MNLCA) and 3',4'-desoxynorlaudanosolinecarboxylic acid (DNLCA) have been shown by computerized mass fragmentography to be trace constituents of normal human and rat brain. Norlaudanosolinecarboxylic acid (NLCA) is a condensation product of dopamine and 3'-4'-dihydroxyphenylpyruvate. Its methylation product, MNLCA, is elevated upon administration of L-dopa (C.J. Coscia et al., Nature 269, 617, 1977). To determine the effects of NLCAs on enzymes of catecholamine metabolism in vivo, an investigation was undertaken using rat adrenal medulla explants maintained in culture. In preliminary trials it was demonstrated that tritiated MNLCA, DNLCA as well as dopamine were taken up by medullae. At the end of the incubation concentrations of the NLCAs within the tissue approached that of the media (0.5 mM). In subsequent experiments medulla explants were cultured in the presence of 0.5 mM MNLCA, DNLCA or NLCA and [1,2-^{3}H]dopamine. After 12 h the medullae were removed from the media, carefully washed and homogenized. Upon addition of carrier catecholamines and their various catabolites, the mixture was subjected to paired-ion reversed-phase high pressure liquid chromatography which afforded complete resolution of the compounds examined (J. Mitchell and C. J. Coscia, J. Chromatogr., 145, 295, 1978). The metabolites were collected and counted. Catecholamine levels were increased over controls in the presence of all three NLCAs and concomittantly, levels of dopamine catabolites (3,4-dihydroxyphenylacetic and homovanillic acids) were depressed by MNLCA. These results suggested inhibition of monoamine oxidase by MNLCA which was also observed in vitro using crude adrenal medulla homogenates. NLCA was not inhibitory. Similarly metanephrine and normetanephrine were elevated over controls in the presence of MNLCA but not NLCA. Previous in vitro data indicated that NLCA is a competitive inhibitor of catechol-O-methyltransferase (COMT)-catalyzed methylation of dopamine and norepinephrine. Metabolism of NLCA upon intravenous injection into mice appeared to occur predominantly by methylation. NLCA is converted into MNLCA, the 7'-O-methyl isomer and the 3',7-O-dimethyl derivative. Thus both in vitro and in vivo effects on catecholamine metabolism by NLCA will depend on the extent of its methylation. (Supported by NIH Grant NS 12342.)

1001 PRESENCE, METABOLISM AND UPTAKE OF PIPECOLIC ACID IN THE MOUSE BRAIN. Ezio Giacobini, Thomas Schmidt-Glenewinkel*, Yasuyuki Nomura*, Y. Okuma* and Tomio Segawa*. Dept. Biobehav. Sci., UConn, Storrs, CT 06268 and Dept. Pharmacol. Inst. Pharmac. Sci., Hiroshima Univ., Sch. Med., Kasumi 1-2-3, Hiroshima, Japan.

Pipecolic acid, an iminoacid related to lysine metabolism, has recently been identified in the mouse brain in our laboratory by means of TLC and mass spectrometry. Endogenous levels of 18±4 nmoles/g pipecolic acid were found in whole brain homogenates of adult mice. Several organs of the adult mouse, including brain, showed in vitro formation of pipecolic acid from L-lysine. The kidney demonstrated the highest rate followed by the brain (Schmidt-Glenewinkel et al., Neurochem. Res. 2: 619-637, 1977). The synthesis of pipecolic acid from L-lysine was studied in whole embryo and brain of mouse and chick. In the mouse brain formation of pipecolic acid could be detected as early as at day 17 of gestation and in the chick embryo head at day 5 of incubation. Synaptosomes isolated from mouse brain showed temperature dependent uptake of (^{3}H)-pipecolic acid at a concentration of $2x10^{-7}$M. The uptake was Na^+ dependent, ouabain sensitive and showed at $K_m=4.17x10^{-6}$M. Structural analogues of pipecolic acid showed a significant inhibitory effect on uptake at a concentration of 10^{-4}M. Release of pipecolic acid could also be demonstrated in rat brain slices. The demonstration of presence, biosynthesis in adult and embryonal brain and high affinity uptake of pipecolic acid, suggests a physiological role of this substance in the central nervous system of the mouse.

1002 PINEAL GLAND PRODUCES ITS OWN S-ADENOSYLMETHIONINE. Ras B. Guchhait and James E. Grau, Jr.* Dept. Epidemiol., Sch. Hyg. and Pub. Hlth., Johns Hopkins University, Baltimore, MD., 21205.

Transmethylation requires S-adenosylmethionine which is catalyzed from ATP and L-methionine by S-adenosylmethionine synthetase (S-adenosyltransferase, EC 2.5.1.6). It is demonstrated that the pineal gland, a tissue highly active in biogenic amine transmethylation, from rat and various other species, contains S-adenosylmethionine synthetase. The synthetase is also present in rat Harderian gland and retina besides other tissues. A product of the enzymatic reaction has been characterized as S-adenosylmethionine which serves as the methyl group donor for the formation of melatonin by the pineal extract. The specific activity of rat pineal S-adenosyltransferase is at least eight times greater than that of the brain, and one-half that of the liver enzyme. Km for L-methionine and ATP has been determined as 6.2×10^{-5} and 6×10^{-4} M respectively. Magnesium at a concentration of 25 mM is required for maximal catalysis, higher concentration is found to be inhibitory. 50 mM potassium ion increases the activity by 30%, however, unlike Mg^{2+}, higher concentrations of K^+ up to 200 mM do not show any inhibition. Omission of a sulfhydryl compound from the reaction mixture causes an insignificant change in overall catalysis.

1003 A SENSITIVE AND SPECIFIC ENZYME LINKED IMMUNOSORBENT ASSAY FOR THE Ca^{2+}-DEPENDENT REGULATOR PROTEIN OF PIG BRAIN. I. Hanbauer, J. Gimble* and H.-Y. T. Yang*. Section on Biochemical Pharmacology, NHLBI, NIH, Bethesda, MD 20014 and Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

The heat stable Ca^{2+}-dependent protein regulator (CDR) from brain was purified according to the procedure of Klee (Biochemistry 16: 1017-1024, 1977). Our CDR preparation was homogenous in 20% polyacrylamide gel electrophoresis in presence of 0.1% SDS. For the production of immunoglobulins directed toward CDR, the purified protein was coupled to hemocyanin with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. The CDR-hemocyanin conjugate emulsified in complete Freund's adjuvant was injected intradermally into the back of rabbits. The injection was repeated 7 to 8 times at 2 week intervals.

Partially purified immunoglobulins [50%$(NH_4)_2$ SO_4 precipitation] were coupled with various concentrations of CDR or unknown CDR. The mixtures were then transferred to CDR coated microplates and incubated for 2 hours. Horseradish peroxidase labeled anti-rabbit globulin was then added and allowed to react. Reaction with o-phenylenediamine produced a colored compound which was measured in a spectrophotometer (488 nm). The enzymatic immunoassay could detect as little as 500 pg CDR. The immunoglobulins directed against CDR were highly specific; the cross-reactivity with Troponin C from rabbit muscle (a gift from Dr. D. M. Watterson) was about 0.5% and that with the Ca^{2+}-dependent activator protein from human erythrocytes (a gift from Dr. F. F. Vincenzi) was 1%.

1004 CHOLINE ADMINISTRATION INCREASES RAT BRAIN DOPAMINE METABOLISM Dean R. Haubrich and A. Barbara Pflueger* Merck Institute for Therapeutic Research, West Point, Pa. 19486

Administration of choline increases the concentration of ACh in brains of laboratory animals [see Life Sciences 20, 1465 (1977)], and alleviates the symptoms of tardive dyskinesia in humans [N. Engl. J. Med. 293: 152 (1975); Ann. Neurol. 1, 418 (1977)]. This neurological disorder is also reversed by administration of the acetylcholinesterase inhibitor physostigmine. These findings suggest that choline administration stimulates the formation and neuronal release of ACh to elicit an increase in central cholinergic function.

To test this hypothesis, central cholinergic function was assessed indirectly by measuring changes in metabolism of dopamine in the corpus striatum of rats. Oral administration of choline Cl (20 mmol/kg, fed rats) enhanced the rate of depletion of striatal dopamine induced by simultaneous administration of α-methyltyrosine (α-MT, 1.2 mmol/kg, i.p.), a tyrosine hydroxylase inhibitor (α-MT alone→50% decrease; α-MT with choline→68% decrease measured 2 hr after treatment). In addition, treatment of fasted rats with two doses of choline (10 mmol/kg, p.o., 1 hr apart) caused an increase (22-54%) in the concentration of the dopamine metabolite, homovanillic acid (HVA) measured 30 min after the second dose. A similar increase in HVA concentration also occurred 30 min after the second of two oral doses of physostigmine sulfate (2.5 μmol/kg, 1 hr apart). Administration of atropine sulfate (8.6 μmol/kg, i.p.) lowered the concentration of HVA measured 90 min after treatment, and this decrease was prevented by simultaneous oral administration of two doses of choline. Treatment of rats with a dose of atropine (2 μmol/kg) which did not affect levels of HVA partially antagonized the choline-induced increase in concentration of the metabolite. These findings show that administration of choline elicits an increase in the metabolism of brain dopamine by a central cholinergic mechanism.

To assess the role of ACh synthesis in mediating this increase in dopamine metabolism, fasted rats were treated with 4-(1-naphthylvinyl)pyridine (NVP), (0.37 mmol/kg, i.p.), an inhibitor of choline acetyltransferase, followed 10 min later by either choline or physostigmine (as above). Treatment with NVP lowered the concentration of HVA and antagonized the choline-induced increase in concentration of the metabolite, but did not prevent the increase in HVA levels induced by administration of physostigmine. This latter finding indicates that choline and physostigmine produce their central cholinergic effects by different mechanisms, and suggests that choline stimulates central cholinergic function by an increase in the rate of synthesis and release of ACh from its presynaptic terminals.

1005 cAMP-DEPENDENT ACTIVATION AND PHOSPHORYLATION OF TYROSINE HYDROXYLASE (TH). John W. Haycock, Michael D.Browning, John A. Meligeni and Jack C. Waymire. Dept. of Psychobiology, University of California at Irvine, Irvine, CA. 92717.

Dopamine synthesis from radiolabeled tyrosine in cultured bovine adrenal chromaffin cells underwent a rapid (less than 2 min.), dose dependent (1.0 mM optimal), pH dependent (pH 5.5 optimal), reversible (20 min. at 37°C) stimulation in response to 8-bromo-3',5'-cyclic adenosine monophosphate (8BrcAMP). TH isolated from activated cells exhibited a higher V_{max} for tyrosine (28.8 vs. 18.6) and pterine cofactor (14.5 vs. 12.6) and a lower K_m for pterine (0.58 vs. 0.36); and TH from control cells exhibited a similar pattern of kinetic alterations when exposed to in vitro conditions optimal for cAMP-dependent phosphorylation. Preincubation of chromaffin cells in the presence of $^{32}PO_4$ to label ATP, followed by 8BrcAMP stimulation and immunoprecipitation with TH specific antisera, revealed a marked stimulation of ^{32}P incorporation into bands which comigrate with more highly purified TH on polyacrylamide slab gels. A similar stimulation of ^{32}P incorporation into immunoprecipitated material occurred upon incubation of TH from the supernatant of control cells with ^{32}P-ATP under cAMP dependent phosphorylation conditions. These results indicate that dopamine synthesis and TH activity may be elevated in situ by a mechanism which involves cAMP dependent phosphorylation of TH molecules.

Supported by USPHS-NS11061-03.

1006 INVOLVEMENT OF LSD-INDUCED HYPERTHERMIA IN THE DISAGGREGATION* OF RABBIT BRAIN POLYSOMES. John J. Heikkila and Ian R. Brown
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We have previously reported that the intravenous administration of LSD to young rabbits induces a transient, organ-specific disaggregation of brain polysomes (Holbrook & Brown, J. Neurochem., 27, 77, 1976). The LSD-induced shift of brain polysomes to monosomes is mediated by the interaction of the drug with neurotransmitter receptors (Holbrook & Brown, Life Sci., 21,1037,1977) and the extent of polysome disaggregation can be accentuated by mild stress and blocked by pre-LSD sedation (Heikkila et al., Life Sci. 22, 757, 1978; Holbrook & Brown, J. Neurochem., 29, 461,1977). We now report that LSD-induced hyperthermia may be involved in the disaggregation of rabbit brain polysomes.

Rectal temperature measurements were taken in conjunction with the examination of brain polysome profiles 1 hr after LSD (10-100 ug/kg i.v.) administration. High rectal temperatures (41-42°C+) prior to sacrifice were always associated with a massive disaggregation of brain polysomes while lower rectal temperatures were correlated with slight polysome shifts. Rabbits given LSD (1-25 ug/kg) plus stress (restraint) consistently showed more extensive brain polysome disaggregation and higher rectal temperatures than animals administered LSD without stress. Pretreatment with neurotransmitter receptor blockers which inhibit the LSD-induced disaggregation of brain polysomes also prevents LSD-induced hyperthermia. If hyperthermia was involved in the series of events leading to brain polysome disaggregation then one might expect that hyperthermia would precede brain polysome disaggregation. Rabbits given 25 ug/kg of LSD showed marked elevations in body temperature within minutes after injection, whereas brain polysome disaggregation is not detectable until 15-30 min later. The administration of 2-bromo-LSD, which does not disaggregate brain polysomes also did not elevate body temperature. These results suggest an involvement of hyperthermia in the LSD-induced disaggregation of rabbit brain polysomes.

(Supported by the Medical Research Council of Canada)

1007 REGULATION OF AEROBIC METABOLISM IN STIMULATED GARFISH OLFACTORY NERVE. L.A. Hershey, S.H. McDougal*, R.V. Dargar*, and D.B. McDougal,Jr.*, Dept. Pharm., Wash. U. Medical School, St. Louis, Mo., 63110.

Metabolic flux is known to accelerate during nerve stimulation. It is not clear how this flux transition occurs in aerobic nervous tissue. Neither is it known how sodium pump activity influences such a transition. Garfish nerve was chosen for this study because the monotonous homogeneity of its non-myelinated fibers assures uniformity in measuring both electrical and metabolic events.

Oxidative metabolism was shown to be necessary in supporting the action potential during repetitive stimulation and in maintaining optimal levels of high energy phosphates in stimulated garfish olfactory nerve. Levels of lactate, pyruvate, acetyl-CoA and α-ketoglutarate were measured fluormetrically in extracts of nerves that had been stimulated in air for various intervals. Changes in the levels of these intermediates during metabolic flux transition gave some clues about control points in oxidative metabolism in this preparation.

When metabolic flux is increased by nerve stimulation, pyruvate dehydrogenase appears to be the first rate-limiting step in the Kreb's cycle. Pyruvate levels increased by 40% after 120 impulses, while acetyl-CoA levels remained unchanged. Next, α-ketoglutarate levels increased by 45% (after 180 impulses), suggesting a second control step beyond this point in the cycle.

There is a fall in the lactate/pyruvate ratio with nerve stimulation that appears to be commensurate with a fall in the ATP/ADP·P_i ratio (maximal after 180 impulses). These ratios return to resting levels in parallel with one another during continued stimulation. This suggests a close association between sodium pump activation and metabolic flux transition in garfish olfactory nerve.

1008 THE ROLE OF pH IN THE ISOLATION OF NERVE ENDING PARTICLES WHICH TRANSPORT GABA. Robert J. Hitzemann* (SPON: Enoch Callaway) Depts. of Pharmacology and Psychiatry, University of California, San Francisco, CA 94143.

Nerve endings were prepared from the whole rat brain using sucrose and sucrose-ficoll solutions buffered to pHs ranging from 6 to 8.2 with 5 mM HEPES. Briefly, a crude mitochondrial fraction was prepared by standard centrifugation techniques using 0.32 M sucrose + 5 mM HEPES (S-H) as the homogenizing medium. After washing the mitochondrial fraction twice with S-H, the pellet was resuspended and layered on a discontinuous S-H-Ficoll gradient containing steps of 15, 12, 8 and 6 percent Ficoll. The gradient was centrifuged for 45 min x 83,000 g. Five fractions (A to E) were harvested from the gradient; each fraction contained the interfacial material plus the material suspended in the layer above each interface. Increasing the pH from 6 to 7.8 resulted in a 460 percent increase in the total [^{3}H]GABA transport appearing in the combined five gradient fractions. Even increasing the pH from 7 to 7.8 significantly increased [^{3}H]GABA transport 60 percent. Changes in pH also affected the distribution of transport sites within the gradient. Below pH = 7, the majority of the transport activity was found in the mitochondrial fraction (fraction E), whereas at a pH of 7 or above the highest density of transport sites was found in fraction C. In fraction C, which is the most enriched in nerve endings, increasing the S-H pH from 7 to 7.8 increased total transport activity 85 percent and transport specific activity 41 percent. A similar effect was observed for [^{14}C]glutamate transport. Total transport activity increased 92 percent and transport specific activity increased 50 percent. Using succinic dehydrogenase and NADPH cytochrome C reductase, respectively, as mitochondrial and microsomal marker enzymes, it was found that increasing the S-H pH to 7.8 did not significantly increase the contamination of fraction C. Finally, it was found that increasing the S-H pH increased the total number of [^{3}H]ouabain binding sites but did not increase binding specific activity in fraction C since the increase in binding was not greater than the increase in protein which occurred in this fraction. Overall, the results of the present study suggest (1) that the pH of the sucrose solutions used to process brain tissue is a critical factor in isolating viable nerve endings, and (2) that when isolating nerve endings to be used for GABA or glutamate transport studies the pH of the sucrose solutions should be about 7.8.

This study was supported in part by Grant NS-13398.

1009 IS THERE ACETYL-COENZYME A HYDROLASE OR CHOLINE ACETYLTRANSFERACE IN MAMMALIAN BLOOD? L. L. Hsu* and J. Claghorn (SPON: T. Samorajski) Texas Research Institute of Mental Sciences, Houston, Texas 77030

In the course of establishing the radiochemical assay for choline acetyltransferase (acetyl-coenzyme A: Choline O-acetyltransfuase E.C. 2.3.1.6., CAT) in mammalian blood, we found that Triton X-100 treated whole blood, red blood cell (RBC) or plasma, catalyzed the hydrolysis of (C^{14}) - or (H^3) - acetyl-CoA either in the presence or in the absence of choline chloride. We have therefore further studied the acetyl-CoA hydrolase in blood fractions. Both the methods of Fonnum (J Neurochem 24, 407-409, 1975) and Brandon and Wu(personal communication) were used. The latter method employs activated charcoal to absorb the unreacted labeled acetyl-CoA leaving products in the supernatant. Whole blood, plasma and RBC from mammals including humans, Rhesus monkeys, rats and mice were homogenized in equal volumes of 0.5% Tritm X-100 and 1 tenth volume of 3M NaCl (Massarelli et al, Neuroscience Letters, 5, 95-101, 1977). Aliquots of blood fractions were incubated in 0.5M potassium phosphate buffer containing 0.2mM (1-C^{14}) - or (H^3) - acetyl-CoA, 2 mM EDTA, 0.1 or 1mM physostigmine, and varying concentrations of choline chloride in a final volume of 0.15 or 0.2 ml at 37° C for various time intervals. At the end of incubation, either 10% acetic acid or 0.01 M potassium phosphate was added to stop the reaction. Either charcoal or Kalignost in acetonitrile (15 mg/ml) was added respectively and radioactivity of the product was measured. The radioactive product is identified by TLC analysis. The enzyme activity is linear with whole blood and plasma up to 50 ul and with RBC up to 25 ul; linear with incubation time up to 1 hr. The enzyme activity is pH dependent and optimal at pH 8.0. The enzyme activity was 2.5 times as high in RBC as that in the plasma fraction. Coenzyme A (0.08 mM) competitively inhibited the enzyme activity in both RBC and plasma. Acetylcholine (0.1 M) slightly inhibited the enzyme activity in RBC but markedly inhibited that in plasma. On the other hand, choline chloride at 4mM did not affect the hydrolase activity. The distribution of acetyl-CoA hydralase in brain has been reported (Matsuda and Yoshida, J. Neurodhem, 26, 817-822, 1976).

1010 DIRECT PHOSPHORYLATION OF TYROSINE HYDROXYLASE BY cAMP-DEPENDENT PROTEIN KINASE: A MECHANISM OF ENZYME ACTIVATION. T.H. Joh, D.H. Park, M.J. Brodsky*, and D.J. Reis, Laboratory of Neurobiology, Cornell University Medical College, New York, NY 10021.

Over the past several years the demonstration that the activity of tyrosine hydroxylase (TH) is altered by cAMP has raised the question as to whether TH may be regulated by phosphorylation, and if so, whether the enzyme itself is phosphorylated. In the present study, we have sought to determine: (a) if TH in rat brain and adrenal medulla can be directly phosphorylated by cAMP-dependent protein kinase (PK); (b) if such phosphorylation increases the catalytic activity of the enzyme; and (c) what the kinetic mechanism is for the increase in the enzyme activity. TH was highly purified from rat brain and adrenal gland by homogenization, centrifugation, and ammonium sulfate fractionation, followed by sequential chromatographies of Phenyl-Sepharose, DEAE-cellulose (stepwise), DEAE-cellulose (gradient), CM-cellulose and Sepharose 4B columns. The highly purified TH was then subjected to conditions for protein phosphorylation, using PK purified from rat heart, and phosphorylated TH (P-TH) isolated. Addition of cAMP to the reaction mixture was necessary for phosphorylation of TH. For the isolation of P-TH, polyacrylamide gel electrophoresis was substituted for the aforementioned final two column chromatographic steps. On a polyacrylamide gel, authentic TH and P-TH showed a single protein band. On an SDS-gel, three protein subunits were seen with MWs equivalent to 52,000, 62,000 and 68,000; the protein band of MW 62,000 represents the catalytically active unit while the other bands probably represent structural or regulatory subunits. When ^{32}P-ATP was used in the phosphorylation mixture, ^{32}P was detected only in the subunit of MW 62,000, indicating that the catalytically active subunit was phosphorylated. The specific activity of the P-TH was two times higher than that of authentic TH. Kinetic analysis of P-TH demonstrated that the increase in TH activity is due to an increase in Vm without change in Km for either substrate or cofactor, reflecting an increase in the amount of enzyme. Since in our system the total amount of TH was fixed, an increase in the amount of enzyme must therefore represent an increase in the amount of the catalytically active enzyme from an inactive form of the enzyme. This concept implies that in tissue the pool of native TH is composed of a mixture of enzyme in both active and inactive forms, that the active form is phosphorylated, and the phosphorylation produces an active form of enzyme at the expense of the inactive form. Interconversion of the forms may be an important mode of regulation of catecholamine biosynthesis in vivo. (Supported by NIH grants HL 18974, MH 24285; and NASA award NSG 2259.)

1011 THE EFFECTS OF LEAD POISONING ON CALCIUM TRANSPORT BY THE BRAIN IN 30-DAY OLD ALBINO RABBITS. C. S. Kim*, L. A. O'Tuama, S. L. Cookson*, and J. D. Mann*. Depts. of Neurology, Pediatrics, Medicine, and the Biological Sciences Research Center. Sch. Med. Univ. N. C., Chapel Hill, N. C. 27514.

We have shown that the extraction of ^{45}Ca from plasma by cerebral gray matter is reduced in lead intoxicated albino rabbits compared to controls. This finding indicates that Pb poisoning may disrupt systems for transport of ^{45}Ca at the blood-brain barrier (Soc. Neuroscience Abs. 3:311, 1977). To investigate the possibility that this effect might also exist at the cellular membrane level, we have studied the uptake and efflux of ^{45}Ca in control and lead treated 30-day old albino rabbits. The clearance of ^{45}Ca from CSF, measured by ventriculo-cisternal perfusion, was sufficiently slow that a ratio between inflow and outflow concentrations of only 0.8 was reached at steady state. These findings are compatible with those of Graziani et al (Am. J. Physiol. 208:1058-1064, 1965) who had likewise noted an extremely slow exchange of ^{45}Ca across the brain-CSF interface. In Pb poisoned animals receiving 165 mgs of $Pb(CO_2)_3$ daily for 5 days, no significant difference was noted in the rate of clearance of ^{45}Ca from the ventricle. The uptake of ^{45}Ca was also studied in brain slices that were preincubated for 30 min in artificial CSF only and then transferred to experimental medium. The T/M ratio was 0.65 $\pm$ 0.08 for controls and 0.86 $\pm$ 0.12 for brain cortex exposed to lead nitrate (5×10^{-6}M) (NS). For the study of ^{45}Ca efflux, brain cortex, paraventricular tissue (PVT) and choroid plexus (CP) slices were rapidly removed and incubated for 30 min in a medium containing ^{45}Ca 350,000 cpm/ml (sp. act. 17.9 mCi/mg). The slices were washed in cold artificial CSF, transferred to unlabeled CSF at 37° and incubated for different periods of time (5 to 45 min). The intracellular retention of ^{45}Ca at 5 min was increased 75% in animals treated in vivo with lead carbonate ($P < 0.01$), 69% in tissues incubated in vitro with Ouabain (10^{-4}M) ($P < 0.01$), and 48% with Sodium Azide (3mM) ($P < 0.05$). However, such treatment did not increase ^{45}Ca accumulation by CP or PVT. The entry of Ca into the cells is down an electrochemical gradient and efflux is by active transport. This study indicates the possibility of interaction of lead and Ca with carrier complexes in the process of active transport. A resultant imbalance of intracellular Ca levels may influence Pb Neurotoxicity. Supported by a grant from the NIH #RO1-ES01151.

1012 IN VITRO INHIBITION OF RABBIT BRAIN MONOAMINE OXIDASE BY NITROBENZENOID COMPOUNDS. Alane S. Kimes and Daniel O.Carr* Dept. Biochem., Ks. Univ. Med. Cen., Kansas City, Ks. 66103.

Several soluble flavoenzymes are known to be inhibited by nitrocompounds. In addition, monoamine oxidase (MAO) from rabbit liver, a membrane bound flavoenzyme, has been shown to be inhibited by nitroaromatic compounds. In the present study, MAO was partially purified and solubilized from rabbit brain and then subjected to inhibition by several nitrobenzenoid derivatives. The results were compared to the inhibition of the rabbit liver enzyme by the nitrobenzenoid compounds in this laboratory. As with the liver enzyme, the nitrobenzenoid compounds were competitive inhibitors of the rabbit brain enzyme when p-dimethylaminobenzylamine, a type B substrate, was used. The nitrobenzene derivatives tested included those with the following substituents: p-carboxy, p-chloro, p-carboxamide, p-carbomethoxy, p-formyl, p-acetyl, p-cyano, m-nitro, and p-nitro. The K_i's ranged from 5.2×10^{-7}M to 1.8×10^{-4}M. The inhibition was greater when the electron withdrawing power of the substituent, as measured by its Hammett sigma value relative to unsubstituted nitrobenzene, increased. p-Dinitrobenzene was the most potent inhibitor and had the most electron deficient ring system. These results were similar to those found for rabbit liver MAO. Some differences were noted, i.e., p- chloronitrobenzene was much more potent with the liver enzyme than with the brain enzyme which may indicate some slight differences in MAO from the two organs.

1013 ANALYSIS OF MAJOR RNA CLASSES IN THE MAUTHNER AXON. Edward Koenig. Dept. Physiol., SUNY at Bflo., Sch. Med., Buffalo, NY 14214.

Work in this laboratory over the years has indicated that myelinated vertebrate axons are capable of a low level of protein synthesizing activity that is extramitochondrial. Recent studies (Frankel and Koenig, 1977, Exp. Neurol., 57:282; 1978, Brain Res., 141:67) have suggested that those few products which appear to be synthesized indigenously in vitro may relate in part to axoskeletal components (e.g., neurofilaments). A major objection to attributing protein synthesizing activity to the axon has been the lack of morphological evidence of ribosomes. A method has been developed for extracting and purifying on a microscale undegraded RNA from myelin-free Mauthner axons of the goldfish. Microextracts were fractionated by microelectrophoresis and showed the presence of major RNA classes indistinguishable from ribosomal classes of fish brain; i.e., 26 S_E and 18 S_E. There was no apparent 5 S_E class and the 4 S class was disproportionately large compared to that of brain tissue. In addition, axonal extracts contained an apparent 15 S_E nonribosomal class that was neither evident in brain extracts, nor in extracts from Mauthner axon myelin sheath samples. Myelin sheath extracts also exhibited a disproportionately large 4 S class. Electron-microscopy showed that the technique of axon isolation yielded samples that were free of significant contamination by myelin. In addition, comparisons between equivalent-sized samples of isolated axons and myelin sheath showed that axon samples yielded significantly more RNA than myelin sheath samples. It is concluded that the Mauthner axon contains a protein synthesizing machinery, which, because of its dispersed deployment, shows relative enrichment of nonribosomal 15 S and 4 S classes.

Supported by P. H. S. Public Health Grant No. 04656 from the NINCDS.

1014 STUDIES ON THE ENZYMATIC DEPHOSPHORYLATION OF TYROSINE HYDROXYLASE. Mitchell A. Lazar*, Joachim D. Raese*, Arthur M. Edelman*, and Jack D. Barchas.

We have previously found that purified tyrosine hydroxylase from bovine corpora striata is a substrate for cAMP-dependent protein kinase. Tyrosine hydroxylase can incorporate 0.7 to 0.9 moles of phosphate per mole of enzyme, and is activated by phosphorylation. We report here that high speed supernatants from rat tissue homogenates can dephosphorylate ^{32}P-labeled tyrosine hydroxylase. The specific activity of tyrosine hydroxylase phosphatase is several-fold greater in corpora striata than in liver, muscle, or kidney. Dephosphorylation of ^{32}P-labeled tyrosine hydroxylase by rat striatal extract is not inhibited by addition of excess unlabeled phosphohistone to the incubation medium. In contrast, unlabeled phosphohistone competes efficiently with ^{32}P-labeled histone as a substrate for phosphohistone phosphatase in rat striata. The data suggest the existence of a phosphatase with higher specificity for phosphorylated tyrosine hydroxylase than for phosphorylated histone.

1015 COMPARISON OF MICROWAVE IRRADIATION AT 986 VS 2450 MHZ FOR IN-VIVO INACTIVATION OF BRAIN ENZYMES IN RATS. Robert H. Lenox, James L. Meyerhoff, Om P. Gandhi*, and John H. Jacobi*, Depts. of Medical Neurosciences and Microwave Research, Div of Neuropsychiatry, Walter Reed Army Inst. of Research, Washington, DC 20012 and Dept. of Electrical Engineering, University of Utah, Salt Lake City, UT 84112.

Our laboratory has previously observed that heat deposition as well as the pattern of enzyme inactivation in the rat brain at 2450 megahertz (MHz) is non-uniform, and that energy distribution is markedly affected by rotation of the rat head. Accordingly we find it necessary to immobilize animals prior to sacrifice by microwave irradiation. Because immobilization is a potent stressor which can cause neurochemical and hormonal changes, a technique of irradiation that was independent of orientation of the rat would be extremely useful if it eliminated the present requirement to immobilize the rat prior to sacrifice. The present study was undertaken to compare the effects of rotation on the geometry of energy distribution in rat brain during exposure at different microwave frequencies. The pattern of energy deposition can be inferred from the pattern of enzyme inactivation using a cytochemical technique. Male 300 gram Walter Reed strain rats were anesthetized with sodium pentobarbital and inserted into a plexiglass cylinder which was placed in one of four degrees of rotation (0^{o}, 90^{o}, 180^{o}, or 270^{o}) within the waveguide chamber. The rats were then exposed to either of two microwave frequencies: 2450 or 986 MHz. An exposure duration was deliberately selected to produce a gradient of enzyme inactivation in the brains of rats exposed to either frequency. Four animals were studied under each of the eight combinations of frequency and rotation. Following exposure to microwave irradiation the rats were decapitated, the brains removed, frozen on dry ice, and 10μ sections cut in a cryostat at -2^{o}C. The pattern of enzyme inactivation was revealed by the cytochemical technique of Nachlas which stains for succinic dehydrogenase activity. The pattern was markedly rotation-dependent at 2450 MHz: absorption of energy was markedly reduced at 90 and 270 degrees of rotation. At 986 MHz, however, the dorsal portion of the brain absorbed most of the energy at all four angles of exposure, and degree of rotation produced almost no effect on the pattern of enzyme inactivation. These data suggest that further exploration of frequency parameters would contribute to development of a microwave inactivation technique more applicable to neurochemical studies in behaving, freely-moving rats.

1016 PURIFICATION OF CHOLINE ACETYLTRANSFERASE FROM CHICKEN BRAINS. Kelvin Ma*, and S.C. Sung. Div. of Neurol. Sci., University of B.C., Vancouver, B.C., Canada, V6T 1W5.

Choline acetyltransferase (CAT), the enzyme responsible for the synthesis of acetylcholine (ACh), has been extensively purified from chicken brains which had their cerebellums removed. Purification procedures included ammonium sulfate fractionation, DEAE-Sephadex (A-25) chromatography, protamine sulfate fractionation, chromatography on hydroxyapatite, sephadex G-150, and affinity chromatography on agarose-hexane-Coenzyme A column. CAT activity was measured radiochemically. Due to the instability of the enzyme in the course of purification, the most active fraction obtained after agarose-hexane-Coenzyme A chromatrography showed a specific activity of only 560 nmoles ACh formed/min./mg. protein which corresponded to a 1000 fold purification from homogenate. However, on non-denaturing polyacrylamide gel electrophoresis at pH 8.8, the highly purified CAT preparation showed 2 distinct bands, and CAT activity was recovered by slicing and assaying the gel, and corresponded to the position of the faster moving band. The same preparation showed 1 major band and 3 minor bands on SDS gel electrophoresis. The estimated MW of the major band was 63500. The lack of carnitine acetyltransferase in the enzyme preparation was indicated by the low specific activity of carnitine acetyltransferase of 0.17 nmoles acetylcarnitine formed/min./mg. protein compared to 24 nmoles/min./mg. in crude extract. The presence of eserine sulfate in the reaction mixture had no effect on the CAT activity of the preparation indicating that it was free of acetylcholinesterase. Studies on the inhibition of ACh on the CAT preparation showed that the CAT activity was not inhibited up to 50 mM ACh (14% inhibition) and that only a 28% inhibition was obtained with 200 mM ACh. The CAT preparation also showed species specificity. By the Ouchterlony double immunodiffusion test, both the highly purified CAT preparation and crude extract from chicken brains diluted to different concentrations did not cross react with rabbit serum (of different dilutions) prepared to purified human CAT. (Supported by the MDAC).

*K. Ma is supported by the MRC studentship.

1017 INORGANIC PHOSPHATE AS A REGULATOR OF BRAIN GLUTAMATE DECARBOXYLASE. S.B. Martin* and D.L.Martin* (SPON. A.T.Campagnoni) Chemistry Dept., University of Maryland, College Park, Maryland 20742.

Previous studies have suggested that inhibition of brain glutamate decarboxylase (GAD) by nucleotides results in the formation of the inactive apoenzyme. The present results suggest that physiological concentrations of P_i oppose this inhibition, at least in part, by promoting the reactivation of apoGAD. In most experiments apoGAD was prepared by incubating GAD preparations with $2x10^{-5}$ M aminooxyacetic acid for about 30 min at room temperature followed by exhaustive dialysis against 0.05 M imidazole-acetate buffer, pH 7.2 containing 1 mM aminoethylisothiuronium bromide. Reactivation of apoGAD by pyridoxal-5'-phosphate (PLP) was studied by incubating apoenzyme at 37° under various conditions. The amount of holoenzyme was then measured by incubating for 5 min with 1 mM 1-^{14}C-glutamate and collecting the $^{14}CO_2$. Incubation of apoGAD with PLP in the absence of P_i resulted in an initially rapid but incomplete reactivation which stopped after 20 min of incubation. The amount of holoenzyme formed after 20 min was proportional to log [PLP] over the range 0.03 to 250 μM. Half-maximal activity was reached at about 10 μM PLP. Addition of 1-10 mM P_i stimulated reactivation. This effect was observed whether P_i was present initially or added after 15 min indicating that incomplete reactivation in the absence of P_i was not due to depletion of PLP. Incubation with 1 μM PLP and 1 mM P_i resulted in partial reactivation which stopped after 10 min. Addition of 10 mM P_i reduced the concentration of PLP giving half-maximal activity to about .07 μM; full activity was reached by about 10 μM PLP. P_i also stimulated reactivation of apoGAD prepared by treatment with glutamate or hydroxylamine or from vitamin B_6 deficient rats. The IC_{50} for several nucleotides including ATP, ADP, GTP, ITP, UTP and CTP is less than 200 μM suggesting that GAD is under continuous strong inhibitory pressure in vivo. In view of this, variations in the level of P_i may be important in controlling GAD activity.

1018 CHOLINE UPTAKE IN THE NEUROBLASTOMA X GLIOMA HYBRID, NG108-15. R. McGee* (SPON: K. L. Dretchen). Dept. Pharmacol., Sch. Med. & Dent., Georgetown University, Washington, DC 20007.

NG108-15 cells possess many neuronal properties, including the ability to form neuromuscular synapses in culture following prolonged exposure to dibutyryl cAMP (Bt_2cAMP). Previous experiments have shown that cells grown in the absence of Bt_2cAMP are unable to release acetylcholine in response to depolarization but gradually develop this capacity during Bt_2cAMP treatment. Since the ability to accumulate choline is essential for the functioning of cholinergic neurons, the choline uptake systems of NG108-15 cells and the effects of Bt_2cAMP on these systems were studied. Cells grown in the absence of Bt_2cAMP exhibit 2 choline uptake systems: a high affinity system, Km = 3.4 µM, Vmax = 40 pmol/min/mg protein, and a lower affinity system, Km = 76 µM, Vmax = 2500 pmol/min/mg protein. The high affinity uptake system (uptake at 0.5 µM choline) was inhibited about 50% by replacement of Na^+ by Li^+ but was unaffected by omission of Ca^{++} or K^+. A tenfold elevation of Ca^{++} or K^+ inhibited uptake by about 20%. In sharp contrast to the high affinity uptake system of brain synaptosomes the high affinity uptake system of NG108-15 cells was not Na^+ dependent since replacement of Na^+ with isosmolar sucrose increased uptake by 60-100%. However, this increased uptake was blocked by elevation of Ca^{++} from 1.8 mM to 18 mM. Also in contrast to syanptosomes, a high concentration of hemicholinium-3 (50 µM) was required to cause 50% inhibition of the high affinity uptake system (0.5 µM choline).

NG108-15 cells cultured with 1 mM Bt_2cAMP for 7 days appeared to no longer possess 2 uptake systems but rather a single system with Km = 46 µM and Vmax = 1800 pmol/min/mg protein. In spite of these changes in kinetic parameters, high affinity uptake at 0.5 µM choline still was increased 60-100% by the replacement of Na^+ with isosmolar sucrose, and 50 µM hemicholinium-3 still inhibited uptake by 50%. Whether or not the changes in kinetic parameters following Bt_2cAMP treatment are involved in the development of depolarization-dependent release of acetylcholine by these cells remains to be determined. However, Bt_2cAMP-treated NG108-15 cells do not appear to have the Na^+-dependent high affinity choline uptake system thought to be associated with cholinergic neurons. Since these cultured cells can form neuromuscular synapses, the results suggest that the Na^+-dependent system is not required for synaptic function. An alternative explanation that cannot be ruled out at this time is that the presynaptic endings of NG108-15 cells do have the Na^+-dependent uptake system but its activity cannot be observed when measuring uptake with intact cells having a high background activity of other choline uptake systems. (Supported by BRSG RR05360 and the PMAF.)

1019 INHIBITION OF MITOCHONDRIAL RESPIRATION BY 3-QUINUCLIDINYL BENZILATE. Brian Melamed* and John J. O'Neill, Temple University School of Medicine, Department of Pharmacology, 3420 North Broad Street, Philadelphia, Pa. 19140.

Potent anticholinergic glycolate esters, in particular the compound Ditran^R (or 30-70% mixture of N-ethyl-3-piperidyl cyclopentyl-phenyl glycolate and N-ethyl-2-pyrrolidyl-methyl cyclopentyl-phenyl glycolate), have marked inhibitory effects upon respiration in stimulated rat brain cortex slices (O'Neill, et al, Adv. Bioch. Pharm. 6: 203, 1972). Furthermore, it was shown that these compounds inhibit mitochondrial respiration when glutamate (10 mM) is the sole substrate; no inhibition of respiration was seen with succinate (10 mM). It was concluded that inhibition occurred at substrate-level phosphorylation. In the present study the effects of Ditran^R on mitochondrial respiration were confirmed.

In light of the current interest in the use of the potent muscarinic antagonist 3-quinuclidinyl benzilate (RO2-3308, QNB) as a marker for muscarinic receptors, we have undertaken an investigation of the effects of this compound on mitochondrial respiration. Mitochondria were obtained from rat brains by a modification of the method of Clark and Nicklas (JBC, 245: 4724, 1970). In these experiments we demonstrated a profound inhibition of state III respiration when QNB was present at similar concentrations to those of Ditran^R in the experiments cited above. Inhibition was observed when glutamate (10 mM) was the sole substrate. No inhibition was seen when either succinate (10 mM) or glutamate (10 mM) plus malate (2.5 mM) were the substrates. Further experiments are planned to elucidate the detailed mechanism of this inhibition. (Supported by NIMH Fellowship 1 F31 MH0733-01.)

1020 EFFECTS OF MILD METHYLMERCURY POISONING ON THE LIPID METABOLISM OF THE DEVELOPING RAT BRAIN. Nirmala K. Menon*, Richard R. Lopez* and R.A. Pieter Kark. Reed Neurol. and M.R. Research Centers, N.P.I., Sch. Med., UCLA, Los Angeles, CA 90024

Injecting CH_3Hg^+ in low doses to pregnant rats on the 4th day of gestation results in inhibition of the incorporation of label from (DL)-3-OH[3-^{14}C] butyrate, but not from [U-^{14}C] glucose, into total extractable brain lipids during the period of active myelination in the virtually asymptomatic pups. Mercury levels and radioactivity of lipids in this model of congenital intoxication have been studied further. Mercury concentrations of the brains, kidneys and livers of the pups were 3.6±0.5µg/g, 5.7±1.0 and 8.0±1.1, respectively, on postnatal day 1; and 0.6±0.2, 1.0±0.3 and 0.6±0.2 on day 21 vs. the asymptomatic dams' concentrations of 20.6±0.3, 42.7±4.0 and 3.0±0.8 in those organs on day 1, and 1.0±0.2, 20.2±4.0 and 2.3±0.9 on day 21. Of the initial mercury, 12% left the pups' brains over the first 21 days vs. 67% from the dams' brains. These data are consistent with evidence of others that the fetus may be more susceptible to methylmercury than are the mothers, and the brain more susceptible than are other organs.

Brain slices from methylmercury-treated pups incorporated significantly less label from 3-OH-butyrate into cholesterol, free fatty acids, phosphatidyl choline and phosphatidyl serine but ten fold more into sphingomyelin at the onset of rapid myelination (day 14), but there were no differences from controls by day 21. However, the inhibition of incorporation into the individual classes of lipids was not enough for changes in any one class to account for the decreased incorporation into total extractable lipids. The data therefore suggest a) inhibition of early steps between the uptake of 3-OH-butyrate by brain slices and its conversion via acetyl- and malonyl-CoA to lipids and b) either inhibition of the conversion of cytidine diphosphate choline to phosphatidyl choline or a defect in the myelination process itself with secondary changes in the metabolism of phospholipids related to myelin.

1021 HYPERTHERMIA IN INFANT RATS: EFFECTS ON IN VIVO PROTEIN SYNTHESIS, ORNITHINE DECARBOXYLASE ACTIVITY AND FREE AMINO ACID LEVELS IN BRAIN AND HEART. N.M. Millan* and F.L. Siegel. Depts. of Pediatrics and Physiological Chemistry, Center for Health Sciences, University of Wisconsin, Madison, WI 53706.

Acute hyperthermia was previously demonstrated to produce a reversible disaggregation of brain polyribosomes and a reversible increase of free amino acid levels in brain (L.L. Murdock, S. Berlow, R.E. Colwell and F.L. Siegel, Neuroscience, In Press). Injection of large amounts of amino acids also produces brain polyribosome disaggregation, but no inhibition of brain protein synthesis occurs in vivo, suggesting that polyribosomes disaggregate only when tissue is homogenzied. In the present investigation, we have determined the effects of hyperthermia on in vivo protein synthesis, ornithine decarboxylase activity and free amino acid levels on 7 and 21 day old rat brain, heart and liver. Our results indicate that pool-corrected in vivo protein synthesis is inhibited by 50% in both brain and liver following a 45 min period of hyperthermia (39.5°C), in 7 day old rats, but no inhibition is produced by hyperthermia in 21 day old rats. Protein synthesis returns to control levels when hyperthermic 7 day old rats are allowed to recover for 2 hr under normothermic conditions. Inhibition of protein synthesis occurs in cortex, midbrain, cerebellum and brain stem and also recovers in all brain areas. Acute hyperthermia for 45 min results in a total loss of ornithine decarboxylase (ODC) in all brain areas and a 39% loss of heart ODC activity. After 2 hr of recovery from hyperthermia, ODC activities in cortex and cerebellum were increased 100% over control; brain stem and midbrain ODC was 30-40% over control, whereas heart ODC activities were 400-600% greater than control values. Cycloheximide administration prevented the increase in heart ODC during the recovery period, suggesting that enzyme induction mediated the increase in enzyme activity. Similar increases in brain free amino acid levels were seen after hyperthermia and cycloheximide administration, suggesting that the increased amino acids result from, rather than contribute to, brain polyribosome disaggregation. β-adrenergic stimulation has been shown to increase heart ODC (J. Bartolme, C. Lau and T.A. Slotkin (1977), J. Pharm. Expt. Therap. 202, 510-518); in the present study pretreatment of infant rats with propranalol (10 mg/kg) or phenoxybenzamine (2.5 mg/kg) failed to prevent the ODC induction during recovery from hyperthermia suggesting that the effects described here do not result from adrenergic stimulation.

Supported by NIH grant HD09045.

1022 BRAIN MEMBRANE PROTEIN PHOSPHORYLATION EXAMINED BY IN VITRO AND IN VIVO LABELING: A FUNCTIONAL ROLE FOR BAND F? Joan C. Mitrius,* David G. Morgan,* Richard G. Conway,* Gary Benson,* and Aryeh Routtenberg. Cresap Neuroscience Laboratory, Northwestern University, Evanston, ILL 60201.

Previous reports from our laboratory have characterized by SDS polyacrylamide gel electrophoresis and autoradiography several synaptosomal phosphoproteins (bands $D_{1,2}$ (MW~83K), D_3 (~67K), E (~53K), F (~47K), G (~34K), and $H_{1,2,3}$ (~18-13K), using in vitro phosphorylation (Conway and Routtenberg, Brain Research, 139, 1978, 366-373) methods. It has been found that band F appears to be especially sensitive to various functional manipulations.

Passive avoidance training affects the in vitro phosphorylation of band F. An increase in band F phosphorylation was found in an osmotically shocked P_2 fraction of rat frontal cortex as a result of experience. Rats were handled daily prior to being trained in a simple learning task. A second group served as yoked-shocked controls, while a third group were handled controls that received no shock. Both trained and yoked-shocked animals displayed significantly greater in vitro phosphorylation of band F than the unshocked controls when sacrificed 24 hr after the experience.

A second approach to assess the functional significance of synaptosomal phosphoproteins was to examine which phosphoproteins were labeled in vivo and the extent of in vivo labeling. Animals with indwelling chronic cannulae injected with 25-100 μCi ^{32}P-orthophosphate into neostriatum were sacrificed by focused microwave radiation (3.0 sec, 2.5 Kw) 2 min to 24 hrs after initiation of the ^{32}P injection. Although the pattern of in vivo phosphorylation varied with time of incorporation, phosphoproteins labeled in vivo were found to co-migrate with phosphoproteins labeled in vitro. Band F demonstrated the most rapid and extensive phosphate incorporation at short time intervals. Indeed, at the shortest time points band F was the major phosphate acceptor.

Finally, subcellular studies indicate that band F is located, at least in part, in the synaptic complex.

Collectively, these data suggest that a synaptosomal substrate, band F, rapidly labeled in vivo and affected by experience may be involved in neuronal function, perhaps at synaptic locations. (Supported by MH25281 and NSF19388 to A. R.)

1023 PHENYLACETIC ACID IN BLOOD AND CEREBROSPINAL FLUID. Aron D. Mosnaim Dept. of Pharmac., Univ. of Health Sciences/Chicago Med. Sch. & Sch. of Grad. and Postdoc. Studies, Chicago, IL 60612 and Daniel L. Feingold* (Sponsor Vel Nair) Dept. of Biol. Sci., Univ. of Illinois, Chicago, IL 60612.

The levels of acid metabolites of biogenic amines have been extensively used to estimate their rate of metabolism. This approach may prove particularly helpful when studying some of the noncatecholic phenylethylamines in view of the relative analytical difficulties found in measuring these substances in biological fluids. We now report the presence of phenylacetic acid (PAAc) in rabbit and human blood and in rabbit, cat and human cerebrospinal fluid (CSF). Using a technique involving solvent extraction-PAAc purification, this acid was reacted with N,N-Bis (Trimethylsilyl acetamide) (BSA) (Madubuike, U., M.Sc. Thesis, UHS Sch. of Grad. & Postdoc. Studies) (1975) and the derivatives estimated by GLC. Radioactive PAAc was added to the initial samples and carried throughout the experimental procedure as internal standard for recovery (PAAc recovery range, as PAAc-BSA, 17-34%). This method provided a detection limit of about 2 ng of PAAc as PAAc-BSA and allowed differentiation of PAAc-BSA (retention time (RT) 18.4 min.) from the BSA derivatives of other possible interfering biogenic acids, e.g., mandelic, homovanillic, benzoic, m-methoxy PAAc and 3,4-dihydroxy PAAc, RT of 13.2, 17.5, 7.4, 27.2 and 17.5 min., respectively. Rabbit and human plasma showed a PAAc concentration of 11.3 ± 2.2 and 13.8 ± 2.7 ng/ml ± S.E.M., respectively. No PAAc could be detected in red blood cells. The levels of PAAc in rabbit, cat and human CSF were 1.13 ± 0.11, 1.17 ± 0.15 and 0.83 ± 0.10 ng/ml ± S.E.M., respectively. Each CSF samples, approximately 10 ml fluid, was obtained by pooling several (3-5) individual samples. Measurement of PAAc could be of importance as one of the parameters used in elucidating the postulated role of PEA in a number of disease conditions, including phenylketonuria and affective and extrapyramidal disorders. Supported in part by NIH General Research Support Grant FR-3566 and by Univ. of Health Sciences/Sch. of Grad. & Postdoc. Studies.

1024 EVIDENCE FOR THE UTILIZATION OF EXTRACELLULAR [γ-^{32}P]-ATP FOR THE PHOSPHORYLATION OF INTRACELLULAR PROTEINS IN SQUID GIANT AXONS. Harish C. Pant*, Susumu Terakawa* and Ichiji Tasaki (SPON: Stuart Judge), Lab of Neurobiology, NIMH, Bethesda, Md. 20014.

The squid giant axons provide a unique model system for the study of biochemical events related to electrogenesis. Some of the special features of this experimental preparation for biochemists is that the sheath can easily be separated from the axoplasm by extrusion.

With this preparation, the radioactive substrate [γ-^{32}P]-ATP, can be applied directly to extruded axoplasm in vitro under rigorously controlled conditions, or alternatiavely it can be injected into the axoplasm in situ, namely, in the excitable axon. In the present studies, we have analyzed the labeled proteins in axoplasm by the polyacrylamide gel electrophoresis method in sodium-dodecyl sulfate (SDS), after phosphorylation in vitro and in situ. The two major phosphorylated regions on the gel had molecular weights of 400,000 and 200,000 daltons. These two peaks appear to be neurofilament proteins of squid axoplasm. In addition, we have made a series of observations which yielded somewhat unexpected results indicating that the same set of proteins were phosphorylated in the axoplasm regardless of whether the [γ-^{32}P[-ATP was applied intracellularly or extracellularly. These results suggest that ATP in the extracellular space is, by some ATP-translocation mechanism, utilized in the process of intracellular phosphorylation. Measurements of the apparent influx of ATP across the squid axon membrane yielded results consistent with the view that ATP in the extracellular fluid could be transported into the axoplasm.

1025 REGIONAL CEREBRAL GLUCOSE METABOLISM DURING HYPOXIA. William A. Pulsinelli and Thomas E. Duffy, Dept. Neurology, Cornell University Medical College, New York, N.Y. 10021

Hypoxic-ischemic insults to the brain usually produce damage to vulnerable areas. Whether such variability reflects regional differences in blood flow or the tissues' metabolic responses remains unclear. We assessed qualitative differences in regional glucose metabolism by the ^{14}C-2-deoxyglucose (2-DOG) technique under conditions of moderate hypoxia in the physiologically-controlled "Levine" rat. Male rats weighing 250-300 gm were paralyzed and ventilated with 70% $N_2$0-30% O_2. The right common carotid artery was ligated and the FiO_2 of the inspired gas lowered to produce an arterial PO_2 of 28-32 mm Hg; arterial blood pressure was maintained above 100 mm Hg. After 10 min of equilibration, 50 uCi of ^{14}C-2-DOG was injected i.v. and the animal decapitated 30 min later. The brains were processed for autoradiography. Because blood flow to both cerebral hemispheres increases (greater contralaterally) in this model (Ginsberg et al. 1976) the insult to brain is one of uncomplicated hypoxia.

Autoradiograms of control rats (normoxic, right carotid ligated) showed no right-left asymmetries and gray matter appeared darker than white matter. Autoradiograms of hypoxic rats revealed alternating light and dark bands of activity in the right cerebral cortex which were not demonstrable in the left cortex. Compared to the left, there was increased activity in the right striatum and hippocampus. Hypoxia induced bilateral increases in 2-DOG metabolism in white matter (corpus callosum, internal cap., anterior commisure) such that they appeared as dark or darker than gray matter.

To determine the relationship of the alternating light and dark cortical columns to the cortical vasculature, hypoxic rats were perfused with black plastic microspheres (15 ±5 u) via the ascending aorta just prior to sacrifice. When the ^{14}C-2-DOG autoradiograms were superimposed upon the brain sections from which they were derived, the microsphere-laden arteries lined up over the light cortical columns; the dark columns were most distant from the arteries. If areas of cerebral cortex between penetrating arteries represent "microwatershed" zones (Welsh et al. 1977), then the cortical columns of higher 2-DOG phosphorylation correspond to regions of lowest tissue oxygen tension.

The hypoxia-induced changes in regional 2-DOG phosphorylation may reflect an increase in energy requirements and/or anerobic glycolysis. If anerobic glycolysis were increased only to meet normal energy demands, one would expect an identical increment rise in 2-DOG phosphorylation in both gray and white matter. The greater degree of 2-DOG phosphorylation noted in white versus gray matter during hypoxia suggests that energy requirements are increased, at least, in white matter structures.

1026 EFFECT OF METAL CHELATES OF L-DOPA ON THE CEREBRAL AND PERIPHERAL METABOLISM IN RATS. K.S. Rajan, R.D. Wiehle*, IIT Res. Inst., Chicago, Ill. 60616, and J.M. Davis, Ill. State Psych. Inst., Chicago, Ill. 60612.

In continuation of our earlier finding regarding the potential application of the metal chelation approach for improved replenishment of the dopaminergic pools of rat brain, studies were undertaken on the metabolic patterns of the tritiated DOPA in the brain and circulating blood after i.v. administration of ^{3}H-L-DOPA and a number of metal-^{3}H-L-DOPA chelates. Among the systems examined, Cu(II)-L-DOPA-ATP chelate was found to be the most satisfactory and consistent one yielding an increase of 130-150% in the overall transport into the brain, a similar increase in the brain catechol amines and a 50% increase in the aromatic aminoacids when compared to the ^{3}H-L-DOPA controls. On the basis of time course studies on L-DOPA, Cu(II)-L-DOPA (1:2), Cu(II)-L-DOPA-ATP (1:1:1), Zn(II)-L-DOPA-ATP (1:1:1) and the combination drug, L-DOPA + RO4-4602, it was found that the Cu(II)-L-DOPA-ATP chelate showed the largest increases in the brain levels of the aromatic aminoacids over long periods of time.

Whereas the brain levels of the aminoacids effected by the combination drug dropped drastically beyond 30 minutes, those of the Cu(II)-chelate remained steady beyond 90 minutes. Detailed results of the metabolic patterns and the time course studies of the different metal-L-DOPA chelates are presented and discussed in terms of the metal chelation approach.

1027 MORPHOLOGICAL LOCALIZATION OF ENHANCED BRAIN ACID PHOSPHATASE RESULTING FROM HYPOCHOLESTEROLEMIC DRUG ADMINISTRATION. R.B. Ramsey and V.W. Fischer. Depts. of Neurology, Anatomy, and Pathology, Saint Louis University, Saint Louis, Missouri 63104.

Previous study has demonstrated that the administration of hypocholesterolemic drugs to developing rats not only affects the lipid metabolism within the central nervous system (CNS) of these animals, but also alters the activity of certain hydrolytic enzymes within the CNS (Ramsey, abs. 6th Int'l Meeting Int'l Soc. Neurochem., Copenhagen, Denmark, 1977, p.42). When two hypocholesterolemic drugs, zuclomiphene (50mg/kg) and AY-9944 (5mg/kg), were used in combination, a pronounced increase in brain acid phosphatase was observed. Neither drug, used individually, was able to elicit a similar response. The response to the drug administration was rapid (within 24 hr), very large (four-fold increase in enzyme activity within 24 hr), and was accompanied by an equally rapid increase in brain total protein. Only minimal numbers of membranous cytoplasmic inclusion bodies were present in the CNS of these animals (Acta Neuropath. 36: 91, 1976), hence, the nature of the cellular and subcellular location of the enhanced acid phosphatase activity was of interest. The CNS of animals 6 days of age (24 hr after treatment) and 20 days of age (treated for 16 days) was examined by electron microscopy for acid phosphatase localization. Ultrathin sections of cerebral and cerebellar tissue of acute and chronically treated rats showed markedly the presence of numerous macrophages within perivascular spaces. These phagocytic cells were conspicuously identifiable by their cytoplasmic vacuolar and electron-dense inclusions, as well as an intense deposition of enzymatic reaction products. Examination of the parenchymal tissue did not reveal the presence of such products to a greater degree than was seen in sections of untreated animals.

Our observations would indicate that when the two drugs, zuclomiphene and AY-9944, are administered together to developing rats, they not only disrupt CNS sterol metabolism, but they also alter the tissue in or around the CNS vessels in such a way that an infiltration of macrophages results. The mechanism by which these drugs bring about this infiltration is yet to be determined.

1028 BRAIN RESISTANCE TO PROTEIN LOSS ON RESTRICTED PROTEIN INTAKE IN WEANLING RATS. Richard R. Rebert, Robert L. Chronister, Herbert E. Longenecker and L. Preston Mercer* Univ. So. Ala., Mobile, AL. 36688

Weanling rats (21 days old) were starved for 24 hours, then put on an ad.lib. standard diet (Purina Rat Chow, 23% protein) for three days. The white male rats were then assigned to one of 12 groups, each group with a different protein level. Six animals were assigned to each group: 0%, 1%, 2.5%, 4%, 6%, 8.5%, 12%, 16% 20%, 24%, 28% and Purina Rat Chow of 23%. Feeding and drinking were ad lib. with each animal in its own cage. Body weights, food and water intake were measured dach day. The diets were isocaloric in that carbohydrate made up any deficiency in caloric intake due to protein deficiency. The protein source was lactalbumin. Twenty-one days after the start of the study the animals were sacrificed. The brains were rapidly removed and prepared according to the procedures outlined for the Falck-Hillarp fluorescent technique. Each brain was sectioned at sacrifice into a frontal one-third, a mid one-third and a hind one-third. Brains from the 0%, 1%, 2.5%, 12%, 24% and 28% were randomly chosen for amino acid analysis. Brain weights did not show any great variation from that of the rats on the 24% or "normal" dietary protein intake. There were variations in free amino acid patterns which could not be statistically validated because of the small number of the sample. The general patterns of brain weights and of free amino acid patterns were within the limits of "normal". Body weights did show a significant change as did the food and water intake of the dietary protein restricted rats. The findings in this study are in accord with the known resistance of the brain to protein loss after the weanling period (e.g. after day 21) when the maturation of the rat brain is more or less completed. The significance of the brain maintenance of weight and general free amino acid patterns compared with that of the body in general under dietary protein limitations is emphasized.

This study was supported by an Intramural Grant (#22610) from the University of South Alabama.

1029 BRAIN ALDEHYDE METABOLISM IN ALCOHOLISM AND IN AFFECTIVE DISORDER. Eli Robins, Evelyn M. Cochran* and Juanita L. Carl.* Dept. of Psychiatry, Sch. of Med., Washington Univ., St. Louis, MO 63110.

The activities of the enzymes responsible for the metabolism of the "biogenic" aldehydes, aldehyde reductase and aldehyde dehydrogenase, have been measured in 12 regions of human brain. These activities were compared in subjects with affective disorder, subjects with alcoholism, and in matched controls. Aldehyde reductase was determined using p-nitrobenzaldehyde as substrate and NADPH as cofactor. Aldehyde dehydrogenase activity was determined using heptaldehyde as substrate and NAD^{+} as cofactor. The activities were determined by measuring the change in fluorescence of the nucleotide. In 11 of the 12 regions, the activity of the aldehyde reductase averaged higher in the alcoholics than in the controls ($p<0.0002$ for the series). These differences reached significance for three individual regions: rostral hippocampus ($p<0.02$), head of caudate ($p<0.05$) and tegmentum of pons ($p<0.05$). The range of activities among controls varied from 52.7 µmol/g/hr for frontal white to 30.5 µmol/g/hr for tegmentum of pons, a 1.7-fold range. For aldehyde dehydrogenase, the activities averaged lower in alcoholics as compared to controls for 10 of the 12 regions ($p<0.02$ for the series). This difference was significant for one individual region, temporal gray ($p<0.01$). The range of activities among controls varied from 5.8 µmol/g/hr for frontal white and for centrum semi-ovale to 16.4 µmol/g/hr for putamen, a 2.8-fold range. These enzymes are involved in the metabolism of the biogenic amines via oxidative (to carboxylic acids) or reductive (to alcohols) pathways. The relatively high levels of aldehyde reductase in white matter suggest that the glial cells play a role in the reductive pathway probably at the expense of the oxidative pathway.

1030 AN AEROBIC AND ANAEROBIC CONTROL OF OXIDO-REDUCTION OF MITOCHONDRIAL NICOTINAMIDE ADENINE DINUCLEOTIDE IN NEURONS OF FROG DORSAL ROOT GANGLION.
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A preliminary report (Rodríguez-Estrada, Soc. Neuroscience vol III Abs #1032) showed that steady state level of reduced nicotinamide adenine dinucleotide (NADH) was changed in vitro preparations of dorsal root ganglion neurons by rising the carbon dioxide partial pressure. This change was similar to that observed in isolated mitochondrial preparation (J.M. Lowenstein & B.Chance, J.Biol.Chem 243:3940,1968) after rising the hydrogen ion concentration. This work studied the effect of changes of hydrogen ion concentration on the steady state level of NADH, it was expected that the protonated form of NADH~X inhibit less the respiratory enzymes components and in anaerobiosis inhibit less its oxidation in the glycolytic stage. Fluorometric determinations of NADH were performed on in vitro preparations of dorsal root ganglion neurons as previously reported. pH change was measured continuously and it was decreased 0.8 unit with any CO_2 mixture used. CO_2/O_2 (2.5%/97.5%), CO_2/N_2 (2.5%/97.5%), N_2, replaced temporarily the O_2 of the chamber. NADH level showed an inmediate and fast decreased (2-3%) after O_2 was replaced with CO_2/O_2 and the NADH level returned slowly after CO_2/O_2 was replaced with O_2. NADH level showed an inmediate and fast decreased (2-3%) after O_2 was replaced with N_2 and the NADH level showed what has been mentioned before and on a prolonging of N_2 after 2-3 minutes a large increase of NADH level (10-20%) was observed. Now replacing N_2 with CO_2/N_2 the NADH level showed an inmediate and fast decrease (10-20%) followed by a later increased that reached a new level which is smaller than that observed in N_2. If CO_2/N_2 is replaced with CO_2/O_2 an oxidation of NADH was observed. When O_2 replaced CO_2/O_2 the level of NADH increased (2-3%). After 2-3 min of CO_2/N_2 replacing O_2 the NADH level increased, this change was smaller than that observed in N_2. The changes observed with CO_2 mixtures are attributed to changes of H^+ ion concentration and H^+ ion concentration controls the steady state rate of oxidation. This results indicate that mitochondrial NADH intervenes in the respiratory chain as well as in the anaerobic stage of glycolysis.

Partially supported by a Grant of Fund.J.M.Vargas.

1031 GENETIC REGULATION BETWEEN NEUROBLASTOMA AND GLIOMA CELLS IN CELL CULTURE AS MEASURED BY THE SYNTHESIS OF SPECIFIC PROTEIN SPECIES.
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Neuroblastoma, glioma and hybrids of neuroblastoma-glioma cell lines were grown in cell culture and protein species were separated and identified in the first dimension initially utilizing isoelectric focusing gels and proteins were separated further in the second dimension by SDS acrylamide gels. Proteins were identified which were dominantly expressed in neuroblastoma and also in hybrid cells and proteins were expressed dominantly in glioma and also hybrid cell cultures. Specific proteins were identified which were significantly expressed in neuroblastoma cells and much reduced in glioma cells, and also conversely so. The hybrid cell line expressed many of the neuroblastoma type proteins and relatively few of the glioma type proteins. Proteins labelled as H, K and E were dominant in neuroblastoma and hybrid cultures and proteins labelled as G, g and gs were dominant to glioma and hybrid cell cultures. A specific protein species (z) was identified in hybrid cells and was not present in either parental neuroblastoma or glioma cultures. Protein z was expressed however by the co-culturing of neuroblastoma and glioma cells strongly suggesting its induction may be dependent on a soluble factor. The z protein is 53,000 dalton and migrates near the tubulin subunits on a two-dimensional gel. Protein z in hybrid cells was demonstrated in both stained gels and by autoradiography. Chromosome analysis of hybrid cells confirmed the presence of both rat and mouse chromosomes. Recently, the protein z species was identified to be present also in homogenates obtained from the Ajax mouse brain. These studies emphasize the point that the determination of an enzyme activity or protein concentration in a homogenate of brain is in actuality the summated expression of both neurons and glia monitoring and regulating the genetic expression of the opposite cell. Biochemical neuronal-glial interaction is in operation in the intact brain as suggested by the finding of the z protein in Ajax mouse brain and may be a central mechanism of differentiation within the mammalian brain.

1032 TURNOVER OF THE FREE GLUCOSE POOL IN BRAIN.
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The recently developed autoradiographic deoxyglucose method can be used to determine quantitatively the rates of glucose utilization in localized regions of the central nervous system. The operational equation of the method originally derived is limited by the requirement that the plasma glucose concentration remain constant throughout the experimental period. A new operational equation has been derived that is free of this restrictive constraint, but it requires knowledge of the half-life of the free glucose pool in brain. A method has, therefore, been developed to measure the turnover rate constant of the free glucose pool. It is based on a kinetic model of the equilibration of the specific activity of the free glucose pool in the tissue with that of the plasma during an infusion of radioactive glucose. With the assumption of a constant arterial plasma glucose concentration during the short experimental period, the following equation has been derived:

$$(SA)_B(T) = Ke^{-KT}\int_0^T (SA)_P\, e^{+Kt}\, dt$$

where $(SA)_P$ = specific activity of the arterial plasma glucose content; $(SA)_B$ = specific activity of the free glucose pool in brain tissue at the time of killing; T = the time of killing; t = the variable, time; and K = the rate constant of the turnover of the free glucose pool in brain.

During a continuous intravenous infusion of [2-^{14}C]glucose over a measured interval from 2 to 3.5 minutes, timed arterial blood samples are drawn. At the end of the interval the animal is killed by microwave radiation. The protein of the blood and plasma samples and the brain tissue is removed by acid precipitation, and the free glucose in the acid-soluble fractions is isolated by ion-exchange chromatography, checked for purity by TLC chromatography, and assayed for glucose specific activity. The rate constant is computed from the equation after correction for the contribution of the glucose in the blood content of brain. The half-lives of the free glucose pool determined thus far in 6 normal conscious rats and 5 rats lightly anesthetized with thiopental are 1.2 (S.D. = ± 0.2) and 1.9 (S.D. = ± 0.4) minutes, respectively. The difference is significant at the $p < 0.005$ level. These results demonstrate that the free glucose pool of brain turns over with a significant half-life that varies with the functional and metabolic state of the brain.

1033 OCTOPAMINE AND SEROTONIN NERVE ENDINGS IN THE LOBSTER.
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Octopamine is synthesized and released from nerve cells in the proximal region of the second roots of thoracic ganglia in the lobster. Structures in the same region have now been found to contain, synthesize, store and release serotonin (5-HT). Tryptophan is taken up by the roots by two processes; one a high affinity (K_m = .3 μM) sodium-independent mechanism and the second a low affinity mechanism. Ninety percent of the tryptophan taken up by the high affinity system is immediately converted to 5-HT unless the synthesis of 5-HT is blocked by a decarboxylase inhibitor. In the latter case, the tryptophan is still taken up and partially converted to 5-HTP. A high affinity (K_m = .8 μM) sodium-dependent uptake of 5-HT is also found in the roots. When animals are treated with 5,7 dihydroxytryptamine, the endogenous 5-HT content and the ability to synthesize and store 5-HT are lost. In addition, the high affinity component of tryptophan uptake disappears while octopamine synthesis from tyrosine or tyramine is unaffected. This suggests that separate systems for the synthesis, storage and release of octopamine and 5-HT are found within the same region of the roots.

To determine what structures might contain the amine systems, electron microscopic studies were performed. Groups of cell bodies having the typical appearance of neurosecretory cells surrounded by numerous granule-filled endings were found in the proximal regions of the roots. We have identified four types of endings: Type 1 contains large (200 nm) dense-core granules and clusters of small (50 nm) round, clear vesicles; Type 2 contains large (200 nm) granules with a distinct crystalline substructure and small clear vesicles; Type 3 is filled with large (200 nm) granules with a pale homogeneous matrix; Type 4 contains small pleiomorphic clear vesicles and abundant glycogen granules.

To determine which type of ending is associated with each amine, roots were incubated with either 2μM ^{3}H-tyramine or 0.2 μM ^{3}H-tryptophan under conditions in which nearly all of the radioactive precursor was converted to either octopamine or 5-HT. The tissues were then processed for EM autoradiography. Newly synthesized octopamine was confined to the Type 2 endings whereas newly synthesized 5-HT was found in the Type 4 endings. When tissues were incubated in low concentrations (0.1 μM) of 5-HT, radioactivity was found again in the Type 4 endings. In animals treated with 5,7 dihydroxytryptamine, a selective degeneration of the Type 4 endings was seen. Thus, our studies indicate that octopamine and 5-HT are synthesized in morphologically different types of endings.

(Supported by NIH).

1034 ACETYLCHOLINE AND SEROTONIN CONTENT IN BRAIN AREAS OF RATS DURING PERIOD OF BEHAVIORAL DEPRESSION FOLLOWING D,L-5-HYDROXYTRYPTOPHAN ADMINISTRATION. P.A. Shea, J.N. Hingtgen* and M.H. Aprison.
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It has been found that administration of D,L-5-hydroxytryptophan (5-HTP) to pigeons working on a food-reinforced operant schedule produces a period of behavioral depression (response rates less than 50% baseline) that is temporally related to increased levels of total serotonin (5-HT) in the telencephalon and diencephalon plus mesencephalon of the brain (Aprison et al., FED. PROC. 34: 1813, 1975 for review). To study serotonergic changes in smaller areas of the rat brain following 5-HTP, as well as possible cholinergic changes associated with this type of depression, injections of D,L-5-HTP (50 mg/kg s.c.) or saline were given to rats working on a VI 1 schedule (milk reinforcement for lever pressing) to establish the average period of suppressed responding. Subsequently, rats were given 5-HTP 15 min after the start of a VI session and were killed at a time equivalent to 30% of their total period of depression (when the response rate had decreased to 0) or at a period when their responding had returned to normal levels following depression. Other trained rats were given saline and killed at times comparable to the 5-HTP treated rats. The method of killing was the modified near-freezing technique of Shea and Aprison (ANALYT. BIOCHEM. 56: 165, 1973). However, instead of the animals being removed from the lever-pressing apparatus and dipped into liquid nitrogen in a separate cage, a new device permitted the entire behavioral chamber to be submerged at any time during the VI session. The following brain areas were dissected at -10°C and were assayed for 5-HT and acetylcholine (ACh) content (Smith et al., ANALYT. BIOCHEM. 64: 149, 1975): hippocampus, striatum (ST), telencephalon minus hippocampus and striatum (TEL), diencephalon, mesencephalon and pons plus medulla oblongata (P+M). Levels of 5-HT were significantly elevated (25% to 84% over controls) in all brain areas except P+M during behavioral depression with levels in the TEL returning to normal by the time response rates had returned to within the normal range. ACh was significantly elevated during depression in the ST (+34%) and P+M (+16%). As in the pigeon studies, 5-HT changes in at least one brain area of the rat appear to be correlated with the behavioral depression following 5-HTP. The observed changes in ACh content during 5-HTP induced depression reported in this study are supportive of the concept of cholinergic involvement (Hingtgen et al., SCIENCE 193: 332, 1976) in certain types of behavioral suppression. (Supported in part by research grant MH-03225-18 from NIMH).

1035 DIFFERENCES IN TURNOVER OF THE BIOGENIC AMINE AND AMINO ACID PUTATIVE NEUROTRANSMITTERS CONCURRENTLY MEASURED IN THE CEREBRAL CORTEX AND STRIATUM OF RAT BRAIN. J. E. Smith, C. Co*, T. R. Mote* and J. D. Lane. Dept. of Psychiat, Louisiana State Univ. Med. Ctr., Shreveport, LA 71130.

The turnover rates of the putative neurotransmitters in various brain regions may be a better measure of the activity of these neuronal systems than are measures of content alone. The turnover of acetylcholine (ACh), dopamine (DA), norepinephrine (NE), serotonin (5-HT), aspartate (Asp), glutamate (Glu), gamma-aminobutyric acid (GABA) and glycine (Gly) were concurrently measured in the cerebral cortex and striatum after injections of radioactive precursors. Ten rats were injected with 0.5 mCi ^{3}H-tryptophan, 1.0 mCi ^{3}H-tyrosine, 0.5 mCi ^{3}H-Choline and 0.2 mCi ^{14}C-glucose through chronic jugular catheters. Five rats were sacrificed by near freezing in liquid nitrogen at 60 min after the administration of ^{3}H-tryptophan, ^{3}H-tyrosine and ^{14}C-glucose and 4 min after ^{3}H-Choline. Five rats were sacrificed at 90 min after the administration of ^{3}H-tryptophan, ^{3}H-tyrosine and ^{14}C-glucose and 7 min after ^{3}H-Choline. The brains were removed and dissected at -18°C into the cerebral cortex (CC) and striatum (Str). The tissue samples were individually pulverized in liquid nitrogen and the biogenic amines (ACh, DA, NE and 5-HT) extracted from one portion of the tissue powder into 1N formic acid-acetone (v/v-15:85) (FA/A). DA, NE and 5-HT were separated from a portion of this FA/A extract using a cation exchange resin, assayed fluorometrically for content and then isolated and assayed for radioactivity with alumina columns and TLC using a previously described procedure (Lane et al, Life Sci 21, 1101, 1977). Acetylcholine was assayed for content and radioactivity by a radioenzymatic method and paper electrophoresis using a previously described procedure (Shea and Aprison, Anal. Biochem. 56, 165, 1973) from another portion of the FA/A extract. The amino acid putative neurotransmitters were extracted into 5% TCA from a 10 mg portion of the powdered tissue samples and their respective dinitrophenyl derivatives separated by two dimensional TLC (Brenner et al, Exper. 17, 145, 1961) and assayed for content and radioactivity. DA turnover in the Str (197.0 pmoles/mg protein-hr) was ten times that seen in the CC (18.9 pmoles/mg protein-hr) while 5-HT turnover was four times higher in the CC than in the Str (28.9 and 8.9 pmoles/mg protein-hr respectively). Asp and Glu turnover in the CC was twice that in the Str (Asp 40.8 and 23.8 pmoles/mg protein-hr; Glu 159.4 and 87.6 pmoles/mg protein-hr). Gly turnover in the Str (24.5 pmoles/mg protein-hr) was three times that in the CC (9.6 pmoles/mg protein-hr). NE turnover in the Str appears to have a completely different time course than the other neurotransmitters investigated. (This project was supported in part by USPHS Grant DA-01999-02.)

1036 MEMBRANE FLUIDITY AND SYNAPTOSOMAL ATPASES. Albert Y. Sun.
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Several experimental data have indicated the dependency of synaptosomal ($Na^{+}+K^{+}$)-ATPase and Ca^{++}-ATPase on the integrity of membrane structure. Low concentrations of detergents and phospholipases treatment interfered with the activities of both enzymes. However, the biphasic response to various concentrations of ethanol was observed only with ($Na^{+}+K^{+}$)-ATPase. At low concentrations (0.1-0.5%) ethanol enhanced ($Na^{+}+K^{+}$)-ATPase, probably as a result of an increase in membrane fluidity. At higher levels of ethanol (1-4%), both ($Na^{+}+K^{+}$)-ATPase and Ca^{++}-ATPase activities were inhibited, probably due to a disturbance of the membrane structure through hydrophobic alcohol-membrane interaction. A sharp decrease in the energy of activation was also observed with the ($Na^{+}+K^{+}$)-ATPase when membrane lipids changed from liquid crystalline state to a more fluid state at transition temperature. It is concluded that ($Na^{+}+K^{+}$)-ATPase may be more sensitive to the microenvironmental changes than Ca^{++}-ATPase. (Supported in part by USPH Grant #AA02054).

1037 INACTIVATION OF BRAIN GLUTAMATE DECARBOXYLASE BY ALKALINE PHOSPHATASE. P.Y. Sze, B.J. Hedrick* and R. Alderson*. Dept. of Biobehavioral Sci., Univ of Connecticut, Storrs, CT 06268

Alkaline phosphatase (AP) is known to dephosphorylate a variety of phosphoproteins as well as low molecular weight phosphate esters. In this study, the effect of the phosphatase on brain L-glutamate decarobxylase (GAD) was examined. GAD was prepared mainly as ammonium sulfate precipitate (30-70% cut) from 100,000g supernatant of mouse brain homogenate. The GAD preparation was incubated in the presence of purified calf intestine AP at 37°C in 50 mM Tris acetate, pH 7.4. The AP was then removed by DEAE-cellulose chromatography, and the activity of GAD was measured radiometrically from 1-^{14}C-L-glutamate. Alternatively, GAD activity was determined directly in the incubation mixture after the AP activity was completely inhibited by 0.2 M phosphate.

Incubation with AP is found to produce a rapid loss of GAD activity. The inactivation follows second order kinetics, with a half-life estimated as 30 min. at the concentration of AP used (80 ug/ml). The ability of AP to inactivate GAD, like its ability to dephosphorylate p-nitrophenyl phosphate, is dependent on zinc and inhibited by inorganic phosphate, EDTA, or arsenate. From Lineweaver-Burk plots, the inactivation of GAD leads to a decrease in V_{max}, with no change in the apparent K_m either for glutamate or for PLP. There is no difference toward AP inactivation between GAD preparations from cell cytosol and synaptosomes, or from various brain regions.

Two possibilities are considered for the action of AP on GAD. First, AP may act on the enzyme-bound cofactor PLP. This possibility, however, does not seem to be compatible with the following data: (1) The inactivated GAD cannot be reversed to activity by PLP; and (2) the inactivation by AP cannot be prevented by the addition of PLP at high concentrations. The other possibility is that AP may act by hydrolyzing protein-bound phosphate, leading to inactivity of GAD. Both possibilities are being tested.

(Supported in part by MH-29237)

1038 MYOSIN ISOLATED FROM BOVINE BRAIN. Michael Toma*, C. Mahendran* and Soll Berl. Dept. Neurology, Mount Sinai School of Medicine, New York, New York 10029.

Kinetic studies have been initiated on myosin isolated from bovine brain. The protein was prepared in the following sequence (modified after Pollard, T.D., et al., Anal. Biochem., 60:258, 1974.):one hour extraction at high ionic strength in 0.6M KCl, 1.6mM $MgCl_2$ and 10mM PPi, precipitation by dialysis to low ionic strength (0.03M KCl), 25-55% ammonium sulfate fractionation, and column chromatography on Biogel A-15M, 200-400 mesh. Fractions having K^+-EDTA ATPase activity were pooled and subjected to low ionic precipitation in the presence of 10mM $MgCl_2$. When treated with sodium dodecyl sulfate (SDS) and 8M urea, this protein reveals a similar electrophoretic pattern to skeletal muscle myosin on SDS polyacrylamide gels. Dialysis at low ionic strength results in the formation of bipolar filaments observed on carbon coated grids stained with uranyl acetate in the electron microscope. These filaments coalesce in a head to head fashion to form periodic arrays. At pH 7.0 and 0.6M KCl, in the presence of Ca^{2+}, the specific activity is 0.406 - 0.600 μmoles inorganic phosphorous mg^{-1} min^{-1}, and in the presence of 2mM EDTA, 0.836 - 1.100 μmoles inorganic phosphorous mg^{-1} min^{-1}. The K^+-EDTA activity of this enzyme reveals typical Michaelis-Mentin kinetics for substrate saturation, and a Km of 10^{-4}M as determined by the Lineweaver-Burke and Eadie-Hofstee plots. The data will be compared with that of rabbit skeletal muscle myosin. Supported in part by NIH Grant NS 11824, The Clinicial Center for Research in Parkinson's and Allied Diseases, NINCDS Grant NS 11631.

1039 NERVOUS SYSTEM CARBONIC ANHYDRASE. Michael C. Trachtenberg and Victor S. Sapirstein*. Neurol. Svc., VA Hosp., Neurol. Dept., BU Sch. Med., Boston, MA 02130 and Dept. Biochem., E.K. Shriver Ctr., Waltham, MA 02154, Dept. Biol. Chem., Harvard Med. Sch., Boston, MA 02115

The activity of carbonic anhydrase (CA) was examined in a wide variety of well defined structures in both the CNS and PNS of the rat. The activity of CA in white matter from forebrain regions (e.g. subcortical white matter, optic nerve) is uniformly higher than that in cortical gray matter (e.g. occipital and parietal corticies). However, retinal-choroid preparations have the highest activity of the structures examined. The difference in activity between gray and white is evident in both the membrane bound and soluble fractions, with membrane bound activity accounting for approximately 60% of the total. By way of a competitive binding procedure utilizing ^{3}H-acetazolamide, it was found that the differences observed in the activity reflects differences in the moles of enzyme present and not differences in the state of activation. The activity in predominantly white matter regions (spinal cord, myelencephalon, pons, mesencephalic tectum and subcortical white) is characterized by a neuraxial progression with activity increasing rostrally. Preliminary evidence suggests that these differences in activity reflect differences in enzyme levels as determined by displacement of ^{3}H-acetazolamide. In gray matter regions from metencephalon to telenecphalon relatively little variation in enzyme activity is seen suggesting that the CA content of glia in gray matter (astrocytes) is constant throughout these areas of the CNS. However, oligodendroglia and astrocytes from white matter differ in their expression of CA along the neuraxis with greater activity in the later developing structures. Our previous report (Neurosci. Abst. 3:1034, 1977) of marked differences in CA in purified myelin derived from spinal cord, lower and upper brain stem suggests that the neuraxial distribution is attributable to variation in the oligodendroglial enzyme. The CA activity in PNS is uniformly low with comparable activity in sciatic nerve, spinal roots, superior cervical and trigeminal ganglia. The values obtained are equal to or lower tan those for spinal cord, lowest of all CNS regions.

1040 EFFECTS OF SODIUM OCTANOATE ON BLOOD-BRAIN BARRIER TRANSPORT. Doris A. Trauner. Dept. of Neurosciences, Sch. Med., UCSD, San Diego, CA 92103.

Short chain fatty acids (SCFA) produce coma, hyperventilation, and seizures in experimental animals within 5-10 minutes after a single intraperitoneal injection. The rapidity of action suggests a direct effect of SCFA on the nervous system, and leads to speculation about changes in blood-brain barrier transport as a potential mechanism. In the present pilot study, effects of the SCFA sodium octanoate on two aspects of blood-brain barrier transport, passive diffusion and active transport, were examined using a double tracer technique.

Experimental rats were given intraperitoneal injections of 1 molar sodium octanoate, pH 7.4, 1 mM/100 gm body weight, at 30, 60, 90, 120, and 240 minutes before sacrifice. Control rats received either normal saline or no injection. Animals were anesthetized with ether and the carotid artery isolated. In the first group, ^{14}C-tryptamine, which crosses the blood-brain barrier by passive diffusion, was injected into the carotid artery simultaneously with ^{3}H-water, which is freely diffusable into brain. The animals received a total volume of 0.2 ml solution. They were then decapitated in 5 seconds, the brains quickly removed, and sections prepared for scintillation counting. The ratio of ^{14}C to ^{3}H in brain compared with the ratio of these isotopes in the original injection mixture provides an index of brain uptake (BUI) of test substance (1). A second test group was given ^{14}C-tyrosine and ^{3}H-water using the same procedure described above. Tyrosine crosses the blood-brain barrier by an active transport mechanism.

The BUI of control animals was 16.1% in the tryptamine/water study. 30 minutes after octanoate injection, the BUI rose to 27.7%, $P<.05$. The peak increase occurred at 1 hour (36%, $P<.01$) and was still elevated at 4 hours after injection (34%, $P<.05$). Similar increases in BUI occurred with tyrosine. A BUI of 43.6% was found in controls, while in experimental animals the BUI rose to 57.7% in 60 minutes and to 56.7% by 2 hours.

These preliminary studies suggest that both passive diffusion and active transport mechanisms which normally protect the blood-brain barrier may be altered in the presence of octanoate. Such alterations in transport would provide one explanation for the rapidity with which coma and electroencephalographic changes occur after injection of sodium octanoate into experimental animals.

This research was supported by the Easter Seal Research Foundation, Grant #N-7704, National Easter Seal Society.

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1041 EFFECTS OF CATECHOLAMINE METABOLITES AND THEIR OXIDATIVE PRODUCTS ON THE UPTAKE OF ^{3}H-NOREPINEHRINE BY A CRUDE SYNAPTOSOMAL FRACTION OF RAT BRAIN. Anthony D. Vanker and Frank L. O'Brien*. Depts. of Biology and Chemistry, Georgia State University, Atlanta, Georgia 30303.

There is evidence that at least some of the effects of 6-hydroxydopamine (6-OHDA) are due to its oxidation to the corresponding quinone which is thought to subsequently undergo nucleophilic reactions (Sachs, C. & Jonsson, G. (1975). Biochem. Pharmacol. 24, 1). The inhibitory effects of 6-OHDA on norepinephrine (NE) uptake into presynaptic elements is well known. Similar quinones could be formed from normal catecholamine metabolites (CAMT). It is, therefore of considerable interest to investigate the effects of CAMT and their oxidative products on the uptake of NE. Controlled potential coulometry was chosen as a means of oxidizing selected CAMT because: 1) there was no contamination by oxidizing reagents 2) there was exact control of potential 3) the corresponding quinone can be formed in the presence of synaptosomes without greatly affecting the synaptosomes. The forebrains of adult female Sprague-Dawley rats were used to isolate a crude synaptosomal fraction which was then resuspended in 0.32 M sucrose. A portion of this suspension was mixed with a physiological salt solution containing 25 mM TES (N-tris [hydroxymethyl]-methyl-2-aminoethane sulfonic acid) at pH 7.4 (NEP). Different portions were then used in each of the following experiments: 1) NEP + stirring 2) NEP + stirring + CAMT 3) NEP + stirring + CAMT + electrolysis. The time interval for each experiment was 10 min. Immediately following each experiment, standard procedures were employed to measure the uptake of ^{3}H-NE into the synaptosomes found in NEP. The effects of the oxidation products of CAMT varied from essentially complete inhibition at 10^{-4} M to almost no inhibition at 10^{-6} M. The corresponding CAMT exhibited considerably less inhibition at most concentrations. At the higher concentrations studied, the electrochemical data indicate substantial secondary oxidation reactions occur in the presence of synaptosomes.

1042 A SENSITIVE METHOD FOR THE SEPARATION AND MEASUREMENT OF S-ADENOSYLMETHIONINE AND S-ADENOSYLHOMOCYSTEINE IN MAMMALIAN TISSUE.

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Relative levels of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) may provide local modulation and regulation of the methyltransferases, t-RNA methyltransferases, protein methyltransferases, and catecholamine methyltransferases. We have been studying the regulation of the catecholamine methyltransferases and have recently found that an endogenous stabilizing factor exists which protects adrenal medullary phenylethanolamine-N-methyltransferase (PNMT) from thermal and tryptic degradation. This stabilizing factor is a small molecule which is dialyzable and has an absorption maximum of 265 nm. Preliminary evidence suggests that this factor may be S-adenosylmethionine. Moreover, while SAM does protect PNMT, SAH does not render protection against thermal and proteolytic denaturation. Our current thinking is that the interconversion of SAM to SAH may modulate PNMT levels *in vivo* by increasing the susceptibility of the enzyme to proteolysis.

Since the endogenous levels of SAM and SAH may be critical to the understanding of PNMT regulation, a sensitive assay system must be developed which allows for the physical separation of SAM and SAH and their subsequent quantitation. Separation of these two compounds is critical, since SAH is an extremely potent inhibitor of SAM-dependent methyltransferases. We have devised an appropriate separation system combined with a sensitive enzymatic assay and have used it to measure tissue levels of SAM. SAM and SAH are separated on an ion-exchange column (DOWEX 1 X 8). SAM is not bound to this column, while SAH is retained and can be eluted with NaCl. The levels of SAM are estimated by reaction with purified hydroxyindole-O-methyltransferase and N-acetylserotonin, modified from a procedure described by Baldessarini and Kopin. This isotope-dilution assay is linear from 0-1.96 nmoles SAM. Tissue levels of SAH are measured by direct inhibition of HIOMT. This assay is linear from 0-0.12 nmoles SAH. Recoveries of both SAM and SAH average 70-75%, with only 1-2% contamination of SAM by SAH.

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1043 DOES APOCRINE SECRETION OCCUR IN THE CHOROID PLEXUS? W.F.Agnew, T.G.H. Yuen*, Cheng, J.T.* and R.B.Alvarez*. Neurological Research Laboratory, Huntington Institute of Applied Medical Research, Pasadena, California, 91105.

Apical blebbing of the choroid plexus has been observed in 8 species in a comparative ultrastructural study which included amphibian, reptilian and mammalian forms. A secretory function for the apical protrusions was suggested by ultrastructurally observed stages including attached and free-floating profiles which underwent swelling and eventual rupture in the CSF space. Membrane dynamics studies using ruthenium red and alcian blue markers, the observation of bleb formation in tissue culture preparations of choroidal epithelium and the stimulation of bleb formation by paracentesis speak against the artefactual origin of these protuberances.

Protein synthesis studies utilizing intravenously administered C^{14}-phenylalanine demonstrated that incorporation of this amino acid in the choroid plexus exceeded that observed in either liver or brain parenchyma. Light and electron microscope autoradiographs following H^3-leucine and H^3-phenylalanine administration have demonstrated preferential intracellular labelling in choroidal epithelium as well as blebs, both in vivo and in tissue culture preparations. These findings, together with previous reports, suggest that the apical blebbing phenomenon represents a physiologically significant source of CSF proteins.

Supported by U. S. Public Health Grant #1 ROl NS12906-01A1.

1044 INTRAVENTRICULARLY ADMINISTERED PUROMYCIN: AN APPLICATION OF THE SWITZER-GOLGI TECHNIQUE TO THE STUDY OF NEUROSECRETORY AND OTHER NEURONS. William E. Armstrong, Steven Warach* and Glenn I. Hatton. Depts. Psychol. and Zool. and Neurosci. Program, Michigan State Univ., E. Lansing, MI 48824.

Switzer's (Neurosci. Lett. 2:301, 1976) technique of puromycin induced argyrophilia was investigated as a means of staining hypothalamic neurosecretory neurons, long noted for their resistance to impregnation with Golgi methods. Injections of 5µl puromycin dihydrochloride (0.1M) into the right lateral ventricle of rats were followed by survival times of 6-48 hours, after which the brains were fixed and sections stained with a modification of De Olmos' (Brain Res. 33:523, 1971) cupric silver technique for degenerating neurons. Optimal impregnation of neurons in several hypothalamic nuclei was observed at 12-18 hr survival. Included were the following neurosecretory nuclei: paraventricular nucleus (PVN), anterior commissural nucleus (ACN), and nucleus circularis (NC). The locations of cells in these nuclei corresponded with those of HRP-labelled neurons in other rats following injections into the neurohypophysis. Thus, they are probably neurosecretory. There is little doubt that our argyrophilic neurons in ACN and NC are neurosecretory, but we are less certain about those in PVN since many unlabelled cells are present there in the HRP material. The results show that, contrary to previous investigations, the neurons across these nuclei are of a heterogeneous morphology. For example, some cells of NC appear monopolar while several of those in PVN and ACN are multipolar with many processes oriented perpendicular to and encroaching upon the third ventricle. A variety of cell types exhibiting a great range in size and number of processes were found in PVN.

Additionally, extensive staining was observed in thalamus, hippocampus, caudate, cerebral cortex, septum, and non-neurosecretory hypothalamic areas, making this approach valuable for impregnating neurons of various types and locations. However, the staining observed in thalamus and hypothalamus is selective, as some areas (e.g., anterior hypothalamus, ventromedial hypothalamic nucleus) stain while others (e.g., suprachiasmatic nucleus, arcuate nucleus) do not. Whether this selectivity is a result of selective availability of puromycin or of its particular interaction with certain neurons has not been determined.

Supported by research grant no. NS09140 from NINCDS.

1045 DIGITIZER ASSISTED QUANTITATIVE STUDY OF BOUTONS CONTACTING NEURONS IN THE SACRAL PARASYMPATHETIC NUCLEUS. H. Keith Brown and Michael F. Nolan. Dept. Anat., Col. of Med., Univ. of So. Fla., Tampa, Fl., 33612.

Methods for quantitative determination of synaptic connections are essential for studies of synaptic reorganization and plasticity. Such methods are already contributing much to the understanding of synaptic population dynamics on spinal motoneurons (Bernstein and Bernstein, J. Neurocytol. 6:85-102 and Conradi and Ronnevi, J. Neurocytol. 6:195-210). The purpose of this study is to describe the frequency of occurance and postsynaptic coverage of each type of bouton found in the spinal nucleus responsible for urinary bladder function.

Adult male and female cats were anesthetized with pentobarbital and perfused through the heart with aldehydes for fixation. Sacral spinal segments were removed and processed for electron-microscopy. The sacral parasympathetic nucleus (SPN) was identified and a total of 455 micrographs (7,100X) were made of the neuropil. The cursor of an Hewlett-Packard 9864A digitzer was used to measure the diameter and perimeter of each dendrite or soma and the length of postsynaptic membrane covered by each bouton profile. These data as well as the number and type of each bouton present were recorded by the interfaced Hewlett-Packard 9815A calculator. This information was subsequently tabulated and provided the following observations.

Boutons which contain clear spherical vesicles (S boutons) occur at the same frequency on somata, large dendrites (>1 µm dia.) and small dendrites (<1 µm dia.). These S boutons average 19 per 100 µm of postsynaptic membrane (19 btn/100 µm). Bouton profiles which contain spherical vesicles and three or more dense core vesicles (GS boutons) are present on somata (3 btn/100 µm) but show a higher frequency on large dendrites (8 btn/100 µm) and small dendrites (10 btn/100 µm). Boutons with flattened vesicles (F boutons) are more numerous on large dendrites (3.19 btn/100 µm) than on the somata (0.9 btn/100 µm). Boutons cover 18% of the surface of somata and 30% of the surface of dendrites. These results demonstrate specific population differences on postsynaptic structures in the SPN and show that GS boutons are primarily applied to small dendrites while most F boutons are associated with large dendrites.

(Supported by NIH 5S01 RR05749-03)

1046 APPARENT PRESYNAPTIC ELEMENTS FORMED ON POLY-BASIC COATED BEADS. Richard W. Burry and John G. Wood, Department of Anatomy, University of Tennessee Center for the Health Sciences, Memphis, Tenn.

In the developing CNS, neuronal processes grow to target neurons and form synaptic contacts. The precise mechanisms that guide these processes and allow selection of proper target neurons are not known. Surface groups of neuronal processes must contain at least some of the information required for establishment of synaptic contacts. We have investigated the effect of modifying surface charges of developing neurons. During synaptogenesis, compounds with different charges were added to cell cultures of the CNS.

Dispersed cell cultures of 2 day old rat cerebellums were prepared as described by Lasher (Brain Res., 69 (1974) 235). In order to stabilize the compounds added to the living cultures, proteins of different charge were coated on to cyanogen bromide activated sepharose 4B beads. The coated beads were then added to cultures at 7 days in vitro, when synaptogenesis had already begun. The cultures were followed using phase optics or fixed for electron microscopy. Beads coated with poly-basic proteins (histone or poly-lysine) attached to the cultures while beads coated with neutral proteins (BSA) or poly-acidic proteins (polyglutamate) did not attach. Beads coated with Con A also attached to the cultures apparently via a sugar binding mechanism and not by the electrostatic mechanism of the poly-basic compounds.

Within 24 hrs., both neuronal and non-neuronal cells had grown up onto the attached beads. Electron microscopic observations showed swellings of neuronal processes which were closely applied to the poly-basic protein coated beads. These swellings contained aggregates of vesicles the size of synaptic vesicles (20 nm) close to the attachment site with the bead. The morphology of this swelling resembled that of a presynaptic element but in place of the postsynaptic element was the protein coated bead. These swellings were called apparent presynaptic elements.

The apparent presynaptic elements were found only as parts of neuronal processes attached to the poly-basic protein coated beads and were not seen with neuronal cell bodies or glial cells attached to the beads. In addition Con A coated beads had many neuronal processes running over them, but no apparent presynaptic elements were seen adjacent to the Con A coated bead.

These results suggest that charged groups on the surface of neuronal membranes carry some of the required information necessary to establish synaptic contacts.

Research support: N.I.H. Training Grant GM-00202, and N.I.H. Grant NS-12590. Alfred P. Sloan Foundation (JGW).

1047 NUCLEAR INVAGINATIONS IN DEVELOPING HAMSTER NEURONS. MB. Tank Buschmann and A. LaVelle. Dept. Anat. & Gen. Nursing, Coll. Med. & Coll. Nursing, Univ. Ill. Med. Ctr., Chicago, IL 60612.

Deep nuclear invaginations have been frequently observed in rapidly growing and in adult neurons undergoing the retrograde reaction. For example, in large motor neurons, as of cranial and spinal nerves, nuclear invaginations appear during the intense, early phase of perikaryal growth when the nucleolar apparatus is also increasing in size; with leveling off of this growth activity, in maturity, the invaginations disappear or are greatly diminished (LaVelle & LaVelle; Tennyson, Dev. Neurobiol., Himwich, ed., 1970). In the retrograde reaction membrane "folding" or invagination has been particularly noted during the peak of chromatolysis (Lieberman, Int. Rev. Neurobiol. 14: 49, 1971). On the basis of such observations, it has been indicated that the invaginations may function in nuclear-cytoplasmic exchange (Hydén, Acta Physiol. Scand., 6: 1, 1943) and their increase may be related to enhanced neuronal metabolism (Lieberman, Int. Rev. Neurobiol. 14: 49, 1971).

In the case of the hamster, in pyramidal cells of layer V, the period of very rapid perikaryal growth is from newborn to 10 days of age by which time adult nuclear and perikaryal volumes are reached. At about 5 days, nuclear invaginations became apparent and their depth and frequency increased progressively with age in the pyramidal cells. However, instead of gradually diminishing following cessation of this rapid perikaryal growth as observed in certain other neuronal types, the depth and frequency of the invaginations continued to increase and persisted at all ages up to 18 months. Illustrations of this sequence of nuclear invaginations of pyramidal cells in layer V of hamster motor cortex will be presented.

It would appear that in these hamster pyramidal cells the persistence of invaginations with age through maturity may indicate a continuation of high metabolic activity which was established during the early growth period. This speculation has yet to be confirmed by more direct metabolic studies.

1048 DEVELOPMENT OF SUPRAEPENDYMAL NEURONS ASSOCIATED WITH THE MEDIAN EMINENCE OF THE HAMSTER. J.P. Card*[1] and J.A. Mitchell, Dept. of Anatomy, Wayne State University School of Medicine, Detroit, Michigan.

In a previous study (Card and Mitchell, Anat. Rec. 187: 544, '77) of the infundibular recess of the adult hamster a well organized cluster of intraventricular nerve cells and processes was consistently found associated with the nonciliated ependyma of the median eminence. The following study was undertaken to examine the developmental features of this cluster during the perinatal period. Brains were collected and fixed with Karnovsky's aldehyde fixative on days 11-15 of gestation and on days 1,2,5,8 and 10 post partum. The floor and walls of the third ventricle in the region of the infundibular recess (IR) were exposed by microdissection and subjected to routine processing for scanning electron microscopic (SEM) examination. Selected specimens at each stage of development were subsequently processed for correlative transmission electron microscopic (TEM) examination. SEM examination of the ventricular surface at 11 days of gestation revealed that the majority of the ependymal lining was undifferentiated, exhibiting only occasional cilia and microvilli. However, in a localized area overlying the anlage of the IR, accentuated convexities clearly demarcated individual cell borders. Correlative TEM confirmed the undifferentiated nature of ependyma and neuropil at this stage of development and demonstrated that the convexities seen in the area of the IR were apical surfaces of individual cells. No intraventricular nerves or processes were present in 11 day specimens. On ensuing days, the ependymal lining was characterized by a progressive differentiation, and supraependymal neuronal elements were encountered with increasing frequency. Independent supraependymal cells and processes were first visible on day 12 of gestation, and a well organized neuronal cluster such as that consistently observed in the adult was observed as early as day 13 of gestation and was always present by parturition (day 16) and thereafter. The ultrastructural characteristics of such clusters closely resembled those seen in the adult. Specifically, each mass consisted of a group of nerve cell perikaria situated peripherally with respect to a central core of nerve cell processes. Studies are in progress to determine the origin of these clusters. [1]C. DeVlieg Fellow.

1049 LARGE FLUCTUATIONS OF MEMBRANE POOLS IN ROD AND CONE TERMINALS OF CHICK RETINA. A THIN SECTION ANALYSIS. Nigel G.F. Cooper* and Barbara J. McLaughlin. (SPON: R. R. Mize). Department of Anatomy, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163

Long term light or dark adaptation of chick photoreceptors causes wide fluctuations in certain membrane pools, (not evident in short term in vitro experiments) which may affect the overall performance of the synaptic terminals, and that are suggestive of a finite lifespan for localised membrane recycling before complete renewal is necessary. 1-2 wk old White Leghorn chicks were dark or light adapted for 1-4 days, the retinas of which were fixed by perfusion and processed for E.M. In the larger cone terminals (first row - double cones) after 48 hours dark adaptation, there is an increase in the numbers of coated vesicles observed, especially along the plasmalemma and also throughout the cytoplasm, but there appears to be an overall reduction in the total number of vesicles per unit area of terminal. This is particularly noticeable in smaller cone terminals (second row - straight cones) where there are few vesicles of any type and where synaptic ribbons can be seen without their usual full complement of synaptic vesicles. In long term light adapted cone terminals there is a significant increase in the amount of internalised plasmalemma that folds around on itself and encloses portions of terminal cytoplasm containing vesicles. Loosely packed membrane whorls form, from which vesicles are gradually excluded. Even after the longest experimental periods of light adaptation significant numbers of synaptic vesicles remain in all terminals examined. There is not such a large population of coated vesicles as seen in the dark adapted state, although some are still evident. In dark-adapted rod terminals there are several complex changes, one of the most noticeable being an increase in the number of densely stained multivesicular bodies. In long term, light-adapted rod terminals there is an increase in the number of large vesicles and dense cores are discernable, especially in these larger vesicles.

In conclusion, there appears to be a loss of synaptic vesicle membrane from cone terminals in long-term dark adaptation when membrane recycling is probably maximal. Coated vesicle re-uptake appears not to lag behind synaptic vesicle release in this condition. There is a maximal internalisation of plasmalemma in cone terminals during long term light adaptation, when coated vesicle re-uptake may be minimal or lag behind synaptic vesicle release. These observations suggest that there may be a diurnal pattern of membrane fluctuations impressed upon terminals which we are currently investigating. Supported by USPHS Grant GM00202 and Fight for Sight, Inc., New York City.

1050 ADAPTATION OF THE DEOXYGLUCOSE METHOD FOR USE AT CELLULAR LEVEL. Michel H. Des Rosiers* and Laurent Descarries, Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada H3C 3T8.

To make the deoxyglucose (DG) method (Sokoloff et al., 1977) applicable at cellular and possibly subcellular levels, a histological procedure compatible with high resolution radioautography after administration of [^{3}H]DG was developed. In adult rats, vascular perfusion of all products required for primary fixation, post-fixation, dehydration and resin embedding of CNS tissue was carried out through the aortic arch, 30-45 min after an i.v. injection of 1-[^{3}H]-2-deoxy-D-glucose. 600 ml of 3.5% glutaraldehyde in 0.05 M phosphate buffer (3-4 min), 500 ml of 1% OsO_4 in 0.15 M phosphate buffer (3-4 min), 3-4 l of absolute acetone (45-60 min) and 3 x 200 ml of acetone-Epon mixtures at increasing concentrations of resin (20-30 min) were perfused in sequence. The whole brain and upper spinal cord were then dissected out, immersed in pure Epon (3-4 hr) and polymerized in toto. One-µm-thick sections were prepared for radioautography according to standard dipping techniques. To evaluate the global retention of [^{3}H]DG and/or its metabolite [^{3}H]DG-6-phosphate, measurements of radioactivity were obtained from several rats in which tissue proteins had been previously labeled with [^{14}C] leucine. Comparable [^{3}H]/[^{14}C] ratios were found in homologous brain regions removed before and after the entire histological processing by perfusion, indicating minimal loss of [^{3}H] radioactivity. In light microscope radioautographs of the cervical cord, the tracer was found to be more concentrated in grey than white matter, and more so in anterior than in posterior horn. The ratios of concentrations between these various areas were nearly identical when determined by grain counts in the Epon embedded sections and by optical densitometry in film radioautographs of fresh frozen samples. Moreover, within given nuclei or tracts, adjacent cellular elements such as neuronal cell bodies, glial cells and myelinated axons exhibited differential labeling. These results strongly suggest that the tracer was retained in situ, which should allow visualization of cellular if not subcellular sites of energy consumption in CNS tissue adequately preserved for light and electron microscopy. Since DG and DG-6-phosphate are water soluble and rapidly lost from brain slices immersed in fixative solutions or acetone, even after primary fixation by perfusion with glutaraldehyde, their retention in situ during the course of histological processing by vascular perfusion might be related to the preservation of unbroken cell membranes in morphologically intact tissue. This histological approach could also be useful for high resolution radioautography of other diffusible compounds. (Supported by the Conseil de recherche en santé du Québec and Medical Research Council of Canada).

1051 IMMUNOPEROXIDASE LOCALIZATION OF MYXICOLA NEUROFILAMENTS (10 nm) AT THE LIGHT AND ELECTRON MICROSCOPIC LEVELS. L. F. Eng, R. J. Lasek, J. W. Bigbee* and D. L. Eng *Dept. Path., VA Hosp. and Stanford U. Sch. Med., Stanford, CA 94305, and the Neurobiology Center and Dept. Anat., Case Western Reserve U., Cleveland,O 44106

Antibodies prepared in rabbits against Myxicola neurofilaments (Lasek and Wu, Neurosci. Abst., 2: 40, 1976) have been employed to stain immunohistochemically neurofilaments in intact Myxicola nervous tissue. The marine polycheate was fixed in 5% buffered paraformaldehyde for 4-5 hours. Paraffin-embedded and frozen sections (5-6 μ) were examined at the light microscopic level with Sternberger's peroxidase-antiperoxidase method, and frozen (5-6 μ) and Vibratome (20-40 μ) sections were studied at the ultrastructural level with Nakane's conjugated peroxidase method. The neurofilament antibody stained only neurons and axons at the light microscopic level. The staining pattern at the electron microscopic level corresponded to the neurofilaments within axons and neurons. Neurons, neurofilaments, glial cells, glial filaments, and non-nervous tissue showed no peroxidase staining when preimmunization rabbit serum, specific antiserum absorbed with neurofilaments, and antiserum to the glial fibrillary acidic protein of mammalian CNS astrocytes were utilized.

Glial cells which surround the axons contain large bundles of filaments which resemble astrocytic filaments in mammalian astrocytes. These filaments do not stain with the anti-neurofilament antibody. This indicates that neurofilaments and glial filaments of Myxicola have different immunologic properties.

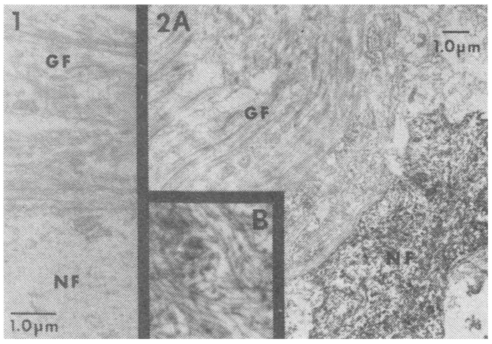

Fig.1,Control section treated with antiserum absorbed with neurofilaments (NF).NF and glial filaments (GF) are unstained.Fig.2A, NF antiserum treated section (1:1000). GF negatively and NF positively Stained.Fig.2B, NF positively stained. High Magnification.

(Supported by the VA, MRIS 2390)

1052 EFFECTS OF SOME MAYTANSINOIDS ON THE CENTRAL NERVOUS SYSTEM NEURONS. B. Ghetti, Dept. of Pathology, Div. of Neuropathology, Indiana University School of Medicine, Indianapolis, IN 46202

Maytansine (MYT) and Maytanprine (MYTPR) belong to a class of naturally occurring ansa macrolide derivatives, the maytansinoids.

MYT binds to tubulin and possesses stathmokinetic properties; it also inhibits DNA synthesis. In nerve cells, in vivo, MYT induces neurofibrillary degeneration (Ghetti, Brain Res., 1978, in press), while in axons, in vitro, it induces a reduction of microtubules and blocks axoplasmic transport (Ghetti and Ochs, Abst. Soc. Neurosci., 1977). The aims of the present communication are 1) to describe the neurocytological changes induced by MYTPR, 2) to analyze the changes preceding and following the appearance of neurofibrillary changes in neurons exposed to these drugs, 3) to describe the topography of the central nervous system (CNS) lesions.

Histological and ultrastructural studies were carried out on the CNS of rabbits sacrificed between 18 hours and 6 days following intrathecal injection of 25, 50 and 100 μg of either MYT or MYTPR. The retina of rabbits intraocularly injected with 3 μg of MYT was studied between 40 hours and 15 days following injection. The results are as follows: 1) MYTPR induced severe neurofibrillary degeneration in neurons of the cervical spinal cord, medulla and pons between 48 and 72 hours following injection. Dendrites were extensively involved; axons were focally distended and filled with neurofilaments. Axonal and dendritic microtubules were drastically reduced. 2) The cell groups known to respond with neurofibrillary degeneration displayed, between 18 and 48 hours, severe loss of microtubules. The cytoplasm showed clumping of the Nissl substance, composed of aggregates of short cisterns of rough endoplasmic reticulum with a large number of ribosomal rosettes. The cytoplasm outside these areas showed clumping of the mitochondria, complete loss of microtubules, dispersed ribosomes and ribosome rosettes, numerous membrane-bound vesicles, rare bundles of neurofilaments and an amorphous floccular material. Also, the retinal ganglion cells responded with neurofibrillary degeneration. The local intraocular injection provided a useful system to study the time course of the cellular changes. 15 days after intraocular injection a large number of retinal ganglion cells had disappeared. The remaining ganglion cells contained scattered neurofilaments and rare microtubules. 3) The neuropathological changes were severe in the cervical cord, in the medulla and pons. Neurofibrillary changes were also present in the Purkinje cells; their axons showed numerous focal swellings. Few neurons with neurofibrillary changes were present in the hippocampus and cerebral cortex.

(Supported by grant IN-46R of the American Cancer Society.)

1053 A RAPID GOLGI STAINING TECHNIQUE FOR BRAIN TISSUE LESS THAN 0.5 mm THICK. Kristen M. Harris. Neural and Behavioral Biology Program, Univ. IL, Champaign, IL 61820.

Recent advances in the use of in vitro preparations to study neuronal plasticity have prompted the development of a golgi technique to stain small pieces of brain. While the technique has not yet been used on tissue maintained in vitro, it has been used successfully on freshly perfused tissue.

All tissue used in the staining procedure is fixed in buffered (0.43% NaOH and 1.88% NaH_2PO_4, pH 7.2-7.4) 4% paraformaldehyde solution for at least 24 hours. The small piece of tissue (0.3-0.5 mm) is then sewn with white polyester thread between two pieces of similarly fixed brain tissue (2 mm). The preparation is then wrapped in cotton gauze and placed in 0.16% OsO_4 and 2.3% $K_2Cr_2O_7$ solution for 5 days. The gauze and tissue is then rinsed in distilled water and 1% $AgNO_3$ solution and placed in 1% $AgNO_3$ solution for 5 days. At the end of this period, the tissue is dehydrated (1:1 acetone-ethanol for 3 hours; 100% alcohol for 30 min; and 1:1 ether-ethanol for 30 min). Then the tissue is carefully unwrapped and the threads are cut so that the small piece of brain tissue can be separated and embedded in celloidin. Tissue is permeated with 8% (W:W) celloidin for 3-8 days, and then transferred to 16% celloidin for approximately 3 days. The small sections are embedded in 16% celloidin and hardened over chloroform vapor for 36 hours. The blocks of embedded tissue are mounted on wooden blocks and microtomed at 60-100 micron thickness. These 60-100 micron sections are mounted in permount under cover glass.

This method has been found to fully stain neurons in preparations from adult rat cortex and hippocampus with sufficiently low staining density to permit qualitative and quantitative analysis. Staining other regions of brain or brains of other animals may require different concentrations or times.

Supported by NSF BMS 75-08596, NSF BMS 77-23660 to W. T. Greenough, and by NSF SER 76-18255.

1054 THE ORDERED TRANSVERSE BRIDGE LATTICE SYSTEM ASSOCIATED WITH NEUROFILAMENTS AND MICROTUBULES IN AXONAL CYTOPLASM OF LOLIGO AND HERMISSENDA. Alan J. Hodge* and W. J. Adelman, Jr. Lab. of Biophysics, IRP, NINCDS, NIH, Woods Hole, MA 02543.

Slender transverse projections radiating from microtubules (mt) and neurofilaments (nf) have been described in thin sections of neural tissue prepared for electron microscopy. In favorably oriented specimens, these projections appear as bridges between parallel neurofilaments and microtubules. This study utilized thicker sections (0.1-0.3 μm) for stereoscopic study of neural networks in conventional TEM (Philips 300) where the upper limit of section thickness is determined primarily by loss of resolution resulting from inadequate imaging of inelastically scattered electrons and by problems associated with heavy metal staining. Improved fixation was observed for squid neural tissue (including giant axons) and for the CNS of Hermissenda crassicornis when the initial glutaraldehyde (2-4%) phosphate-buffered fixative (pH 7.2) contained EGTA (2-40 mM) and Mg^{++} (1-5 mM) with the total osmolarity adjusted with sucrose to not less than 1100 mosm. Initial fixation (30 min.-2 hours) was followed by cold OsO_4 treatment (30 min.-2 hrs.). Specimens were dehydrated and embedded using Epon 812. Specimens were stained en bloc with either aqueous or alcoholic solutions of uranyl acetate, sometimes followed by alcoholic PTA. Often these thicker sections required further staining, usually alcoholic uranyl acetate and aqueous lead citrate for adequate contrast.

When thicker sections (0.1-0.3 μm) of longitudinally oriented axons were examined, a distinct transverse striation could be discerned in the mass of parallel neurofilaments. Close stereoscopic inspection revealed an extensive ordered network of thin (3 nm) transverse elements arranged in planes approximately normal to the fiber axis and spaced about 40 nm apart along this axis. Thin elements often appear to bridge neighboring neurofilaments and to connect the lattice to axolemmal membrane surfaces. It thus appears that neurofilaments and microtubules together with thin transverse elements form a highly ordered lattice structure or gel intimately connected to the neurolemmal surface. This ordered lattice was confirmed by image enhancement using multiple image superposition techniques. The neuronal lattice system is presumably the equivalent of the microtrabecular system found by Porter and colleagues in cultured cells. The ordered lattice system might be involved in axoplasmic flow and in the transport mechanism proposed by Schmitt for transmitter substances and enzymes. The small diameter (2-3 nm) of transverse elements suggests the possibility that they may be composed in part of a tropomyosin-like protein, which, in striated muscle, acts as a bridging component, at least in Z-bands.

1055 CYTOPLASMIC CHANNELS OF THE MAMMALIAN SCHWANN CELL: A FREEZE-FRACTURE STUDY. Lawrence Kruger and Aidan S. Breathnach*. Dept. Anat. and Brain Research Inst., UCLA Med. Center, Los Angeles, CA 90024, and Dept. Anat., St. Mary's Hosp. Med. Sch., London, W 2, England.

The distribution of Schwann cell cytoplasm was examined in spinal and trigeminal nerves and dorsal roots of rat, rabbit and human fetus preserved in aldehyde mixtures or unfixed and using a variety of cryoprotective agents before rapid freezing for purposes of electron microscopic examination of freeze-fracture replicas.

The surface plasmalemma displays broad cytoplasmic bulges extending beyond the perikaryal region and is extensively studded with caveolae, although these are less numerous in the region of the outer mesaxon. The outer layers of "semi-compact" or "loose" myelin are permeated by an interlacing network of trabecular cytoplasmic channels of approximately 0.1 μm diameter. Channels of similar diameter can be followed through "compact" myelin layers, as Schmidt-Lanterman incisures, some ending blindly, others continuing as longitudinal tubules terminating as loops at the paranodal zone.

The inner surface of the Schwann cell contiguous with the axolemma usually consists of a single "semi-compact" layer with less regular expanses of trapped cytoplasm except for zones containing a uniform spiraling multiple tubular network corresponding to the "axon-Schwann" network described in thin sections by Berthold. The similarity in diameter for the inner tubules, paranodal terminal loops, nodal microvilli, incisural channels and the outer Schwann trabecular network, suggests a parsimonious principle. This form of sequestration in Schwann cells might underlie the specialized needs of cytoplasmic communication in a cell with large domains in which cytoplasm has been extruded to form compact myelin.

(Supported by NIH grant NS 5685, Fogarty Senior International Scholar Award TW-125 and the Wellcome Trust.)

1056 PROLIFERATION OF FIBROUS ASTROCYTES IN RESPONSE TO BRAIN INJURY: SIMULTANEOUS LOCALIZATION OF GLIAL FIBRILLARY ACIDIC PROTEIN BY IMMUNOPEROXIDASE TECHNIQUE AND OF ^{3}H-THYMIDINE UPTAKE BY RADIOAUTOGRAPHY. Norman Latovitzki*, Gajanan Nilaver*, Earl A. Zimmerman, Ann-Judith Silverman, William G. Johnson*, Richard Defendini*, and Lucien Cote. Departments of Neurology, Anatomy and Neuropathology, College of Physicians and Surgeons, Columbia University, New York City, N.Y. 10032.

Astrocytes proliferate and hypertrophy in response to many different kinds of CNS injury. The acutely reactive astrocyte develops large numbers of intracellular gliofibrils and is rich in glial fibrillary acidic protein (GFAP). Since the production of GFAP by an astrocyte denotes a further degree of cell specialization or differentiation and since highly differentiated cells often cease proliferating, the ability of the GFAP-containing astrocyte to undergo division was examined by combining radioautography of radiolabelled thymidine with localization of GFAP by immunoperoxidase technique.

A needle stab wound in the cerebrum was produced transcranially in mice. Three days later 100μCi of ^{3}H-thymidine/animal was injected intraperitoneally and the animals sacrificed one hour later. Formalin fixed, paraffin embedded, 6 μm brain sections were immunostained by the peroxidase-antiperoxidase bridge method using rabbit antihuman GFAP antiserum as the first antibody. Large numbers of GFAP reactive cells were present in the lesion, while only occasional ones were encountered in normal tissue. ^{3}H-thymidine radioautographic products on the subsequently emulsion coated sections were located in a significant number of nuclei of GFAP reactive astrocytes in the lesion. These findings suggest that astrocytes sufficiently differentiated to produce GFAP retain the ability to divide and that such cell division may be a significant source of astroglial proliferation in response to tissue injury.

1057 IMMUNOHISTOCHEMICAL LOCALIZATION OF ACTIN IN IDENTIFIED NEURONS OF *APLYSIA*. Beverly W. Lubit, Ariel A. Sherbany*, and James H. Schwartz. Dept. Physiol., Div. Neurobiol. & Behav., Columbia Coll. Phys. & Surg., New York, N.Y. 10032.

Because contractile proteins are likely to be involved in the intracellular movement of neuronal components by axonal transport, we have developed a radioimmunoassay for *Aplysia* actin and have used rabbit anti-actin antiserum for immunohistochemical localization. Actin was extracted differentially from *Aplysia* body wall muscle and purified by Mg^{++}-polymerization, ion-exchange chromatography on DEAE-cellulose or affinity chromatography on bovine pancreatic DNase I-Agarose. Rabbits were immunized for 4 weeks with 30 μg per week of the actin contained in a suspension of polyacrylamide from a final purification by slab gel electrophoresis in sodium dodecyl sulfate. Antibody was first detected by Ouchterlony immunodiffusion a week after the last immunization.

Immunofluorescence studies were initially carried out on squashes of the isolated cell body of R2, the giant neuron dissected from the abdominal ganglion. In these whole mount preparations, actin was found in fluorescent strands along the surface of R2's cell body. Because the neuron is so large (0.5-1mm in diameter), 10-μm cryostate sections were necessary to reveal a granular cytoplasmic localization *within* the cell body and axon. Although the perinuclear area was the brightest, the nucleus itself was only faintly fluorescent. Connective tissue and glial cells appeared to contain only small amounts of actin, if any. As expected, cryostat sections of muscle from body wall, buccal mass and heart were highly fluorescent. Incubation of sections in the presence of 0.2% *Aplysia* tropomyosin did not affect the patterns observed. A similar distribution of actin was obtained using Sternberger's PAP-immunoperoxidase method.

The specificity of the anti-actin antibody was assessed by immunoadsorption and by radioimmunoassay. Fluorescence was not obtained with antibody from which anti-actin had been removed by passage through actin-AH Sepharose; after elution, the adsorbed anti-actin provided strong fluorescence. The antiserum contained a negligible cross-reacting activity to tropomyosin detected by radioimmunoassay using the double antibody method to precipitate actin labeled by reaction with ^{3}H-acetic anhydride or with the ^{125}I-Hunter-Bolton reagent. The latter probe is of sufficiently high specific radioactivity to permit assay of actin in isolated nerve cell bodies.

1058 ULTRASTRUCTURAL LOCALIZATION OF CALCIUM BY ELECTRON PROBE ANALYSIS IN PRESYNAPTIC NERVE TERMINALS. C.F. McGraw, A.V. Somlyo*, and M.P. Blaustein. Dept. Physiology and Biophysics, Washington University Sch. Med., St. Louis, MO 63110, and Pennsylvania Muscle Institute, Philadelphia, PA 19104.

Presynaptic nerve terminals (synaptosomes) were isolated from adult rat brains according to the method of Hajos (Brain Res. 93: 485, 1975). Saponin-disrupted synaptosomes were incubated in Ca-containing, K-rich media in order to load the intraterminal Ca-sequestering sites with Ca (Blaustein, et al., J. Gen. Physiol., in press); the incubation solutions all contained 10-30 mM K oxalate. Some preparations were fixed with aldehydes and postfixed with OsO_4; all fixatives contained oxalate. Other synaptosome preparations were processed by a freeze-substitution method. All tissue samples were dehydrated and embedded in plastic; unstained, thin sections were examined in the electron microscope. Many of the terminals contain flattened cisternae and tubular profiles which resemble smooth endoplasmic reticulum; these structures are frequently observed adjacent to mitochondria. In the aldehyde-fixed preparations, electron-dense deposits, probably calcium oxalate, are localized in the structures resembling smooth endoplasmic reticulum. Electron probe X-ray microanalyses of these structures verify the presence of calcium at a significantly higher concentration than in the cytoplasm. Mitochondria, and synaptic vesicle-rich areas of the cytoplasm, also contain significant concentrations of calcium. Electron probe analyses of the freeze-substituted preparations confirm that the flattened cisternae in these terminals also contain calcium at a higher concentration than is observed in the cytoplasm.

Presynaptic nerve terminals contain non-mitochondrial, ATP-dependent calcium storage sites; the uptake of calcium by these sites, which are exposed in disrupted terminals, is enhanced in the presence of oxalate (Kendrick et al., Nature 265: 246, 1977). The cisternal and tubular structures in which calcium has been localized by electron probe analysis may be the organelles which are responsible for the non-mitochondrial, ATP-dependent calcium storage. [Supported by USPHS grant NS-08442 and HL-15835].

1059 CYTOCHEMICAL EVIDENCE FOR PHAGOCYTIC CELLS UNDERLYING THE CEREBRAL VENTRICLES. Olivia C. McKenna. Department of Biology, City College, City University of New York, New York, N.Y. 10031

A population of cells which rapidly incorporate extracellular material has been identified lying immediately beneath the ependymal cells of the cerebral ventricles of the toad (Bufo marinus). The ventricular system was perfused with 1% horseradish peroxidase (HRP) in Ringer's solution and tissue sections processed for electron microscopy using the DAB cytochemical method. It was found that HRP readily penetrates the ependymal lining filling the extracellular spaces of the underlying tissue. More notably, within two minutes after the perfusion reaction product is incorporated into subependymal cells where it is found within cisternae, vesicles and multivesicular bodies. These cells characteristically contain membrane-limited dense bodies of various sizes and shapes, long strands of granular endoplasmic reticulum, lipid bodies and nuclei with clumped chromatin. Microtubules and filaments are notably absent. The cells also give rise to many pseudopodia which contain a uniformly dispersed flocculent material and are free of organelles. Cells with similar cytoplasmic components but lacking clearly defined pseudopodia are found in untreated tissue. Other cells also incorporate HRP but to a much lesser degree than the subependymal cells. As a result of the presence of pseudopodia and dense bodies and the ability to incorporate HRP the subependymal cells are readily distinguished from other central nervous system cell types.

The finding of a population of cells with phagocytic activity beneath the ependymal lining suggests that, although a barrier equivalent to the blood brain barrier does not exist at the cerebrospinal fluid brain interface, the brain has a population of cells which function to protect the brain by engulfing foreign materials entering the brain tissue from the cerebrospinal fluid.

(Supported by grants from NSF and from the City University PSC-BHE research award program)

1060 KAINIC ACID LESIONS OF THE SUPERIOR COLLICULUS: HISTOLOGICAL CHARACTERISTICS AND INCIDENCE OF INFARCTIONS. Björn H. Merker*. (SPON: R. Held). Dept. Psychol., M.I.T., Cambridge, MA 02139.

Kainic acid (KA) was injected into the anterior part of one superior colliculus (SC) of 17 adult Syrian hamsters via a modification of the Eide et al. (Neurosci. Lett. 2: 51, 1976) thermal microinjection method (0.15 or 0.2 ul of 2.5-10ug KA/ul in normal saline). After 6 to 15 days survival, brains were studied by light- and darkfield microscopy on adjacent serial sections stained for Nissl substance by cresyl violet, myelin by the Loyez hematoxylin stain, and normal axons and degeneration products by modifications of the Fink-Schneider and Fink-Heimer I methods, respectively. KA injections produced a remarkably benign tissue reaction characterized by drastic though subtotal neuronal destruction in the absence of severe gliosis, fibrosis or macrophage invasion. Neuronal destruction around the injection site tended to be more severe in the deep layers of the SC than in the superficial grey (SG). Central grey was always conspicuously spared even when drastic SC cell loss extended to its borders. In the SC large neurons were invariably destroyed. The density of surviving medium and small neurons was somewhat variable, but in the deep SC it approached complete neuronal destruction in some cases. Though the region of cell loss included the stratum opticum, the distribution of optic tract and other fibers throughout the SC behind the lesion was indistinguishable from the control side in the myelin and normal axon stains. The sparing of fibers of passage was further supported by cases in which both eyes were injected with ^{3}H-leucine and ^{3}H-proline 24 hr prior to killing. In 2 of these cases autoradiographically assessed labelling of the SC was bilaterally symmetrical throughout its extent, though a substantial portion of the optic tract passed through the region of cell loss. Within the region of cell loss a number of cases showed loss of variable amounts of fibers in the SG, particularly when cell loss there was more severe. This might represent a secondary reaction on the part of afferent fibers to loss of terminal sites. In 5 of the 17 animals, local elongated patches of total necrosis with the radial orientation of the collicular vascular system were associated with the lesion, and could extend beyond the region of cell loss into the central grey. Such damage, apparently caused by vascular infarction, was virtually nonexistent in 77 animals injected with a variety of metabolic inhibitors, though many of these cases had tissue reactions far more severe than those induced by KA. This incidence of infarctions therefore appears to be associated with the tissue effects of KA rather than with the injection procedure or nonspecific reactions to tissue damage.

Supported by NIH grant EY00126 to G. Schneider, and by a Whitaker-Health Sciences Fund Fellowship.

1061 GENETIC ENZYME VARIANTS AS CELL MARKERS IN STUDIES OF NERVOUS SYSTEM DEVELOPMENT. Mary Lou Oster-Granite and John Gearhart*. Dept. Anat., Univ. Md. Sch. Med., Baltimore, MD 21201.

Glucosephosphate isomerase (GPI, E.C. 5.3.1.9) is a ubiquitous soluble, glycolytic enzyme which is expressed early in mammalian embryogenesis. The structure of this enzyme is specified by a single autosomal locus with at least four different alleles (Gpi-1^{a}, Gpi-1^{b}, Gpi-1^{c}, and Gpi-1^{d}) in mice. These alleles are expressed as electrophoretically distinct allozymes of GPI (GPI-1A, GPI-1B, GPI-1C, and GPI-1D). We have isolated and purified two of these allozymes, GPI-1A and GPI-1B, from mouse liver, brain, and skeletal muscle. The GPI-1A allozyme was isolated from Balb-c and AKR mice, the GPI-1B allozyme from C57Bl/6J, C57Bl/6J (pcd), and C57Bl/6J (bg^{J}) mice. Purified allozymes have been used in xenogeneic, syngeneic, and allogeneic immunizations involving goats and mice. Goat antisera to GPI have been absorbed with allozyme-conjugated Sepharose columns and the allozyme specificity of these absorbed antibodies determined by radioimmune assay. Such purified allozyme-specific antibodies have been used in an indirect fluorescent assay to determine the presence of GPI in paraffin sections of various parts of the immature and mature nervous system to test the usefullness of this enzyme as a genotype specific cell marker. We are currently extending this procedure to the EM level by use of an indirect staining procedure utilizing a horseradish peroxidase (HRP)-antibody conjugate. Such allozyme-specific antibodies may be very useful for studies of the developing nervous system examining 1) cell interactions between various populations of neurons or between neurons and glia; 2) the origins of various cell populations in the nervous system; 3) the locus of action of mutant genes which produce neurologically mutant mice; 4) the circumstances necessary for the expression of mutant phenotype by genetically mutant cells; and 5) aspects of regeneration and plasticity in the immature nervous system in combinations of both genotypes in chimeric mouse systems. The usefullness of this enzyme as a marker of cell genotype is illustrated primarily in the spinal cord and cerebellum, but its general applicability to other areas of the nervous system will be demonstrated.

This research was supported by a grant from the National Science Foundation (PCM-16763). MLOG is a Fellow of the Alfred Sloan Foundation. JG is the recipient of a Basil O'Connor Starter Award from the National Foundation - March of Dimes.

1062 PERSISTENT CELL MULTIPLICATION IN THE SPINAL ROOTS OF ADULT DYSTROPHIC MICE. C. Suzanne Perkins*, Garth M. Bray and Albert J. Aguayo. Department of Neurology and Neurosurgery, McGill University and the Montreal General Hospital, Montreal, Quebec, Canada.

In spinal roots of dystrophic mice, there are bundles of large, unensheathed axons (J. Neurol. Sci. 18:227, 1973). This abnormality is associated with a neonatal deficit of Schwann cell multiplication (J. Neurol. Sci. 32:203, 1977). In the present study, adult dystrophic (C57BL/6J dy^{2J}/dy^{2J}) and control (C57BL/6J +/+) mice were injected with tritiated thymidine (4μCi/g body weight) daily for 10 days and sacrificed on the 10th day by systemic perfusion of fixative solution. Labelling indices in the lumbar ventral roots were determined by radioautography.

No labelled nuclei were observed in the spinal roots from control animals but in dystrophic roots, up to 20% of nuclei were labelled. This abnormal cell proliferation could be caused by myelin debris which is present in the dystrophic roots. Thus, in addition to the initial deficiency of axonal ensheathment, there may also be a failure to sustain myelin in the abnormal segments of these nerves.

1063 NEURONAL UPTAKE OF HORSERADISH PEROXIDASE IN MECHANICALLY BRAIN INJURED CATS. J.T. Povlishock,* D.P. Becker, and J.D. Miller* (Spon: J.A. Astruc). Departments of Anatomy and Neurological Surgery, Medical College of Virginia, Richmond, Virginia 23298.

Recently in an analysis of central nervous system vascular permeability alterations in response to low-grade concussive injuries uncomplicated by either microscopic intraparenchymal hemorrhages or other relevant neuropathological change, we have described at both the light and electron microscopic level an increased vascular permeability to intravenously injected horseradish peroxidase within the raphe and reticular core. In these animals the protein apparently reached the parenchyma through a dramatic form of transendothelial vesicular transport (Brain Research - In Press). Concomitant with the spread of the peroxidase into the brain stem parenchyma, numerous neurons became inundated with the tracer and as such appeared reminiscent of cells visualized in Golgi preparations. In that such inundation with peroxidase most probably reflects altered neuronal membrane permeability, the question arises as to what is the fate of these neurons.

Toward the resolution of this question these flooded neurons were studied in serial ultrathin sections over a 24h period following mechanical brain injury. Immediately after injury ultrastructural examination of the flooded neurons demonstrated peroxidase diffusely spread throughout their cytoplasm as well as being localized within vesicles and vacuoles. Careful examination of the neuronal cell membranes failed to reveal any evidence of physical damage, and all other aspects of cellular morphology appeared unremarkable. Within 6h of brain injury, no neuronal flooding with peroxidase was observed. No evidence of cellular degeneration was noted and thus, the fate of the flooded neurons appeared questionable. However, in those same loci, which immediately after trauma displayed inundated neurons, numerous neurons displayed a cytoplasm laden with peroxidase containing vesicles. Within these same group of neurons, after 12h of survival, the peroxidase containing vesicles assumed a perinuclear orientation and within 24h of the injury the peroxidase reaction product was observed to be both diffusely spread in the nucleus as well as being selectively sequestered within nucleolar vacuoles. In conjunction with this nuclear and nucleolar peroxidase uptake, no ultrastructural manifestation of any cellular alteration was observed.

Such neuronal peroxidase uptake appeared to be directly related to the traumatic injury and was not secondary to various traumatic hemodynamic episodes.

Supported by PHS Grant NS-12587-02S2

1064 DEPLETION OF DENSE-CORED VESICLES IN AXON TERMINALS OF CAT SUPERIOR CERVICAL GANGLIA FOLLOWING ELECTRICAL STIMULATION. J.J. Pysh and Daniel G. Nehls*. Dept. Anat., Northwestern Univ. McGaw Med. Ctr., Chicago, IL 60611.

Cholinergic nerve terminals in sympathetic ganglia contain small numbers of large dense-cored vesicles (LDCV's) dispersed amongst the larger population of small clear-cored vesicles. The function of LDCV's in cholinergic nerve terminals is unknown. The objective of this study was to determine whether the concentration of LDCV's in nerve terminals of cat sympathetic ganglia is altered during synaptic activity.

Cervical sympathetic trunks of cats were stimulated electrically at 10 Hz for 2, 5, or 30 minutes and nictitating membrane contractions were recorded to monitor the effectiveness of stimulation. Animals were perfused intra-aortically with an aldehyde fixative. Subsequently, stimulated and contralateral control ganglia were processed simultaneously for electron microscopy.

A morphometric analysis was carried out on electron micrographs of randomly selected axo-dendritic synapses identified by the presence of characteristic synaptic densities and the presence of clear-cored vesicles. The concentrations of LDCV's in axon terminals ($\#/\mu^2$) was computed from counts of LDCV's and measurements of terminal profile areas. Paired comparisons between control and stimulated ganglia were made to obtain percentage differences of LDCV concentration for each animal.

The mean concentration of LDCV's in presynaptic terminals decreased significantly after 5 minutes of stimulation and continued to decline to reach a concentration 31% of controls after 30 minutes of stimulation. In ganglia allowed to rest for 1 hour after 30 minutes of stimulation, the concentration of LDCV's in axon terminals recovered to 47% of controls, whereas the concentration of clear-cored vesicles completely recovered as shown in a previous study (Pysh and Wiley, 1974).

The present data offers evidence that the contents of LDCV's may be released from cholinergic nerve terminals during functional activity.

Pysh, J.J., and Wiley, R.G. Synaptic vesicle depletion and recovery in cat sympathetic ganglia electrically stimulated in vivo. J. Cell Biol., 60:365-374, 1974

(Supported by NIH Grant #NS 11325)

1065 INCORPORATION OF NEWLY FORMED ETHANOLAMINE PHOSPHATIDES INTO PERIPHERAL NERVE (PN) MYELIN: AN ELECTRON MICROSCOPE (EM) AUTORADIOGRAPHIC ANALYSIS. F.A. Rawlins, Dept. Biophysics, IVIC, Apartado 1827, Caracas 101, Venezuela.

Recent EM autoradiographic studies indicate that PN shows different mechanisms of uptake for various of its lipid components. Thus, cholesterol rapidly enters the forming myelin sheath (MS) almost simultaneously through its inner an outer edges. A movement of cholesterol from axon into MS was also suggested (Rawlins, F.A. J. Cell Biol. 58: 42, 1973). Uptake of newly formed lecithin by the adult MS occurs almost exclusively through its outer edge (Rawlins, F.A. 8th Int. Cong. Elect. Micros. 2: 288, 1974: Gould and Dawson, J. Cell Biol. 68: 480, 1976). Phosphoinositides are first concentrated within the axon and afterwards appear incorporated into the MS and Schwann cell (SC) (Gould, R.M. Brain. Res. 117: 169, 1976). In the present work we have studied the site of synthesis of ethanolamine phosphatides, one of the major myelin lipid components, in PN and its route of incorporation into the MS of young and adult mice. The animals were injected i.p. with 500 µCi of ethanolamine hydrochloride (1,2-^{14}C), sacrificed at various times after injection and the sciatic nerves processed for EM autoradiography. The autoradiograms were analyzed following the method of Salpeter et.al. (J. Cell. Biol. 41: 1, 1969). Each nerve fiber (cross and longitudinal sections) was divided into 3 main compartments: myelin, axon and SC. Each compartment was subdivided into several subcompartments of 2000 Å wide (one ^{14}C half distance). The results indicate that in both young and adult mice, the label was initially concentrated in the SC cytoplasm mainly over the smooth endoplasmic reticulum. In the young animals "filling" of the MS was attained by two radial movements of the labelled molecules: one from the SC cytoplasm through the outer edge and another from the adaxonal SC cytoplasm through the inner edge. In the adult mice only the first type of movement could be detected. Although some label was confined to the axon, no evidence of an axon to myelin movement of molecules was obtained. It is suggested tha while growth of the MS implies a simultaneous incorporation of molecules at the outer and inner mesaxon, maintenance of the adult MS is achieved mainly through its outermost lamellae.

1066 ABNORMALITIES IN THE PLASMA MEMBRANE OF CENTRAL MYELINATED AXONS OF "QUAKING" MICE AS SEEN IN FREEZE-FRACTURE REPLICAS. Jack Rosenbluth. Departments of Physiology and Rehabilitation Medicine New York University School of Medicine, New York, N.Y. 10016.

Previous freeze-fracture studies of myelinated nerve fibers have shown that the axolemma is regionally specialized in relation to the surrounding myelin sheath. Paranodal junctional membrane specializations are prominent, and nodal intramembranous particle accumulations may represent ion channels important in saltatory conduction. "Quaking" is a mutant mouse strain which exhibits "hypomyelination" as well as a variety of irregularities in myelin sheath structure. Thin sections of central nervous system white matter reveal thin myelin sheaths whose lamallae often terminate irregularly along the internode. The paranodal region may appear normal, but irregularities in the pattern of the terminal loops occur here as well. "Transverse bands" are present at some paranodal regions, but are absent or only partially present at others. The "radial component" of myelin is conspicuous even in very thin sheaths,and myelin debris occurs commonly as well as redundancies and aberrant formations around cell bodies and processes other than axons. In freeze-fracture replicas the outer leaflet of the paranodal axolemma sometimes exhibits a faint diagonal pattern characteristic of the axoglial junctional specialization normally found in this region. However, in the quaking animals the paranodal membrane specialization is neither consistent nor regular. This is particularly evident in etched preparations where the pattern in the specialized axolemma stands out distinctly and exhibits a rope-like configuration. Abnormal angulations in the pattern occur in which the orientation of the component strands changes abruptly forming ~30, 60, or 90° angles, and islands of non-specialized axolemma are also present. Large intramembranous particles, like those normally found concentrated in the nodal axolemma, are found in the axolemmal E face within the broad areas between paranodal "grooves" to a much greater extent than normally, and the concentration of large particles in the outer leaflet of the nodal axolemma is sometimes reduced markedly below normal. The P face of the membrane of the paranodal glial loops facing the axolemma often exhibits discoid aggregates of closely packed large particles instead of the regularly spaced diagonal rows of particles seen normally in the junctional membrane. Freeze-fracture analysis thus shows that in addition to the previously described abnormalities of myelin, the quaking nervous system exhibits abnormalities in both glial and axonal membranes in the region of the paranodal axoglial junction and in the nodal axolemma as well. These alterations in structure in the axonal plasma membrane, whether secondary to glial abnormalities or not, could account in part for the neurological deficit seen in these animals. Supported by grant NS 07495 from the NIH.

1067 RAPID RETROGRADE DEGENERATION IN THE OLFACTORY BULB OF MICE. David W. Samanen* and P.P.C. Graziadei. Dept. Bio. Sci., Florida State Univ., Tallahassee, FL 32306.

Axotomy of the olfactory mitral cells was performed 1) in the lateral olfactory tract (LOT) at the level of the caudal margin of the anterior olfactory nucleus, or 2) by direct lesion of the olfactory bulb with a wedge-shaped lesion in the coronal plane at its mid-length. Animals at postoperative intervals of 12 hrs - 180 days were sacrificed and the brains serially sectioned and stained by the Nissl and Holmes methods. Several changes in the arrangement of Nissl substance and the argyrophylia of the neurons were observed. These changes occurred very rapidly, as early as 12 hrs postoperatively in the animals with bulbar lesion and at 48 hrs for animals with LOT lesion. Disappearance of the lesioned neurons occurs from both lesions thereafter.

The distribution of the mitral cells undergoing retrograde degeneration was different between the two lesions. It was rather diffuse, as observed in coronal sections, scattered about the entire perimeter of the mitral cell layer, in animals with LOT lesions. It was restricted to a rather discrete sector of the bulb in animals with the direct bulbar lesion.

The mitral cells caudal to the direct bulbar lesion also sustained degenerative changes. While these neurons were not affected by axotomy, the axons of their associated primary neurons may have been cut during the surgery performing the wedge-shaped lesion. We believe that their degeneration may have been induced by the deafferentiation of their apical dendrite at the glomular level. It is interesting to observe that the time course of this putative transneuronal degeneration follows the same rapid temporal pattern of the retrograde degeneration observed in the mitral cells rostral to the lesion.

Supported in part by NSF grant BNS 77-16737 to P.P.C. Graziadei and by NIH training grant 5T32NS 07010.

1068 THE EFFECT OF HYPERGLYCEMIA ON BRAIN CAPILLARY PERMEABILITY IN A LIZARD, ANOLIS CAROLINENSIS. A FREEZE-FRACTURE STUDY. Richard R. Shivers. Dept. Zoology, Univ. Western Ontario, London, Canada

The anatomical basis of the blood-brain barrier in the American chameleon, Anolis carolinensis, is the tight junction system that "seals" brain capillary endothelia to systemically injected horseradish peroxidase (HRP) and trypan blue. These inter-endothelial junctions, when examined in freeze-fracture replicas, consist of a highly complex network of anastomosing continuous PF membrane face ridges and complementary EF membrane face grooves. Many experimental protocols and pathological states are known to alter brain capillary permeability and are assumed to act by disrupting the structure of brain capillary endothelial tight junctions. In the present study, systemic injection of 5 mg of glucose into lizards resulted in increased brain capillary permeability as evidenced by escape of trypan blue or HRP (Sigma Type VI), which were included in the glucose injection, into the intercellular spaces of central neuropil. Freeze-fracture analysis of brain capillaries of glucose-hyperglycemic lizards showed no alteration in the ridge and groove construction of endothelial tight junctions, indicating that although the blood-brain interface becomes leaky during hyperglycemia, the capillary occluding junctions are not affected. Evidence obtained in this study strongly supports the notion that the increased capillary permeability is the result of amplified trans-endothelial transport that is facilitated by and manifest as formation of chains of pinocytotic vesicles derived from the luminal surface of the endothelial cells, which then fuse to create open trans-endothelial channels. It is likely that formation of open conduits across the brain capillary endothelial cells, as a response to hyperglycemia, could allow unrestricted passage of a wide range of molecules, some potentially toxic, into the CNS extracellular milieu. Careful examination of the EF fracture face from many control and experimental brain capillary endothelia failed to show any increase in the number or distribution of pinocytotic vesicles as a response to hyperglycemia. This is the first report to unequivocally document, with freeze-cleave techniques, that abnormally elevated levels of blood sugar can affect blood-brain barrier permeability, and because of this, suggests that similar consequences probably result from diabetic hyperglycemia in humans. Results of this study also suggest that amplification of pinocytotic activity,with formation of trans-endothelial channels,may constitute the usual consequence of most of the experimental and disease-related insults known to affect the barrier.Further studies are required to determine if this condition contributes to impairment or death of CNS neurons and their processes. (Supported by the National Research Council of Canada)

1069 MORPHOLOGICAL BASIS FOR CROSSED MOTONEURONAL INTERACTIONS IN FROG SPINAL CORD. R. W. Soller* and S. D. Erulkar (SPON: G. B. Koelle). Department of Pharmacology, University of Pennsylvania, School of Medicine, Philadelphia, Pa. 19104.

Recently, we reported that lumbar motoneurons on opposite sides of the frog spinal cord show electrically- and chemically-mediated synaptic interactions (Soc. Neurosci. Abst. III: 514, 1977). In order to identify the morphological substrates of these crossed interactions we used LM and EM methods to examine spinal cords whose ventral roots were loaded with horseradish peroxidase (HRP).

HRP was applied topically to the cut ends of ventral roots of segments 7-10. After two days spinal cords were prepared according to the HRP-diaminobenzidine method. Dendritic processes were identified at the EM level by the presence of synaptic input, endoplasmic reticulum and microtubules.

At the LM level the distribution of HRP-positive processes confirmed the motoneuronal dendritic profile described in Golgi studies (Liu and Chambers, Anat. Rec. 127: 326, 1957). In addition, medially-directed dendrites were seen to terminate in an ipsilateral field that was ventrolateral to the central canal or to cross the midline in the anterior commissure and terminate in the ventral gray matter or, in some cases, the lateral funiculus. Crossing dendrites emanated from both lateral and medial motor groups of segments 7-10, but were most numerous in segments 7 and 8. A notable specialization of these processes, particularly in the anterior commissural region of rostral lumbar segments, was the appearance of localized enlargements (8-15 um in diameter).

At the EM level, HRP-positive processes, presumably motoneuronal dendrites, were observed in (1) the anterior commissural region as localized enlargements that receive synaptic input and that appear in close apposition (17 nm separation) to non-reactive dendritic processes, (2) dendritic thickets (see Stensaas and Stensaas, Brain Res. 31: 67, 1971) of the ipsilateral gray matter, where they closely apposed other HRP-positive processes, (3) otherwise non-reactive dendritic thickets of the contralateral gray matter and (4) close apposition to neurons of unknown origin on the contralateral side. As yet, we have not seen gap junctions associated with HRP-positive processes.

These results suggest that motoneurons of lumbar segments receive contralateral synaptic inputs on their crossed dendrites and that crossed interactions between lumbar motoneurons may be mediated at sites where dendrites from one side are closely apposed to dendrites and neuronal perikarya of the other side.

(Supported by USPHS grant NS 12211)

NEURO-ENDOCRINOLOGY

1070 PINEAL INFLUENCE ON ADRENOCORTICOTROPIC HORMONE (ACTH) SECRETION IN THE RAT. J.P. Allen, G.M. Vaughan* and P. Starr*. Dept. Neurosci., Peoria Sch. Med., Peoria, IL. 61605, and Dept. Med., Un. TX. Hlth, Sci. Ctr., San Antonio, TX. 78284.

Because the pineal gland may modulate pituitary-adrenal function in the rat, we measured plasma ACTH and corticosterone (B) in pineal-stimulated (blind-anosmic) and pinealectomized male Sprague Dawley rats housed in a 12-hr light/12-hr dark environment. We compared these data to plasma testosterone (T) levels in the same groups. Rats were sham-pinealectomized (ShPx), pinealectomized (Px), optic-enucleated olfactory-bulbectomized (blind-anosmic [BA]), or a combination BAPx in the fifth week of life. Five weeks later, half of each group was adrenalectomized (Adx), and after three more weeks the animals were decapitated under basal conditions 100 min after the onset of the light phase. Trunk blood was collected and analyzed for ACTH, B and T using sensitive and specific radio-immunoassays. The inter-assay and intra-assay variations for each radioimmunoassay was <5%. In the non-adrenalectomized groups, plasma B levels were significantly higher ($P<0.01$) following either BA or BAPx compared to either ShPx or Px. Significant ($P<0.01$) suppression of mean plasma T levels occurred only in the BA group. In contrast, mean plasma ACTH levels were slightly but significantly elevated ($P<0.05$) in the BA group compared to the ShPx, Px or BAPx groups. In the adrenalectomized groups, the post-Adx rise in mean plasma ACTH level was significantly higher ($P<0.01$) in the BAPx animals than in ShPx, Px or BA groups.

Our data suggest that in the male rat: (1) deprivation of sight and smell either stimulates B secretion or alters its circadian rhythmic secretion. Moreover, there is possible non-concordance between basal ACTH and B concentrations after this dual sensory deprivation; (2) either the pineal gland or intact sight/smell can separately inhibit the post-Adx rise in plasma ACTH levels with release of this inhibition after the combination of pineal ablation, optic enucleation and olfactory bulbectomy; (3) intact sight and smell facilitates T secretion. In marked contrast to the pineal-adrenal inter-relationships, the effect of sight/smell on the testes is through the blocking of inhibitory influences from the pineal on the gonads. However, in both pineal-adrenal and pineal-gonadal systems, the pineal acts as an inhibitory influence.

1071 LOCALIZED UPTAKE OF (^{14}C) DEOXYGLUCOSE BY THE PREOPTIC AREA OF FEMALE RATS IN RESPONSE TO VAGINOCERVICAL STIMULATION. Theresa O. Allen* and Norman T. Adler, Dept. Psych., Univ. Penn., Phila. PA 19104.

Stimulation of the vagina and cervix by the intromissions of the male rat during copulation is required to initiate the neuroendocrine events (progestational state) necessary for successful pregnancy (Adler, J. Comp. Physiol. Psychol. 69:613, 1969). In the present study, we used the (^{14}C)-2-deoxyglucose (2DG) method (Kennedy, DesRosiers, Jehle, Reivich, Sharpe & Sokoloff, Science 187:850, 1975) to identify brain areas which increased functional activity in response to vaginocervical stimulation.

Unanesthetized, behaviorally receptive female rats were gently restrained and stimulated with a smooth metal rod attached to a vibratory engraving tool. The vaginocervical stimulation was intermittent (on for 15 sec/min) and was applied for 5 minutes preceding and for 45 minutes following the 2DG injection. The pulse of 2DG was delivered intracardially via a chronically implanted jugular catheter. There were two groups of control animals: (1) females receiving no vaginocervical stimulation, and (2) females receiving the stimulation after cervical denervation by bilateral excision of the pelvic nerves.

In the experimental animals, x-ray autoradiographs revealed increased concentration of the label in the medial preoptic area of the hypothalamus. Autoradiographs from both groups of control females did not show this activation. Nonspecific activation of other brain areas (e.g., auditory structures) was similar in all three groups. These results confirm the involvement of the preoptic area in the response to vaginocervical stimulation (Carrer & Taleisnik, Endocrinol. 86:231, 1970) and reveal the value of the 2DG method to investigate neuroendocrine events.

(Supported by NIMH grant I T32 MH 15092, NIH grant HD-04522, and NSF grant BNS-76-01098).

1072 SEXUAL DIFFERENCES IN PATTERN OF HORMONE ACCUMULATION IN THE BRAIN OF A SONG BIRD. Arthur P. Arnold and Albert Saltiel*. Dept. Psychol., UCLA, Los Angeles, CA 90024.

The autoradiographic method was used to determine the distribution of hormone-accumulating cells in brain after injection of tritiated testosterone into gonadectomized adult male and female zebra finches (Poephila guttata). Significant sex differences in accumulation are found in two brain regions involved in control of song: Hyperstriatum ventrale pars caudale (HVc), and magnocellular nucleus of the anterior neostriatum (MAN). In the female HVc, only a very small number of cells are labelled, many fewer than in the male. Since the female HVc is much smaller than the male HVc, the paucity of hormone-accumulating cells in female HVc may be a reflection of the small number of female HVc cells in general. However, in female MAN, there are cells which appear to accumulate a small amount of radioactivity, but this accumulation is strikingly less in amount per cell than in male MAN. This suggests that hormone-accumulating cells are present in both male and female MAN, but the MAN cells differ between the sexes in their ability to accumulate testosterone or its metabolites. The implications for mechanisms of sexual differentiation in the brain will be discussed.

In certain other brain areas, including regions related to song, we did not detect sexual differences in distribution of hormone-accumulating cells. The following areas contain labelled cells in both sexes: the hypoglossal motoneurons controlling the muscles of the vocal organ (syrinx), nucleus intercollicularis of the midbrain (related to vocalization), medial preoptic area, periventricular magnocellular nucleus of the hypothalamus, and infundibular nucleus of the posterior hypothalamus.

1073 EFFECTS OF CASTRATION AND ESTRUS CYCLE STATE ON MONOAMINE-STIMULATED ADENYLATE CYCLASE ACTIVITY IN RAT HYPOTHALAMUS AND AMYGDALA. G. A. Barr, H. S. Ahn*, J. L. Gibbons, and M. H. Makman*. Depts. of Psychiatry, Biochemistry and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

There is increasing evidence that both normally occurring and surgically induced changes in endocrine systems can alter hypothalamic monoamine functions. For example, castration of male rats increased dopamine (DA) turnover, tyrosine hydroxylase (TH) activity, and steady state serotonin (5-HT) levels in certain hypothalamic nuclei. In the cycling female rat, dopamine turnover is lowest and norepinephrine (NE) turnover highest during proestrus, while steady-state levels of DA and NE peak during proestrus.

Adenylate cyclase (AC) activity in homogenates of rat anterior and medial hypothalamus or amygdala was measured as ATP dependent formation of cyclic AMP as previously described (Brain Res., 138, 125-138, 1977). In castrated males, there was significant stimulation of adenylate cyclase in the medial hypothalamus by both DA (100 μM) and 5-HT (100 μM) that did not occur in either intact or sham operated controls. In addition, there was no significant stimulation by NE (100 μM) or by the NE agonist methoxamine (100 μM) for any group. Using GPP(NH)P, a nonspecific stimulator of AC, there were no differences between groups. There was no significant stimulation in the anterior hypothalamus except for GPP(NH)P. In the amygdala, stimulation of AC by DA was unaffected by the endocrine status of the males.

In cycling females, AC was stimulated in the medial hypothalamus by dopamine, 5-HT, and isopropylnorepinephrine (100 μM) only during proestrus, and not during other stages. Furthermore, in the anterior hypothalamus, only GPP(NH)P stimulated AC. Amygdaloid AC activity was increased by several amines and GPP(NH)P but this stimulation was unaffected by differences in estrus cycle.

In summary, we have shown changes in monoamine stimulated AC activity in the medial hypothalamus due to alterations in endocrine systems for both male and female rats. These effects on AC systems are anatomically specific, occurring only in the medial hypothalamus and are pharmacologically specific, occurring only in response to specific monoamines and not to GPP(NH)P. These changes may reflect postsynaptic events that are either primary or secondary to alterations in presynaptic activity.

(Supported by Grants MH 06418, NS 09649, and RR 05397)

1074 EFFECT OF ANTERO-VENTRAL THIRD VENTRICLE LESIONS ON ANTIDIURETIC RESPONSES TO CENTRAL ANGIOTENSIN II. Steven L. Bealer, M. Ian Phillips and Phillip Schmid*. Depts. Psychology, Physiology and Internal Med., Univ. Iowa, Iowa City, IA 52242.

Electrolytic lesions of periventricular tissue of the antero-ventral third ventricle (AV3V) typically render rats adipsic for a period of one to 10 days following surgery, with no apparent primary aphagia, hyperemotionality, or other behavioral disturbances. In addition, during the period of adipsia, animals continue to excrete dilute urine, indicating a failure to initiate an appropriate antidiuresis which would be expected in view of their accumulating fluid deficit. Consequently, AV3V lesioned animals rapidly become hypernatremic and hyperosmotic (Johnson & Buggy, Am. J. Physiol., 1978, 234, R122-R129). The continued diuresis during lesion-induced adipsia indicates that antidiuretic hormone (ADH) mechanisms of fluid conservation have been compromised by AV3V ablation. The present experiment was designed to determine if AV3V lesions attenuate ADH release in response to intraventricular (IVT) injections of angiotensin II (AII), hypertonic NaCl, and phenylephrine during the adipsic period.

Following electrolytic ablation of the AV3V region or sham lesioning in 20 rats, each animal was implanted with a cannula in the lateral cerebral ventricle. On the day following surgery, all animals were prepared with arterial, venous and bladder catheters. Blood pressure, urine conductance, and urine flow rate were monitored in awake, unrestrained animals during a continuous intravenous infusion of a hydrating solution administered to produce a diuresis. Changes in these blood and urine parameters were recorded following IVT injections of 100 ng and 500 ng AII, 1 ul 3% NaCl, and 50 ug phenylephrine.

In the AV3V lesioned rats increases in blood pressure and urine conductance, as well as the decreased urine flow rate normally evoked by AII and hypertonic NaCl were significantly attenuated relative to the responses of sham operated controls. However, there was no difference in the pressor and antidiuretic responses evoked by IVT infusion of phenylephrine between lesioned and sham lesioned animals. In addition, a water deprived control group was tested to determine the effect of adipsia on ADH release in intact animals. This group had responses similar to water replete shams for all stimuli tested.

These data suggest that the integrity of the AV3V region is essential for the ADH release response to AII and osmotic stimuli IVT. Whether this is due to impaired synthesis or release of ADH remains to be shown. Finally, the lower blood pressure response in the presence of an attenuated ADH release is consistent with a role for ADH in the pressor response to AII.
(Supported by grants from NIMH and NSF)

1075 CORTICOSTERONE INDUCED PROTEINS IN THE PITUITARY OF ADRENALECTOMIZED MALE RATS. Margery C. Beinfeld* and Paul M. Packman* (SPON: R. A. Cohen). Dept. Psychiatry, Washington U. Med. Sch., St. Louis, MO 63110.

Corticosterone is known to be taken up and retained in the liver, pituitary, hippocampus and hypothalamus. The possibility that the synthesis of specific soluble proteins follows this uptake has been investigated using mature and immature Sprague-Dawley rats. The rats were given either steroid or the same volume solvent and sacrificed after one or three hours. Tissues from steroid treated animals were incubated with ^{14}C labeled leucine while controls were incubated with ^{3}H labeled leucine at 37°C for one hour. The steroid treated and control tissues were pooled separately, homogenized, and spun at 105,000 x g for one hour. The pellets from the high speed centrifugation were extracted with high salt and spun to clarify. The soluble protein extracts were analyzed on SDS-polyacrylamide gels.

Using this technique we have confirmed the result of Shelton and Alfrey (Nature 228:132, 1970) that corticosterone induces the synthesis of a protein in the liver with a subunit molecular weight of about 42,000 daltons.

We find no corticosterone-induced protein in the hippocampus, hypothalamus, cortex, or cerebellum in either the immature or adult rats at either one or three hours after steroid injection. Also, there is no induced protein in the high salt extracts of the pellets from these tissues.

However, in immature rats we do find that corticosterone induces the synthesis of two proteins with molecular weights of about 17,000 and 19,500 daltons. In mature rats corticosterone induces the synthesis of a single protein with a molecular weight of 17,000. There have been several reports recently that corticosterone causes growth hormone to increase in rat pituitary tumor cells (J. Martial, et al., PNAS 74:4295, 1977). Since the larger protein, 19,500 daltons, is sufficiently close in size and mobility to rat growth hormone, it is possible that one of the induced proteins is growth hormone. The experimental identification of the induced proteins will be described.

This work was supported by grants MH30140 and MH19624 and NIMH National Research Service Award (M. Beinfeld).

1076 ALTERED NEUROANATOMICAL ORGANIZATION IN THE CENTRAL NERVOUS SYSTEM OF THE GENETICALLY OBESE (ob/ob) MOUSE. David A. Bereiter and Bernard Jeanrenaud*. Univ. of Geneva, Laboratoires de Recherches Medicales, Geneva, SWITZERLAND.

The genetically obese (ob/ob) mouse is characterized by excessive body weight gain, hyperphagia and hyperinsulinemia. These and other endocrine-related abnormalities have suggested a generalized central nervous system (CNS) disorder, possibly hypothalamic. The following results have been obtained from nine week old male genetically obese (C57BL/6J ob/ob) mice and lean (C57BL/6J ++) control mice: (1) Ob/ob mice have greatly reduced total brain wet weight (-14.6%) that is not due to altered water retention. (2) Using a planimetric technique, ob/ob neurons demonstrate significantly reduced soma cross-sectional areas in eight of nine brain regions sampled, including the ventromedial hypothalamic nucleus. (3) Only lateral hypothalamic area neurons in ob/ob mice have soma cross-sectional areas as large as those of control mice. (4) A Golgi-Cox study of ventromedial and lateral hypothalamic neurons reveal no dramatic difference in dendritic organization between these two mouse strains.

Conclusion. The reduced total brain weight coupled with the morphometric data suggest that the ob/ob mouse brain differs significantly from that of lean controls. These differences may underlie the abnormal adult endocrine status of this genetic obesity model. (Supported by Swiss National Science Foundation, Berne.)

1077 RATE OF SYNTHESIS OF NEUROSECRETORY PROTEINS IS NOT INFLUENCED BY SOMATIC POOL SIZE. Robert W. Berry. Dept. Anat., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Feedback inhibition is one conceivable method by which neurosecretory cells could adjust their production of secretory proteins in accordance with physiological demand. Under this hypothesis, the size of the somatic storage pool of proteins destined for axonal transport would influence the rate of synthesis of such proteins. I have tested this hypothesis in cells L11 and R15 of Aplysia. Each of these cells produces a group of low molecular weight proteins which leave the soma by axonal transport, and in each cell these peptides account for 40-50% of the total incorporation of ^{3}H-leucine into protein in a two-hour period. These peptides leave the soma with a half life of approximately 4 hours at 20-25°C, and this decay can be completely abolished by exposure to 0.1 mg/ml vinblastine. Exposure to vinblastine for 4 hours at this temperature does not inhibit incorporation of ^{3}H-leucine into total protein, and can thus induce a doubling of the somatic content of these peptides. However, the extent of incorporation of ^{3}H-leucine into these species during a 2 hour labeling period following 4 hours in vinblastine is identical to that of controls kept in seawater. Furthermore, somatic accumulation of these peptides can also be induced in L11 by crushing its axon close to the soma, and again, this treatment does not affect the relative rate at which it synthesizes the transported proteins. These data indicate that feedback inhibition is not an important mechanism in the short-term regulation of the synthesis of neurosecretory proteins. (Supported by NIH grant NS-11519).

1078 PROGESTIN RECEPTORS IN GUINEA PIG BRAIN: CHARACTERIZATION AND RELATIONSHIP TO LORDOSIS. Jeffrey D. Blaustein, Institute of Animal Behavior, Rutgers University, Newark, NJ 07102.

The synthetic progestin, 17α, 21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione (R 5020), which binds with higher affinity and dissociates less rapidly than progesterone from the progestin receptor, was used to measure progestin receptors in the high speed supernatant of guinea pig central nervous system and pituitary gland. The receptor has an apparent dissociation constant of .2 nM as measured by LH-20 gel filtration. It is progestin-specific; 100x excess of radioinert progesterone, 5α-dihydro-progesterone, or d-norgestrel inhibits ^{3}H-R 5020 binding by more than 50%, whereas corticosterone, cortisol, testosterone, estradiol, dexamethasone and several other steroids do not. The receptor is partially protein in nature; the binding is destroyed by pronase, not DNase or RNase. Although the progestin receptor is detectable in the hypothalamus, preoptic area-septum, cortex, midbrain, amygdala and pituitary gland of ovariectomized guinea pigs, estradiol pretreatment increases the concentration only in the hypothalamus (150%), preoptic area-septum (57%), midbrain (28%) and pituitary gland (144%). The increase in a pooled sample of hypothalamus-preoptic area-septum (HPS) is dependent on the dose of estradiol benzoate injected.

Sequential administration of estradiol benzoate and progesterone results in sexual receptivity in ovariectomized guinea pigs. Following subcutaneous injection of 1.6 µg of estradiol benzoate (slightly above the threshold dose for inducing lordosis when followed by progesterone in our guinea pigs) the HPS progestin receptor content increases between 14-24 hr, peaks by 40-64 hr and decreases by 88 hr. This time course correlates well with the previously reported time course for the induction of sexual receptivity by estradiol benzoate and progesterone. The concentration of cytosol receptor in HPS and midbrain, measured by this assay, is decreased in vivo by behaviorally effective doses of progesterone. Following termination of heat, a refractory period is seen during which time an additional injection of progesterone does not facilitate sexual receptivity. The failure of progesterone to facilitate lordosis correlates with an absence of the progestin receptor in HPS and midbrain. The lack of cytosol receptor may prevent progesterone from exerting its facilitatory effect on sexual receptivity. These experiments are consistent with the notion that brain progestin receptors may mediate at least some of the behavioral effects of progesterone.

1079 INCREASED SERUM GROWTH HORMONE (GH) AND SOMATIC GROWTH IN ADULT HAMSTERS WITH HIPPOCAMPAL TRANSECTIONS. K.T. Borer, R.P. Kelch*, and M.E. Trulson, Departments of Phys. Educ. and Pediat., Univ. Michigan, Ann Arbor, MI 48109 and Dept. Psych., Princeton Univ., Princeton, N.J. 08540.

Lesions of rostral septum lead to increased serum GH and somatic growth and to decreased pituitary GH content and concentration in adult hamsters (Borer et al., Neuroendo., 1977, 23, 133). We examined the hypothesis that this effect was due to damage to fibers of passage by producing bilateral transections of dorsal hippocampus in 30 adult female hamsters (HIPPO) and of overlying cerebral cortex in 30 control animals (CON) with a retractable wire knife. Ponderal and skeletal growth and percentage of body fat were determined in 10 HIPPO and 10 CON hamsters allowed to survive 90 days after surgery. Endocrine changes associated with somatic growth were examined by measuring the concentration of serum GH and insulin with heterologous radioimmunoassays for hamster GH and rat insulin, respectively, and changes in brain neurotransmitter pathways were determined in four brain regions: hippocampus (HIP), cerebral cortex (CC), corpus striatum ST) and diencephalon (D) by the spectrofluorometric method in 20 HIPPO and 20 CON animals killed on 12th postoperative day.

HIPPO hamsters gained weight 10.7 times faster ($p < .001$) between days 3 and 35 and had a 2 to 7% greater ($p < .01$) skeletal growth than CON hamsters. In HIPPO hamsters serum GH was increased regardless of the prandial state (9.6 ± 1.9 vs 2.1 ± 0.2 ug SHAP/ml, $p < .01$) and serum insulin was increased in animals killed ad libitum (16.4 ± 4.2 vs 5.2 ± 1.3 ng/ml, $p < .01$). HIPPO hamsters were significantly fatter than CON (17.9 vs 11.0%, $p < .01$) with obesity accounting for 25% of weight differences between the groups. HIPPO hamsters had significant depletions of 5-HT in HIP (-50%, $p < .001$) and CC (-12.4%, $p < .02$) and of NE in HIP (-37.9%, $p < .001$) and in CC (-16.6%, $p < .01$)

We conclude that 5-HT, NE and/or other nerve fibers travelling through rostral septum to HIP inhibit GH secretion and somatic growth in adult hamsters.

1080 INVOLVEMENT OF ADENOHYPOPHYSIAL DOPAMINE IN THE REGULATION OF PROLACTIN RELEASE. Sara R. Chiocchio*, Miguel A. Cannata*, Jorge R. Cordero Funes* and Juan H. Tramezzani* (SPON: D.A. Pasquier). Instituto de Neurobiología, Serrano 665, 1414 Buenos Aires, Argentina.

Dopamine (DA) was measured in the pituitary anterior lobe and median eminence from lactating rats using a modification of Coyle and Henry's method which increases its sensitivity. The effect of pup separation and suckling was studied in order to correlate changes in DA levels with changes in serum prolactin.

In lactating rats that were separated from their pups low levels of circulating prolactin were found at 2, 4 and 8 hours. DA levels in the median eminence showed a decline at 2 hours; at 4 and 8 hours of separation a significant increase was observed. In the pars distalis the concentration of DA increased with the length of non-suckling interval. Suckling induced a rapid rise in serum prolactin levels in rats that were separated from their pups 4 hours earlier. Under these conditions a significant decrease on DA levels in the median eminence and pars distalis was observed as early as 5 min after the onset of suckling; at 30 min the DA levels were still low. In the situations studied, suckling and pup separation, a negative correlation between serum prolactin and DA levels both in the median eminence and pars distalis was always found. These data suggest a role for adenohypophysial DA in the regulation of prolactin release.

1081 CHARACTERISTICS OF THE ARCUATE NEURONS IN RESPONSE TO MEDIAL PREOPTIC STIMULATION. Carlos M. Contreras* and Ei Terasawa (SPON: R. W. Goy). Wis. Regional Primate Res. Center, Madison, WI 53706.

The medial preoptic nucleus (MPN) is necessary for the release of LH-RH in rats. However, the mode of innervation from this structure to the basal hypothalamus (MBH) remains unknown. A neurophysiological attempt was made to clarify this relation in ovariectomized (OvX) or ovariectomized estrogen primed rats (EB - 5 µg, 2 days prior to recording). A concentric electrode for stimulation was stereotaxically implanted in the MPN under urethane anesthesia. Glass pipette microelectrodes for single unit recording were inserted into the MBH by a parapharyngeal approach. A side-by-side electrode for stimulation was visually placed on the surface of the stalk median eminence (ME) for identification of the recording neurons.

A total of 68 neurons from the OvX rats and 59 neurons from the EB rats were recorded. In both groups about 52-60% of the neurons were recorded from 0.5-1.5 mm depth of the ventral surface (where the arcuate nucleus is located), 32-42% of the neurons were from 1.6-2.5 mm depth and 6-8% of the neurons were from the superficial layer (0-0.5 mm depth). Distribution of neurons responding to ME stimulation in OvX and EB rats was similar: 1) neurons of 19.1% (13/68, mean latency 8.9 msec) in OvX and 18.6% (11/59, mean latency 8.3 msec) in EB were antidromically identified; 2) the remaining 80.9% (55/68) in OvX and 81.4% (48/59) in EB were not antidromically identified, but they were classified as paucisynaptic neurons (latency 15-30 msec, 47.1% in OvX and 35.6% in EB) and as polysynaptic neurons (latency over 30 msec; 33.8% in OvX and 45.8% in EB); 3) the majority of antidromically activated neurons and paucisynaptic neurons were recorded from the arcuate nucleus and a higher percent of polysynaptic neurons with long latency were recorded at 1.6-2.5 mm from the ventral surface. 4) MPO stimulation induced more activation in the antidromically identified neurons than polysynaptic neurons, and MPO stimulation induced more depression in the polysynaptic neurons than antidromically identified neurons in both OvX and EB groups. 5) During MPO stimulation slightly higher percentages of activated neurons and lower percentages of depressed neurons were recorded in the EB group. 6) EB treatment induced prolonged MPO activation and shortened MPO depression in the antidromically identified and the paucisynaptic neurons, while EB treatment produced shortened MPO activation in the polysynaptic neurons.

It is concluded that 1) a major portion of antidromically identified and paucisynaptic neurons originate from the arcuate nucleus, 2) those neurons were activated rather than depressed by MPO stimulation, and 3) this activation is facilitated with EB treatment. (Supported by NIH Grant RR-00167.)

1082 EFFECTS OF BODY WEIGHT VARIATION ON ESTRADIOL INDUCED ACTIVITY. Verne C. Cox and James M. King.[1] Dept. Psychol., Univ. Texas, Arlington, TX 76019.

Previous work (Neurosci. Abs., 1978, 3, abs. no. 1111) has shown that estradiol benzoate (EB) acts on the medial preoptic - anterior hypothalamic area in the female rat to produce increments in wheel running activity. In female rats which have not sustained brain lesions, the increments in activity produced by EB are accompanied by substantial changes in body weight (BW). If EB induced activity is BW regulatory, like estrogenic suppression of food intake, then the EB induction of activity should be modulated by BW level. The present study explored the effects of BW variation on EB induced wheel running activity in female rats. Twenty-four female Holtzman albino rats were maintained in activity wheels for the duration of the study. After a 21 day baseline period, the subjects were ovariectomized, and allowed to recover for 19 days. During this recovery period, 1/2 of the subjects were maintained at their preoperative BW level by restricting their food intake while the rest of the subjects were given unrestricted access to food. At the end of the recovery period, 1/2 of the subjects in each BW condition were given daily subcutaneous injections of 3.0 µg/day of EB for 14 additional days. The postsurgical activity decrement was greatest in those animals given unrestricted access to food. However, EB treatment significantly enhanced activity in both hormone treated groups, and this effect was independent of BW level. When animals in the EB unrestricted group are matched for BW level, before and during EB treatment, it was found that activity levels were higher during hormone treatment. Thus, the activity increments induced by EB are independent of BW level.

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1083 NORADRENERGIC CONTROL OF PITUITARY OXYTOCIN (OXT) AND ARGININE VASOPRESSIN (AVP): EFFECTS OF ESTROUS CYCLE, OVARIAN HORMONE TREATMENT AND VENTRAL NORADRENERGIC BUNDLE LESIONS. W. R. Crowley, T. L. O'Donohue*, J. M. George* and D. M. Jacobowitz (SPON: M. B. Carpenter). Lab. Clin. Sci., NIMH, Bethesda, Md. 20014 and Dept. Med., Ohio State Univ., Columbus, Ohio 43210.

Several lines of evidence suggest a role for posterior pituitary hormones in reproductive neuroendocrine processes. The present studies employed radioimmunoassays to measure pituitary OXT and AVP over the course of the four day estrous cycle and after ovariectomy and subsequent ovarian hormone treatment in female rats. Because norepinephrine (NE) has been implicated in feedback actions of gonadal steroids, changes in OXT and AVP were correlated with NE levels and turnover in the paraventricular and supraoptic nuclei. Maximal levels of OXT and AVP in the pituitary were attained on proestrus and lowest levels on metestrus. NE levels in the paraventricular nucleus were high during metestrus and diestrus and low during proestrus and estrus. NE in the supraoptic nucleus did not vary during the cycle.

Pituitary OXT levels were elevated 54 hr. after a single injection of estradiol benzoate (5 µg) in ovariectomized rats. Subsequent administration of progesterone (1.5 mg) or a second estradiol injection 48 hr. later did not further increase or decrease OXT levels. A single estrogen injection did not affect AVP levels but subsequent treatment at 48 hr. with progesterone or estrogen significantly elevated pituitary AVP levels when measured 6 hr. later. AVP was significantly decreased 30 hr. after a second estrogen injection. Steroid effects on AVP could be related to NE changes in the paraventricular nucleus because NE levels and turnover were decreased by the treatments that elevated pituitary AVP content. The ovarian steroids did not affect NE in the supraoptic nucleus.

Bilateral transection of the caudal ventral noradrenergic bundle reduced NE in the paraventricular (-30%) and supraoptic (-50%) nuclei, decreased pituitary AVP (-70%) and markedly elevated pituitary OXT (+ 710%).

These studies suggest that pituitary content of OXT and AVP is modulated by central noradrenergic neurons and that this control may mediate the effects of ovarian steroids on posterior lobe hormones.

1084 UPTAKE OF IODOTHYRONINES INTO SYNAPTOSOMES: EVIDENCE FOR A HIGH-AFFINITY TRANSPORT MECHANISM. F. L. Crutchfield*, M. B. Dratman, Veterans Administration Hospital and Medical College of Pennsylvania, Phila., Pa.

Intravenously administered ^{125}I-labeled iodothyronines (T_3*, T_4*) reaching the rat brain become highly localized in synaptosomes (SYN). Although both hormones are taken up to the same degree in brain cytosol, T_3* is approximately 2-fold more concentrated than T_4* within nerve terminals. Measurement of endogenous hormone concentrations in SYN (isotope equilibrium studies) confirm significant enrichment in T_3 relative to T_4. To determine whether different rates of SYN membrane transport of the two hormones might account for the observations, and to gain information regarding mechanisms of iodothyronine transport into SYN, enriched nerve ending preparations, separated from other particulate fractions (on discontinuous sucrose density gradients), were incubated under 95% O_2:5% CO_2 in bicarbonate buffered salt solution (artificial CSF, Glowinski) containing $1x10^{-9}$M T_3* or T_4*. To verify uptake into, as distinguished from binding to, SYN vesicles, portions of prelabeled SYN were treated with either CSF or H_2O; intact and osmotically shocked particles were recovered by centrifugation and identity of iodocompounds* in each pellet determined by means of paper chromatography (2 systems). Results: Uptake rates were highly temperature-dependent, were maximum during the first 3 min (0.2-0.3 pmol/min/mg SYN), reached a plateau in 10 min and were similar for both T_3* and T_4* throughout the 30 min period of observation. At 30 min, iodothyronine* was 40-50 fold more concentrated in SYN than in medium. Addition of increasing concentrations of unlabeled hormone, over a range of $1x10^{-7}$ to $5x10^{-6}$M, progressively decreased labeled hormone uptake. Hypoosmolar conditions produced approximately 40 % loss of iodothyronines* from SYN; most individual metabolites were lost to an equal or greater degree. Conclusions: No differences in rates of T_3* and T_4* transport into SYN were observed in these experiments. Therefore differences in concentrations of the two hormones may be due to differences in their disposal rates. Features of iodothyronine uptake thus far measured: high initial velocity at low amino acid concentrations, saturability, and marked temperature dependence, resemble the high-affinity SYN membrane transport characteristics described for other aromatic amino acids. Supported by funds from the Medical Research Service, Veterans Administration Hospital, Philadelphia, Pa.

1085 PITUITARY-DEPENDENT CHROMATOPHORE CHANGES FOLLOWING FROG DIENCEPHALIC ROOF ELECTRICAL STIMULATION. M. Duff Davis* and Mac E. Hadley* (SPON: P. E. Pickens). University of Arizona, Tucson, Arizona 85721.

Melanophore stimulating hormone (MSH) is known to effect melanin dispersion within pigment cells for adaptive color change in most lower vertebrate species. In higher mammals, including humans, MSH is a modulator of selective attention and other behavioral parameters. This hormone is secreted from the pars intermedia lobe of the pituitary gland. Severing the hypothalamic connections leading into the pituitary releases neuronal inhibition of the pars intermedia with a resulting spontaneous secretion of MSH.

It has long been reported that the sensory input controlling MSH secretion is from photic cues processed through the lateral eyes. However, Oshima and Gorbman (Gen. Comp. Encocrinol. 13: 98-107, 1969) propose that the pineal complex is the light transducer which regulates MSH release via neural tracts through the hypothalamus which then innervate pars intermedia secretory cells.

We have been able to induce darkening of Rana pipiens melanophores in vivo by electrically stimulating the surface of the diencephalon on and immediately surrounding the pineal gland. The latency between onset of stimulation and skin darkening, as measured by a photoelectric reflectometer, is between one and three minutes. Stimulation after hypophysectomy did not lead to melanin dispersion. Continuous stimulus pulses were 1 milliamp in current, 0.5 msec duration and 100 Hz through bipolar stainless-steel electrodes (50 mic.) separated by 0.2 mm.

It is concluded that stimulation of the pineal region influences hypothalamic neurons either through a humoral step via the cerebrospinal fluid in the third ventricle or possibly by direct neuronal connections to the pars intermedia.

(USPHS Grant AM-16282)

1086 LOCALIZED BEHAVIORAL EFFECTS OF TRITIATED ESTRADIOL IMPLANTS IN THE VENTROMEDIAL HYPOTHALAMUS OF FEMALE RATS. P. G. DAVIS*, B. S. McEwen, D. W. Pfaff, Rockefeller Univ., New York, NY 10021.

The ventromedial nucleus (VMN) of the hypothalamus has been implicated consistently in the control of feminine sexual behavior. The broad objective of the present experiments was to determine if estrogenic stimulation of the VMN of ovariectomized (OVX) rats is sufficient to activate lordotic behavior. For this purpose, it was important to determine first the amount of hormone contained within a cannula, the amount delivered to the brain, and the degree of spread from the site of implantation. Thirty gauge implants of undiluted estradiol contain ≃ 10 μg of steroid, which appears to be a considerable excess. Therefore, we chose to use a refined implant technique in which crystalline ^{3}H estradiol (^{3}H-E$_2$) was diluted with cholesterol in a ratio of ≃ one to 300. Such cannulae contain 9-15 ng of ^{3}H-E$_2$. Similar cannulae were implanted unilaterally into the VMN of OVX rats (n=12). Progesterone was administered to the animals on days 3 and 6 post-implantation and tests for feminine sexual behavior were conducted. Seven of the 12 females exhibited lordotic behavior on at least one of the tests ($\bar{X}$ LQs: Test I=38; Test II= 68). Following the last test, animals were decapitated, their brains removed and dissected and whole tissue radioactivity determined by scintillation counting. High levels of radioactivity were recovered from the half of the hypothalamus containing the cannula (350 pg/gm tissue); whereas virtually no radioactivity was recovered from the contralateral side of the hypothalamus, or the preoptic area, amygdala, cortex, pituitary, or uterus (≤ 3% of hypothalamic levels). Determinations of the radioactivity remaining in the cannulae indicated that ≃ 30% of the total was delivered in a 7-day period. Subsequently we showed that similarly prepared bilateral implants produced a reliable degree of lordotic responding without increasing the spread of hormone to regions outside the hypothalamus (whole tissue homogenates: hypothalamus, 990 pg/gm tissue; all other regions, 0.6-3.2% of hypothalamic levels). Determinations of nuclear bound radioactivity in animals with either unilateral or bilateral implants in the VMN revealed a pattern of distribution of radioactivity essentially identical to that shown in whole tissue (individual hypothalami: 17-25 fmoles/mg DNA; other regions: ≤ 6% of hypothalamic levels). These results suggest that behaviorally effective, dilute implants of estradiol exert their actions primarily within the region of implantation. (Supported by MH05781 to P.D., NS07080 to B.Mc., HD 05751 to D.P., and a Rockefeller Foundation Grant, RF70095.)

1087 PROGESTERONE INHIBITION OF ANDROGEN- OR ESTROGEN-INDUCED SEXUAL DIFFERENTIATION IN THE FEMALE HAMSTER. Joseph F. DeBold and Lynwood G. Clemens*. Dept. Psych., Carnegie-Mellon Univ., Pittsburgh, PA 15213 and Dept. Zool., Michigan State Univ., East Lansing, MI 48824.

Progesterone can inhibit a number of central effects of estrogen and androgen in adult rodents. This inhibitory action may be an important component in the ability of progesterone to coordinate sexual receptivity and ovulation and reduce the probability of receptivity in the absence of ovulation. Another possible role of progesterone could be to protect the female fetus or neonate from androgenic or estrogenic stimulation during sexual differentiation. The present study examined the effect of progesterone (P) on sexual differentiation and its protective ability against the action of testosterone propionate (TP) and estradiol benzoate (EB) in developing hamsters.

Entire litters were treated 24hr after birth with either 10ug TP, 2ug EB, 500ug P, 2mg P, 10ug TP + 500ug P, 10ug TP + 2mg P, 2ug EB + 500ug P, 2ug EB + 2mg P or the oil vehicle. In another set of litters the pups were treated with 500ug P, 10ug TP + 500ug P, or 2ug EB + 500ug P 24hr after birth and then also given 500ug P 48 and 72hr after birth. High or repeated dosages of P with or without TP or EB resulted later in some acyclicity and gonadal abnormalities. TP or EB alone did not have either effect. After gonadectomy at 70 days of age the animals were tested for female and male patterns of sexual behavior with appropriate hormone replacement. Neonatal treatment with either EB or TP decreased adult sexual receptivity (defeminization) and induced the capacity for masculine sexual behavior (masculinization) in female hamsters. Neonatal treatment with 2mg P or 500ug P/d x3 (but not a single injection of 500ug P) reduced the defeminizing effect of early EB or TP. None of the P treatments reduced the masculinizing action of EB or TP. Neonatal treatment with P alone had no effect on the adult sexual behavior of either female or male hamsters.

Although P was found to be capable of inhibiting defeminization induced by either EB or TP, this required large dosages of P. Even these large amounts of P failed to reduce the masculinizing influence of EB or TP. However, the effective P treatments did result in higher pup mortality and adult gonadal abnormalities. This weak behavioral action of P coupled with its side effects does not support an important protective role of endogenous P during sexual differentiation in hamsters. In addition, a comparison of the dose-effectiveness of P inhibition of EB induced neonatal sexual differentiation and of EB and P induced adult sexual receptivity seen in earlier studies suggests that these two inhibitory effects of P are subserved by different mechanisms of action.

Supported by USPHS Grant HD-06760.

1088 DOPAMINE LEVELS IN HYPOPHYSEAL STALK BLOOD OF THE RAT DURING SURGES OF PROLACTIN SECRETION INDUCED BY CERVICAL STIMULATION. W.J. de Greef* and J.D. Neill*. (SPON: K.V. Anderson). Physiol. Dept., Emory Univ., Atlanta, GA. 30322.

Stimulation of the rat's uterine cervix establishes a pseudopregnancy and the secretion of two daily prolactin (PRL) surges. One PRL surge is called nocturnal and is secreted from 0100-0700 hr and the other is diurnal and is secreted from 1600-2000 hr (lights on 0600-1800 hr). These surges also can be induced in long-term ovariectomized rats and last for 6 days. We found in the present study that urethane anesthesia (1.1 g/kg BW) did not abolish the PRL surges induced by electrical stimulation of the uterine cervix 16-24 hr before treatment with urethane. Under urethane anesthesia, PRL levels during the nocturnal surge (0130-0430 hr) were 11.8±3.0 ng/ml vs 2.9±0.5 ng/ml in unstimulated rats (mean ± SE, n=6). During the diurnal surge (1530-1830 hr), PRL concentrations were 7.4±1.2 ng/ml vs 2.0±0.6 ng/ml in unstimulated rats (n=6).

There is now strong evidence that dopamine (DA) is a physiological prolactin inhibiting factor. The purpose of this study was to measure DA levels in hypophyseal stalk blood during the PRL surges in urethane-anesthetized, ovariectomized rats. Blood from the hypophyseal stalk was collected for 3-one hr periods during both the nocturnal and diurnal surges. During the nocturnal PRL surge, stalk plasma DA levels were 7.5±2.6 (0230 hr), 6.1±1.3 (0330 hr) and 5.7±0.5 (0430 hr) ng/ml (n=10) whereas in unstimulated rats DA levels at the same time intervals were 10.3±4.0, 12.5 ±4.8 and 8.8±2.6 ng/ml (n=10). During the diurnal PRL surge, stalk plasma DA concentrations were 4.1±0.9 (1630 hr), 3.7±0.7 (1730 hr) and 4.3±0.8 (1830 hr) ng/ml (n=10), whereas in unstimulated rats DA levels at the same time intervals were 7.7±1.9, 6.0 ±1.2 and 6.7±1.3 ng/ml (n=10).

Statistical analysis revealed that DA levels were not significantly different between stimulated and unstimulated rats at any of the individual time intervals. However, when the data from the 3 individual hourly collections during the diurnal surge were pooled, DA levels were significantly lower in stimulated than in unstimulated rats ($P < 0.05$). The correlation coefficient (-0.41) between mean DA levels in hypophyseal stalk blood and PRL levels in peripheral blood at the individual time intervals, was not significant ($P > 0.05$).

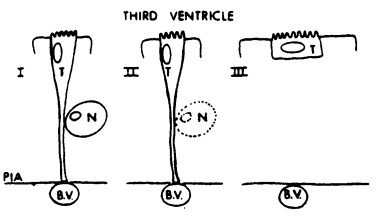
Thus, although hypothalamic DA secretion is important for the tonic inhibition of PRL secretion (Gibbs and Neill, ENDOCRINOLOGY 102:1895, 1978) it seems unlikely that changes in DA secretion alone can account for the changes in PRL secretion observed during pseudopregnancy. (Supported by grants from the NIH, HD 04312, and from ZWO, The Netherlands).

1089 EFFECT OF MONOSODIUM GLUTAMATE ADMINISTRATION ON THE TANYCYTES OF THE ARCUATE NUCLEUS. Manuel del Cerro, Edith Hurst (1)*, Kirk I. Moldoff* and Karl M. Knigge. Dept. Anat. and Brain Res. U. of Rochester, Rochester, N.Y. and Dept. Biology, Eastern Mich. U., Ypsilanti, MI (1).

It is well known that the administration of high doses of monosodium glutamate (MSG) to perinatal animals induces widespread neuronal death within the hypothalamic arcuate nucleus. Recently we have shown (del Cerro and Knigge, 77,78) the existence of intimate contacts between the tails of the tanycytes of the third ventricle and the somas of arcuate neurons (Fig. I). Thus we decided to investigate what cytological alterations occur in the tanycytes when the adjacent neurons are destroyed by MSG.

Newborn rats and mice were injected with MSG, at a dose of 4 MG/KG of body weight. The animals were allowed to survive from 6 hrs to 18 months, and studied by optical and electron microscopy. Controls included littermates injected with NaCl and non-injected animals. 24 hrs after injection most of the arcuate neurons show signs of advanced degeneration. By 48 hrs the neurons undergo lysis, but the adjacent tanycytes do not show any obvious damage. Their tails extend unaltered across pools of liquified material resulting from the dissolution of the neuronal elements (Fig. II). However, in animals sacrificed months after the injection, the tanycytic somas appear distorted and their nuclei abnormally orientated in relation to the ventricular surface. The more striking finding, however, is that the tanycytic tails have disappeared. This causes a profound change in the geometry of the tanycyte, which loses its contact with pial and neural elements and adopts the configuration of a low cuboidal epithelium (Fig. III).

These experiments show that if the neural-glial associations in the arcuate nucleus are disrupted, by destruction of the neural elements, the glial component undergoes a slow but extensive morphological regression. This result is consistent with the idea, postulated by this and other laboratories, that the tanycytes may functionally link hypothalamic neurons with the CSF of the III ventricle.



(Supported by NIMH H34.6236-JHU-1 and BRSG RR 05043).

1090 HETEROGENEOUS AXOPLASMIC RETICULUM (AR) IN PEPTIDERGIC NEUROSECRETROY (NS) NEURONS: POSSIBLE ROLE IN HORMONE TRANSPORT AND VESICLE FORMATION. H. Dieter Dellmann*, Mona Castel* and John G. Linner* (SPON: D. G. Emery). Department of Veterinary Anatomy, Pharmacology and Physiology, Iowa State University, Ames, IA 50011 and Zoology Department, Hebrew University of Jerusalem, Israel.

Ultrastructural studies of the hypothalamo-neurohypophysial system (HNS) in several rodent species and in the frog reveal extensive AR extending from the NS perikarya into the axon terminals. This reticulum consists of anastomozing tubes often containing electron-dense material similar to that in the neurosecretory granulated vesicles (NGV). Constrictions and dilatations are seen along the tubes from which NGV may bud. NGV bearing protrusions, like vestiges of reticulum, are apparent and reticular connections may exist between NGV (Castel, M.: Gen. Comp. Endocr. 31: 63, 1978).

AR is scarce in the HNS of normal adult animals, but increases after water deprivation,hypertonic saline administration, excessive doses of HRP, stalk transection or compression and after adrenalectomy. The HNS of fetal and neonatal rats is particularly well endowed with AR. We suggest that this electron-dense AR may transport hormone and carrier or precursor, representing an extension of the Golgi complex into the axon. The NGV may be formed not only in the perikaryon but also along the axon and even in the axon terminal.

The AR of the HNS is heterogeneous. In addition to that described above, electron-lucent tubules of AR are seen, often fragmenting into vesicles. Furthermore, a type of narrow and extensively anastomozing AR is present under some experimental conditions, but is usually not associated with NGV or vesicles. These other types of AR in NS axons are possibly concerned with anterograde and retrograde axonal transport of substances, other than hormones.

The electron dense AR with which the NGV are associated may represent the avenue of fast axonal transport postulated by Droz (In: The Nervous System, D. B. Tower, ed., Vol. 1, pp. 111-127, Raven Press, New York, 1975). In the stressed HNS and in the fetal and neonatal HNS it becomes abundant, demonstrating a remarkable adaptation of the neuron to the organism's increased hormone demand.

Supported by NIH grant 1 RO1 NS14062 (HDD) and in part (MC) by BNSF grant 200.

1091 SEROTONERGIC MODULATION OF THE EFFECT OF STRESS ON THE PLASMA ACTH AND CORTICOSTERONE RESPONSES TO SUBSEQUENT STRESS. Errol B. De Souza* and Glen R. Van Loon* (SPON: **D.R.Crapper**). Depts. of Medicine and Physiology, University of Toronto, Toronto, Canada.

The possibility that plasma ACTH and corticosterone responses to stress may be modified by prior exposure to stress was examined in male Sprague-Dawley rats using a 2 min. immobilization stress. The discrete 2 min. immobilization stress is a low to medium intensity stimulus to the adrenocortical system which produces a submaximal plasma corticosterone response that peaks at 15 min. (35.8±3.6 ug/dl; mean ± S.E.M.) and returns to the control range by 90 min. (12.5±3.4 ug/dl). The peak ACTH response to a single stress occurs at 5 min. (117.0±11.0 pg/ml) and returns to basal levels by 30 min. (62.0±13.5 pg/ml). A second stress was then applied at varying intervals (30, 60 and 90 min.) after the initial stress in an attempt to study the effect of stress-induced ACTH and corticosterone secretion on subsequent stress responses. Peak ACTH and corticosterone responses always occurred at 5 and 15 min. respectively following the second stress. The level of the peak responses were similar in magnitude to the corresponding levels following a single stress, but the duration of elevation was less in the 30 and 60 min. stress groups. The plasma corticosterone time course of the second stress applied at 90 min. was almost identical to that following a single stress. The time courses of plasma ACTH and corticosterone following four repeated stresses at 90 min. intervals (stress at 0, 90, 180 and 270 min.) reflect those to a single stress. Incremental (base to peak differences) plasma ACTH and corticosterone responses were similar in rats that received 1 to 7 repeated stresses at 90 min. intervals. These data suggest that prior exposure to discrete stresses does not modify ACTH and corticosterone responses to subsequent stresses, provided that the stresses are separated by an interval (90 min.) allowing plasma corticosterone to return to control levels. A second stress applied during the recovery period (refractory period) results in inhibition of the total corticosterone response (integrated area). Correlation was demonstrated between plasma ACTH and corticosterone responses to stress in refractory versus responsive periods with changes in concentrations of serotonin and 5-hydroxyindoleacetic acid in discrete brain regions. These data suggested that during the refractory period hypothalamic and cerebral cortical serotonin turnover and release were increased, and suggested further that brain serotonin may play an inhibitory role in modulating the ACTH and corticosterone responses to a subsequent stress applied during the refractory period.

(Supported by MRC MA-5183)

1092 FEMALE FROG REPRODUCTIVE BEHAVIOR ELICITED IN THE ABSENCE OF THE OVARIES. Carol Diakow, Josiah N. Wilcox*and Richard Woltmann*. Biology Department, Adelphi University, Garden City, N.Y. 11530.

A release call is emitted by unreceptive female frogs when they are clasped around the trunk; this call is inhibited in receptive females. A recent study indicates that water accumulation induced by arginine-8 vasotocin causes a mechanical distension that inhibits the call (Diakow, Sci. 199: 1456, 1978). This finding, that a posterior pituitary hormone is important for mating behavior, leads to the question of the role of ovarian steroids in the reproductive behavior of the female frog, and the experiments reported here address this question.

In one experiment, ovariectomized female Rana pipiens given subcutaneous estradiol and/or progesterone continued to call at a rate equivalent to that of unreceptive females. These results fail to provide evidence that ovarian steroids inhibit the release call.

In another experiment, the release call was inhibited in the absence of the ovaries by distension of ovariectomized Rana pipiens with intraperitoneal fluid. These results are consistent with the interpretation that the release call is inhibited by a mechanism that involves water accumulation, but not ovarian participation.

1093 CONTROL OF RELEASE OF ACTH AND VASOPRESSIN BY SUPRAOPTIC AND PARAVENTRICULAR NUCLEI. Anne Dornhorst*, Drew E. Carlson*, Said M. Seif*, Alan G. Robinson*, Earl A. Zimmerman and Donald S. Gann. The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205, Univ. Pittsburgh Sch. Med., Pittsburgh, PA 15261 and the College of Physicians and Surgeons, Columbia Univ., New York, NY 10032.

To determine the relative roles of the supraoptic (SON) and of the hypothalamic paraventricular (PVN) nuclei in the control of release of vasopressin and of ACTH, we have examined the hormonal responses to electrical stimulation of these regions. Cats (38) were anesthetized with chloralose-urethane, artificially respired, and immoblized with gallamine. Electrical stimulations (200μA, 0.2msec, 100 HZ, 20sec) were delivered with bipolar coaxial electrodes, positioned stereotaxically. Blood samples were taken 30 seconds prior to stimulation and 1.5 minutes poststimulation. Hypovolemia was prevented by simultaneous infusion of equal volumes of warmed isoncotic dextran. ACTH and vasopressin were measured in plasma by radioimmunoassay. Stimulation sites were determined histologically. Eleven brains were immunostained for vasopressin using the triple-layer peroxidase-antiperoxidase method of Sternberger. The relative vasopressin content of neurons was measured as the mean optical density of stained cells by means of a microdensitometer. Stimulation of the lateral portion of the supraoptic nucleus (SON) increased vasopressin (2.1±0.5μU/ml, N=14, $p<0.01$) and decreased ACTH (-26±4 pg/ml, N=13, $p<0.01$). In contrast, stimulation of the hypothalamic paraventricular nucleus (PVN) increased vasopressin (2.2±0.7μU/ml, N=7, $p<0.02$) and increased ACTH (107±20 pg/ml, N=32, $p<0.01$). Stimulation of either nucleus decreased the vasopressin content of neurons adjacent to the electrode (-30.5±3.5%, N=8, $p<0.001$). In addition, the immunostaining of the descending projections from these nuclei became more prominent. Stimulations in regions which led to no hormonal changes yielded no cytochemical differences. Previous work has shown that vasopressin neurons of PVN, but not of SON, project to the zona externa of the median eminence. Others have suggested that retrograde flow of blood from the neural lobe to the median eminence and thence to the anterior lobe would allow vasopressin to influence the release of ACTH. The present results indicate that both SON and PVN control the release of vasopressin. However, PVN facilitates, but SON inhibits, release of ACTH. These findings suggest that a projection from PVN to the median eminence mediates release of ACTH, and that retrograde flow from the neural lobe is not important in the short-term control of ACTH.

(Supported in part by NIH grant AM 14952)

1094 EFFECT OF OVARIAN HORMONES ON NEURONAL MEMBRANE SENSITIVITY TO HYPOTHALAMIC-RELEASING HORMONES. Carol A. Dudley* and Robert L. Moss. Dept. of Physiology, Univ. of Texas Health Science Center at Dallas, Dallas, Texas 75235

The ability of ovarian hormones to affect neuronal membrane responsiveness to iontophoretically applied luteinizing hormone-releasing hormone (LRH) and LRH analogs was evaluated in ovariectomized (OVX) non-primed and OVX, estrone (E)-progesterone (P) primed female rats. Extracellular action potentials were recorded from 274 medial preoptic (MPO) neurons. The spontaneous activity of these neurons was monitored to detect changes resulting from microelectrophoretic deposition of 1mM solutions of LRH, LRH agonist analog (D-Trp^6, Pro^9 NHEt[LRH+]), and/or LRH inactive analog (des-Pro^9-Gly^{10}[LRH^o]). One hundred and seventy MPO units (n=170) were recorded in OVX rats while the remaining units (n=104) were found in OVX, E-P primed females. A summary of the results is presented in the table below.

Agents Microelectrophoresed	N	Response In Untreated Ovariectomized Female Rats			N	Response In Estrogen-Progesterone Treated Ovariectomized Female Rats		
		↑	↓	→		↑	↓	→
LRH	170	52 (31%)	22 (13%)	96 (56%)	104	51 (49%)	3 (3%)	50 (48%
LRH$^+$	135	49 (36%)	15 (11%)	71 (53%)	103	57 (55%)	2 (2%)	44 (43%
LRH^o	98	30 (31%)	14 (15%)	52 (54%)	87	43 (50%)	3 (3%)	41 (47%

In the OVX rat, the majority of cells recorded were found to be unresponsive to the microelectrophoresis of the releasing hormones. Of the responsive neurons, more displayed excitation than inhibition. In contrast to the relative inability of these hormones to effectuate changes in spontaneous activity in non-primed females, the predominant response to LRH and its analogs in E-P primed females was excitation. A 2 x 3 x 3 chi square analysis conducted on responsiveness to LRH and its analogs in primed and non-primed rats yielded a significant association between hormonal status and response. Primed animals were more likely to respond to a given LRH drug with excitation, regardless of the LRH drug tested. Although the excitatory effects produced by LRH^o are perplexing, preliminary experiments with an LRH antagonist analog in OVX rats have resulted in more inhibition than excitation. The observation that neuronal responsiveness to LRH is dependent on the hormonal status of the animal is consistent with LRH's postulated role in mediating gonadotropin secretion and mating behavior.
Supported by research grant NIH-USPHS-10434 END.

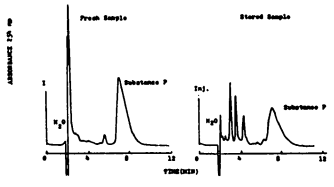
1095 POSTNATAL DEVELOPMENT OF THE LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) SYSTEM IN NORMAL AND NEONATALLY ESTROGENIZED RATS. Karen E. Elkind*, Joan C. King, and Arnold A. Gerall. Dept. Psychol., Tulane University, New Orleans, LA., 70118.

The postnatal development of the luteinizing hormone-releasing hormone (LHRH) system was studied in normal and neonatally estrogenized rats using the peroxidase-antiperoxidase immunocytochemical technique of Sternberger et al (J. Histochem. Cytochem. 18: 315, 1970). Thirty-three male and 33 female albino rat pups were randomly assigned to one of 4 experimental treatments on day 2 (day 1= day of birth): the high dosage group was injected with 1000µg of estradiol benzoate (EB) dissolved in 0.05 cc sesame oil, the low dosage group with 1 µg EB in 0.05 cc sesame oil, the control group with an equivalent amount of the oil vehicle and a handled control group which received no injection. Two to 3 animals from each group were perfused on days 6, 9, and 11 after birth and the brain prepared for immunohistochemical study. The distribution of LHRH processes in the neonatal animal resemble that described for the adult; a rostral pathway is located close to the organum vasculosa of the lamina terminalis (OVLT) in the vicinity of the rostral preoptic area (POA) and another caudal one extended throughout the arcuate-median eminence (ARC-ME) region. LHRH immunopositive cell bodies were found in the rostral POA but not in the ARC-ME region. The pattern of development of LHRH positive perikarya is to be described for the first time in the neonatal rat.

The present study provides immunocytochemical evidence for salient maturational differences between males and females in the distribution of LHRH perikarya. Both the intensity of positive stained processes and the number of LHRH cell bodies localized are greater in the normal male than in the female neonatal rat at all ages. The high dosage of neonatal estrogen reduced the number of immunoreactive perikarya visualized and the intensity of stained processes in the male while estrogen enhanced the staining of LHRH elements in the female. The results also indicate that early estrogen treatment alters the LHRH system in a dose dependent manner. The data are consistent with the hypothesis that the hypothalmic-pituitary-gonadal inhibitory feedback system is functional earlier in the male than in the female rat. It is concluded that the administration of exogenous estrogen neonatally modifies the organization of the hypothalamic LHRH system as well as the mediation of reproductive function in adulthood.

1096 HYPOTHALAMIC PEPTIDES - PURITY, STABILITY AND MEASUREMENT BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC). Joseph A. Feldman*, Major L. Cohn and Dorothea Blair* (SPON: Richard W. Keller, Jr.) Duquesne Univ. and Dept. Anes. Magee-Womens Hosp., Univ. Pgh. Sch. Med., Pittsburgh, PA 15123.

Although radioimmunoassay has permitted detection of the neuropeptides in brain structures, the technique is associated with serious shortcomings. Until recently, available methodology was not sensitive enough to efficiently determine purity and stability of synthetic neuropeptides. We are now reporting our new HPLC method which separates, identifies, quantifies and purifies the tripeptide thyrotropin releasing hormone (TRH), the pentapeptides met and leu enkephalin, the decapeptide luteinizing hormone releasing hormone (LHRH), the eleven amino acid peptide substance P and the tetradecapeptide somatostatin. Our equipment consists of a Waters Associate HPLC System with a M-6000 A pump, a U6K septumless injector, a reversed phase analytic column and a model 450 variable wavelength U.V. detector. The reversed phase solvent system varied according to specific peptide tested. The eluting system was run isocratically at room temperature at a flow rate of 1.5 ml/min. With detector set at 254 nm and the eluting solvent system 50% MeOH/50% HAc (8%) pH 2.9, K' values for LHRH, substance P and somatostatin were recorded respectively at 2.10, 6.08 and 10.19. Under identical conditions, met and leu enkephalin were separated; K' values for the peptides were recorded at 1.17 and 1.67 respectively. For TRH, which does not absorb at 254 nm, with the detector set at 215 nm and an eluting system of 50% MeOH/50% HAc (0.1%), a K' value of 1.23 was obtained. Our HPLC technique demonstrates that 1) several peptides lack chromatographic homogeneity; 2) dissolved in water several peptides are not stable; new absorbance peaks appear while the peak representing the parent compound disappears. For example LHRH in aqueous solution is stable if kept for 3 months at 8°C while TRH, substance P (Fig) and somatostatin show significant structural alterations in a short period of time.



1097 CORRELATION OF HYPOPHYSIAL PORTAL PLASMA DOPAMINE AND PERIPHERAL PLASMA PROLACTIN: EFFECTS OF INTRAVENTRICULAR HISTAMINE AND ACETYLCHOLINE. D.M. Gibbs*, P.M. Plotsky, and J.D. Neill*, (SPON: J. W. Manning). Dept. Physiol., Sch. Med., Emory U., Atlanta GA 30322

Dopamine secreted by the hypothalamus into the hypophysial stalk vessels has a physiologic role, at least tonically, in the inhibition of pituitary prolactin secretion (ENDOCRINOLOGY 102:1895 1978). The role of dopamine in the dynamic regulation of prolactin is still unknown. Histamine is one of many putative hypothalamic transmitters which stimulate prolactin secretion when injected into the cerebral ventricles. Histamine antagonists interfere with a number of physiologic and pharmacologic stimuli of prolactin secretion suggesting that the functional site of histamine action is relatively close to the final hypothalamic output to the pituitary. We studied the effect of 10µg histamine injected into the lateral ventricle of urethane anesthetized, diestrous rats. Prolactin levels in 5 control rats injected with acidified saline increased only to 111% ± 5 (mean ± SE) of baseline values while prolactin levels in 6 rats injected with histamine increased to 692% ± 134 of baseline ($P < 0.005$). Stalk plasma dopamine levels in 7 control rats injected with acidified saline increased to 117% ± 16 of baseline values while dopamine levels in 8 rats injected with histamine decreased to 74% ± 6 ($P < 0.025$). While it appears that the effect of histamine to increase prolactin secretion may be mediated by a decrease in hypothalamic dopamine secretion, it remains to be determined if the observed 35% decrease in stalk plasma dopamine concentration can account for a 7 fold increase in prolactin release.

ACh is another putative hypothalamic transmitter that may be involved in the regulation of prolactin secretion. In most previous studies, ACh caused a decrease in prolactin secretion in unanesthetized rats. We were surprised to find that in 5 urethane anesthetized diestrous rats, 50µg ACh injected into the lateral ventricle caused prolactin to increase to 2580% ± 410 of baseline values. This stimulation of prolactin by ACh was seen over a wide range of ACh doses under urethane and pentobarbital anesthesia, and in urethane anesthetized, ovariectomized estrogen-progesterone treated rats. However, stalk plasma dopamine levels in 8 diestrous rats injected with ACh were 93% ± 17 of baseline. This response was not significantly different from the response in rats injected with saline.

These data suggest that the effects of histamine on prolactin secretion may be mediated by dopamine, but that the effects of ACh probably involve some other hypothalamic factor(s).
(Supported by USPHS NIH Grant HD 04312).

1098 IMMUNOCYTOCHEMICAL STAINING OF DOPAMINE RECEPTORS ON MAMMOTROPHS. Paul C. Goldsmith*, Michael J. Cronin*, Robert J. Rubin* and Richard I. Weiner. Dept. of Ob., Gyn. and Reprod. Sci., U.C. Sch. Med., San Francisco, CA. 94143, and Dept. Psych., U.C.L.A. Sch. Med., Harbor Gen. Hosp., Torrance, CA. 90509.

Dopamine in physiological concentrations inhibits prolactin secretion. A high affinity, saturable dopamine receptor has been characterized in crude homogenates of the anterior pituitary with various radioligands including haloperidol (Hal). In the present study we have demonstrated immunocytochemically that the majority of these receptors are located on mammotrophs.

Anterior pituitaries from mature female rats were dispersed using the technique of Hopkins and Farquhar. Intact cells were separated from debris by centrifugation through 4% BSA. The cells were incubated overnight at 37°C in Medium 199 containing Earle's salts, glucose, HEPES buffer, BSA and antibiotics (CM199) with 14% fetal calf serum added. Ninety percent of these cells appeared viable by the exclusion of trypan blue. The presence of functional dopamine receptors was verified by the ability of 10^{-5}M dopamine to inhibit 50% of the prolactin released.

Aliquots of unfixed or lightly fixed cells were washed in CM199 and incubated in $2x10^{-7}$M Hal in CM199. Following intervening washes and centrifugation in CM199, cells were suspended in 1:20 to 1:100 rabbit antiserum to Hal-BSA conjugate (R-a-Hal), 1:100 goat antirabbit IgG, and finally 1:100 rabbit PAP. After reaction with substrate, cells were fixed with dilute Karnovsky mixture and post-fixed in osmium. Pellets were embedded in agar, dehydrated, stained with uranyl acetate, embedded in Araldite, and thin sectioned for electron microscopy. Two or more PAP complexes at membrane sites were interpreted as positive binding. Cell types were identified on a basis of established ultrastructural criteria for the rat anterior pituitary.

Patches of characteristic PAP complexes appeared on the plasmalemma of the vast majority of mammotrophs. PAP complexes were preferentially observed on microvilli and in plasmalemmal invaginations. Immunostaining was also observed on the inner face of apparent endocytotic vesicular membrane. Staining was infrequently observed on somatotrophs, to a lesser degree on gonadotrophs, and was not seen on other pituitary cell types. Controls included elimination of Hal, co-incubation of cells with Hal and 100-fold excess of d-Butaclamol, substitution of CM199 for one of the three antisera, or use of R-a-BSA in place of R-a-Hal or R-a-Hal absorbed with excess Hal. These controls had reduced or absent staining on mammotrophs and other cell types. Dopamine receptors on mammotrophs support the function of dopamine as prolactin inhibitory hormone. Supported by USPHS Grants HD 10907, HD 08924, and the Rockefeller Foundation.

1099 EFFECTS OF ESTROGEN ON BRAIN AND PITUITARY POLYAMINES IN THE OVARIECTOMIZED RAT. H.E. Gray, T.W. Jasper*, W.G. Luttge, J.B. Shukla* and O.M. Rennert*. Dept. Neuroscience, Univ. Fla. Coll. Med., Gainesville, FL 32610 and Dept. Pediatrics, Univ. Oklahoma Health Sci. Center, Oklahoma City, OK 73190.

The natural polyamines, especially putrescine (PUT), spermidine (SPD) and spermine (SPM) have been implicated in a variety of essential cell functions in several tissues including the mammalian central nervous system. For example, recent studies using peripheral steroid target tissues have suggested that polyamines are involved in the mediation of steroid-induced alterations in RNA and protein synthesis. Since many estrogenic effects within the central nervous system also appear to require ongoing RNA and protein synthesis, we have thus initiated an investigation of the effects of estradiol on the levels of endogenous polyamines in several brain regions of adult female rats.

Two weeks following ovariectomy rats were injected with 20 µg estradiol benzoate (EB) or the oil vehicle and killed by perfusion 12, 24, or 36 hrs later after the removal of a blood sample. PUT, SPD and SPM are now being determined by high-pressure liquid chromatography (Durrum D-500) in extracts of plasma, pituitary, cerebral cortex, whole hypothalamus and amygdala. Preliminary results from the group receiving EB or oil 24 hrs prior to killing reveal significant estrogen-induced alterations in polyamine metabolism in both brain and pituitary. In the pituitary, concentrations of all three polyamines were elevated by EB priming. However, in the hypothalamus only SPD and SPM were elevated, while PUT showed a significant decrease in endogenous concentration. Amygdala and cortex showed small, marginally significant changes in SPM and SPD, respectively. Polyamine concentrations in plasma were unaltered by EB treatment.

Thus, although polyamine metabolism in the hypothalamus and pituitary are both responsive to EB priming, the patterns of the responses, at least in the 24 hr group, may be fundamentally different and may reflect the different functional roles of estrogen in these very dissimilar tissues. When completed, the analysis of samples from the rats receiving 12 and 36 hrs of EB priming will provide a time course for the hormone-induced changes.

1100 CELLULAR ANALYSIS OF A SEXUALLY DIMORPHIC REGION OF THE PREOPTIC RAT BRAIN. R.E. Harlan*, C.D. Jacobson*, J.E. Shryne* and R.A. Gorski. Dept. Anat., Sch. Med., UCLA, Los Angeles, CA 90024.

In the preoptic area (POA) of the rat brain, a distinct area of increased nissl staining is found, the volume of which is sexually dimorphic (larger in males). To examine the cellular characteristics of this area and to determine possible bases of this sex difference, cresyl-echt violet stained 10µ sections were projected through a closed circuit television camera and monitor so that three investigators could view and characterize the same cells simultaneously. A fourth person measured the longest diameter of each cell. Nearly 5000 cells were evaluated. An area encompassing the sexually dimorphic region and regions adjacent to it was systematically analyzed. One coronal section was viewed in each of 4 male and 4 female gonadectomized rats, without the investigators' knowledge of the sex of the subjects. Each cell was rated by each investigator according to the following parameters: intensity of staining (light, medium or dark), shape (round, medium or angular), the presence of processes extending from the soma, and the presence of a nucleolus. The rating score for each cell was defined as that score agreed upon (in subsequent data analysis) by at least two investigators. After completion of the cell characterization, outlines of the sexually dimorphic area were drawn for each animal. Comparison between the drawings and the area viewed through the TV monitor allowed the assignment of each cell to a position outside or inside the sexually dimorphic area. Since the mean density ($\bar{x}$ number of cells per 37.5µ grid) of cells inside the drawn borders was significantly greater than the density outside, in both sexes, the sexually dimorphic region can be described as a nucleus, here called the Sexually Dimorphic Nucleus of the POA (SDN-POA). The density of cells either outside or inside the nucleus was not sexually dimorphic. The area of the SDN-POA was about 60% larger in males than in females. Inside the nucleus, the mean cell size of males (10.1µ) was significantly ($p < 0.01$) larger than that of females (9.2µ; Mann-Whitney U test). After dividing the cells into three size groups, we found that, of those cells inside the SDN-POA, there was a significantly greater proportion of relatively large cells (>12.5µ) in males than in females; 22.6% were large in males compared to 13.6% in females. Although no significant sex differences were found in any other cellular characteristic, in males a larger proportion of cells were angular inside than outside the nucleus. In summary, 1) the sexually dimorphic region of the POA is indeed a nucleus, 2) within this nucleus the mean cell size is larger in males than in females, apparently due to an increase in the proportion of large cells, and 3) in males, the frequency of angular cells is greater inside than outside the nucleus. (Supported by HD 01182.)

1101 INTRACELLULAR RESPONSES FROM THE PARAVENTRICULAR NUCLEUS IN SLICES OF RAT HYPOTHALAMUS. G.I. Hatton, B.A. MacVicar and F.E. Dudek. Depts. of Psych. and Zool., Mich. State Univ., E. Lansing, MI 48824, and Dept. of Zool. and Erindale Coll., Univ. of Toronto, Mississauga, Ont.

Little is known about the physiology of intrahypothalamic cellular interactions, in part because of the difficulty of obtaining intracellular recordings from hypothalamus. Recent work (Hatton et al., Neurosci. Abstr. 3:345, 1977) demonstrating the viability of hypothalamic slices and the physiological nature of the spontaneous unit activity in such preparations suggested the feasibility of studies of cellular physiology and synaptic relationships (or lack thereof) within relatively circumscribed hypothalamic regions. The paraventricular nucleus (PVN), due to recent anatomical findings of far-reaching and varied connections, is among the more interesting of hypothalamic nuclei, especially since no intracellular data have been reported for this nucleus and only two reports have been made of intracellular recording in mammalian magnocellular neurosecretory neurons of which PVN, in large part, consists.

Intracellular recordings were obtained from the PVN in 400 µm thick slices of rat hypothalamus. Activity patterns observed included cells that were either silent, slow-irregular or bursting. The action potentials of some of these cells were characterized by long durations (2.5 msec at ½ amplitude or 4-5 msec at threshold) and followed by afterhyperpolarization. Other cells in the PVN displayed short-duration action potentials (1-1.5 msec at ½ amplitude). Small depolarizing potentials with a fast rising phase, slow decay, and graded in amplitude up to 11 mV also occurred spontaneously. Since weak hyperpolarizing current injection could increase the amplitudes of these depolarizations, they probably arise from chemical synaptic inputs within the slice. Extracellular stimulation lateral to the PVN appeared to evoke excitatory postsynaptic potentials, which also increased in amplitude during small hyperpolarizing currents. These results suggest the presence of local circuits at the level of PVN. The long- and short-duration action potentials probably represent magnocellular neurosecretory and parvocellular non-neurosecretory cells, respectively. The electrophysiological characteristics of these intracellular responses in vitro are consistent with earlier in vivo and in vitro studies.

Supported by NIH grant NS09140 to G.I.H. and NRC grant to F.E.D.

1102 MAGNITUDE OF THE RESPONSE OF THE PITUITARY-ADRENAL SYSTEM IS A FUNCTION OF THE DEGREE OF STIMULUS UNFAMILIARITY. J. P. Heybach*†, M. B. Hennessy*‡, J. Vernikos-Danellis*† and S. Levine‡ (SPON: W. R. Mehler). †NASA/Ames Res. Ctr., Moffett Field, CA 94035; ‡Dept. of Psychiatry & Behav. Sci., Stanford Univ., School of Med., Stanford, CA 94305.

Measurement of fluctuations in adrenal corticoid levels has been widely used as an index of emotional state. Yet it has been recently argued that the adrenal response is insensitive (i.e., cannot distinguish various levels of emotional state) and therefore is an ineffective measure of emotionality. This conclusion was based on studies in which rats exposed to a range of intensities of stimulation never showed more than two levels of corticoid elevation. However we have recently shown that the corticoid response of mice can accurately reflect various intensities of psychological stimulation, i.e., degrees of unfamiliarity. The present study extends these findings to the rat and examines concomitant changes in ACTH.

Rats were exposed for 15 min to three apparatus which differed in their degree of unfamiliarity. A linear increase in plasma corticosterone concentrations over the resting level was found as a function of the degree of unfamiliarity. A control group which received the same handling as did the experimental animals showed corticosterone concentrations which were significantly greater than the basal control group, and significantly less than any of the experimental groups. Plasma ACTH concentrations displayed a pattern similar to that found for plasma corticosterone. Although ACTH concentrations obtained here are probably not maximal, the values are interpreted as reflective of group differences at the time of peak circulating levels. Thus overall, four distinct levels of pituitary-adrenal activity were observed in the present study. These data confirm the capacity of the pituitary-adrenal system to differentiate various levels of stimulus intensity under appropriate conditions.

1103 DIURNAL CHANGES OF MELATONIN IN RETINA, PINEAL GLAND, SUPRACHIASMATIC NUCLEUS, COLON, AND DUODENUM OF THE RAT. W. R. Holloway*, L. J. Grota, and G. M. Brown. Dept. Psych., Sch. Med., U. Roch., Roch., N. Y. 14642, and Dept. Neurosci., McMaster U., Hamilton, Ontario, Canada.

Melatonin(M) has been localized immunohistologically in a number of extrapineal areas (Br. Res. 118:417, 1976; Exp. 33: 662, 1977). Although known to exhibit a diurnal fluctuation in the pineal, daily rhythms of M in other organs have not been established. Because of its potential role in physiological functioning, relative M levels were determined using quantitative immunohistology in several tissues of the rat during the 24 hour day. Adult male Charles River rats, kept on a 12:12 light:dark cycle, were decapitated at 3 hr intervals beginning 1.5 hr after light onset. Unless noted, n = 4 per time period. Trunk blood was collected and the pineal gland(P), eyes(R), suprachiasmatic area of the hypothalamus(SCN), duodenum(D), and descending colon (C) were removed and frozen on dry ice. Tissues were sectioned in a cryostat at 10um. The presence of M was determined with a modified Coons double antibody technique, using a highly specific anti-M antibody (sheep) and FITC labeled anti-sheep IgG (Miles). Fluorescence intensity was measured with a CdS photocell having its maximum spectral sensitivity (515nm) close to the emmission peak of FITC (520nm). The photometer reading differences between sections stained with anti-M serum and adjacent sections stained with normal sheep serum were used in all statistical analyses.

Plasma corticosterone exhibited a normal rhythm with peak and trough 1.5 hr after lights-off(L-off) and 4.5 hr after lights-on (L-on), respectively. M content of the P had a single broad peak, beginning late in the light period and falling sharply to background 7.5 hr after L-off. The SCN showed a similar pattern, although only 2 animals were observed at each point. Three tissues exhibited double peaks, or troughs. The C, with intense fluorescence in the epithelial layer, had a major peak 7.5 hr after L-on, with a smaller increase 4.5 hr after L-off. The R, with fluorescence in both the inner and outer nuclear layers, mirrored the C pattern. There was a large drop in intensity 7.5 hr after L-on, with a smaller decrease 4.5 hr after L-off. The D, with specific fluorescence in the lamina propria of the villi, had 2 minima; the first 7.5 hr after L-on, the second 4.5 hr after L-off.

These results indicate the usefulness of this method for measuring M. Possible influences of M from these areas on rhythmic processes and functions in the specific tissues studied will be discussed.

Supported in part by NIMH grant MH-14650.

1104 PERSISTANCE OF A CIRCADIAN RHYTHM OF N-ACETYLTRANSFERASE IN THE CHICK PINEAL IN VITRO. C.A.Kasal*, M.Menaker, and J.R. Perez-Polo. Dept. Zool., Univ. Texas, Austin, TX 78712 and Dept. of Human Biol. Chem. and Gen.,Univ. of TEX Med Branch,Galveston, Tx 77550.

A circadian rhythm in melatonin production has been demonstrated in the pineal gland of birds and mammals. Examination of levels of N-acetyltransferase (NAT), the enzyme involved in melatonin production, also reveals a circadian rhythm. The location of the oscillator (clock) driving these rhythms appears to differ between birds and mammals. In rats, the oscillator is located somewhere outside the pineal gland and controls the levels of NAT via adrenergic innervation. In contrast, experiments in sparrows have produced evidence supporting the hypothesis that the oscillator controlling melatonin production resides within the pineal gland.

The present study was carried out to determine whether the rhythm of NAT activity will persist under constant conditions in vitro. White Leghorn chicks were raised from one day of age under the same lighting regime (12 hours light-12 hours dark). At three weeks of age animals were sacrificed, pineals removed and placed into culture. Examination of days one and two in culture entailed placing cultured glands directly into constant darkness(DD) and sampling glands every four hours. A significant rise and fall in NAT activity occured during the projected night of day one, remained low during the projected day period of day two and rose again on the night of day two. Experiments on days three and four entailed placing glands into culture for two days under the continued lighting regime of the intact animal. Media was changed on day three and then the glands were placed into DD at which time sampling began at four hour intervals. Again levels of NAT increased during the projected night period of day three, fell and remained low during the projected day period of day four, rose again in the projected night period of day four and fell again during the day period of day five.

These findings of a perisitant rhythm of NAT in vitro support the propostion that the avian pineal gland is a source of endogenous oscillation in the bird.

Supported by NINDS grant NS14034 and Research Career Award K04 NS 00213 to J.R.P.

1105 QUANTITATIVE AUTORADIOGRAPHIC ANALYSIS OF ESTROGEN CONCENTRATING CELLS IN HYPOTHALAMUS, PREOPTIC AREA AND AMYGDALA. M.S. Krieger*, J.I. Morrell, and D.W. Pfaff. (SPON: R. Norgren). Rockefeller University, New York, NY 10021.

The anatomical locations of estrogen-concentrating cells have been described for the major classes of vertebrates (for review, see Morrell and Pfaff, Am. Zool., 1978). In this study quantitative analyses of the peak areas of hormone concentration were done to determine whether there are different modes of hormone concentration and to investigate possible area-to-area differences in hormone concentration.

The basic methods used in this study were those previously used in this laboratory (Pfaff and Keiner, 1973) with modifications to facilitate the interpretation of apparently unlabelled cells. The dose of ^{3}H-estradiol administered was increased to the nuclear saturating level of 2.7 μg/kg and was administered by an intravenous route. This hormone infusion maintained blood levels at or above proestrus levels for 3 1/2 to 4 hours. Tissue was sectioned at 2 μ.

A cell-by-cell analysis of 32 sections through the preoptic area (POA), the ventromedial nucleus (VMN), arcuate (ARC), or medial nucleus of the amygdala (AMYG) was performed. Each cell was designated as either labelled or unlabelled. The percentages of labelled cells were calculated, and a representative section was chosen of each area for each animal. In these sections the number of silver grains per cell was counted. The total number of cells in which grains were counted was 314 in POA, 453 in VMN, 485 in ARC and 654 in AMYG.

Frequency distribution histograms of the number of silver grains per cell were analyzed. The shape of the frequency distribution curve was similar for all four areas. Even under these conditions of high, maintained blood levels, there were still unlabelled cells visible. The large number of unlabelled cells and the wide range in the number of silver grains per cell resulted in curves which differed significantly from the Poisson distribution. In addition, these frequency distribution curves were smooth; modes in the curve, as might reflect quantal uptake processes, were not seen.

Supported by NIH grants HD-10655 and HD-05343.

1106 MATURATION OF CNS CONTROL OF GROWTH HORMONE SECRETION IN RATS. Cynthia M. Kuhn* and Saul M. Schanberg. Department of Pharmacology, Duke University Medical Center, Durham, N. C. 27710.

The concentration of growth hormone (GH) in serum of neonatal rats was determined after administration of various drugs that are known to alter serum GH in adult rats. Administration of the serotonin precursor 5-hydroxytryptophan, morphine or TRH increased while treatment with the serotonergic antagonist cyproheptadine decreased serum GH in pups 15, 20 or 35 days old but had no effect on serum GH in younger animals. Clonidine decreased serum GH in pups 10, 14 or 20 days old and increased GH in older animals. Urethane produced its characteristic decrease in serum GH in 20 day old pups but had no consistent effect on GH in younger animals. These results suggest that the serotonergic system involved in regulation of GH secretion in rats and the midbrain and/or pituitary mechanism through which morphine affects GH secretion are functional within the first postnatal week. In contrast, the extrahypothalamic mechanisms through which pentobarbital and urethane exert their effects do not mature until 2-3 weeks after birth. Finally, the inability of clonidine to stimulate GH secretion before day 35 suggests that the noradrenergic mechanism which generates the characteristic "surges" of GH secretion observed in adult rats does not mature until after puberty. (Supported by NIMH grants MH-13688 and MH-06489 and NIH Fellowship NS0-5152.)

1107 STEROID HORMONES AND DOPAMINERGIC ACTIVITY IN THE STRIATUM. P. Langelier, J. Dankova, R. Boucher and P. Bédard, SPONS. by L.J. Poirier, Lab. Neurobiologie, Univ. Laval, Québec, G1K 7P4,Canada.

Estrogens inhibit dopaminergic transmission at the level of the hypothalamus and hypophysis resulting in an increased secretion of prolactin (Raymond, V., Labrie, F. and others, Science, in press). We have recently reported a beneficial effect of estrogens in patients suffering from L-DOPA-induced-dyskinesias and neuroleptic-induced tardive dyskinesias, two conditions which appear to result from overstimulation of certain dopaminergic receptors in the striatum (Bédard et al., 1977, Lancet 2 (8052): 1367-1368). In a group of 25 female rats a radiofrequency lesion of the left entopeduncular nucleus was performed stereotaxically and the animals were tested several times for circling after apomorphine 0.5 µg s.c. General motility was also tested with a motility box after saline and apomorphine 0.5 µg s.c.

The animals were then divided into four groups, kept in constant lighting conditions and treated twice a day with one of the following: 1) 17-β-estradiol 2 µg s.c.; 2) R-5020 (a progestative coumpound) 200 µg s.c.; 3) a combination of 1) and 2); 4) vehicle.

During treatment, the animals were tested three times for circling and motility. The animals were then castrated and after a period of recovery the same procedure was applied. Our results show a clear effect of the hormone treatment on both apomorphine induced circling and motility, which are decreased by 40% with respect to the control group. The three hormone treated groups behaved in the same fashion. Our results suggest that both estrogens and progesterone exert an inhibitory action on dopaminergic receptors in the striopallidal system. This hormonal influence on the motor system contributes to our understanding of the mechanisms which modulate the sensitivity of dopaminergic receptors in the striatum. This may have very interesting clinical applications.

1108 MELATONIN INTERACTIONS WITH THE CENTRAL NERVOUS SYSTEM. Robert M. Levy*, Huda Akil, Stanley J. Watson, Pamela Angwin*, Laughton Miles*, and Jack D. Barchas.

Melatonin, N-acetyl-5-methoxytryptamine, a product of the pineal gland, has been shown to have a number of significant endocrine and behavioral effects. Our laboratory has recently developed a highly sensitive radioimmunoassay (RIA) which when coupled with various other indicators of melatonin action provides new information on the interaction of this indoleamine with the central nervous system.

One RIA, developed by our laboratory utilizes tritium-labeled melatonin and has a sensitivity of 25-50 pg per assay tube. Antibodies have been raised to both BSA- and thyroglobulin-coupled melatonin, although the thyroglobulin-coupled antibody is slightly more potent. A second RIA, using an ^{125}I-labeled trace and an antibody from Rollag and Niswender (Endocrinology 98:482-489, 1976), has a sensitivity of 2.5 pg per assay tube, an effective range into nanogram levels, and has also been used in these studies.

Bovine and rat tissue studies investigating both the effects of various pharmacological treatments on melatonin levels and activity, and the effects of melatonin, by pinealectomy and administration via various routes of infusion, on the neuroendocrine axis are currently underway. Human clinical studies involving medicated psychiatric and medical patients are also being performed. These studies include patients receiving monoamine-altering agents, as well as one group receiving mega-propranolol therapy (increasing doses up to 3 g/day). Further studies of melatonin interaction with the CNS are in progress.

1109 EFFECTS OF SEPTAL LESIONS ON LORDOSIS BEHAVIOR AND MALE SEX BEHAVIOR IN FEMALE AND MALE RATS. M.Y. McGinnis*& R.A. Gorski (SPONS A.M. Adinolfi). Dept. Anat., Sch. Med. UCLA, Los Angeles, CA. 90024.

Septal lesions have been shown to facilitate lordosis behavior in ovariectomized rats when primed with estradiol benzoate(EB) only. The first study was designed to test whether this increase in hormone sensitivity would also manifest itself in a facilitation of male sex behavior. Ovariectomized rats were given septal lesions (SL) or sham surgery(no current passed) and 3 weeks later tested for lordosis behavior following 2ug EB/day for 3 days and tested on day 4. Three weeks later they were given a pretest for male sex behavior and tested on days 4,7,11 & 15 of 150ug daily testosterone propionate(TP) treatment. Mount latency and mount and intromission frequencies were measured. Mean lordosis quotients(LQ) were higher in SL(74±7) than in sham operated(12±4) rats($p<.05$). However there was no difference between SL and sham groups on any test for male sex behavior. Thus the septal lesion effect in females appears to be specific for female sex behavior.

Unless SL male rats are chronically exposed to EB for 2-4 weeks during the immediate post-lesion period, septal lesions do not facilitate lordosis behavior in males. The purpose of the second study was to determine whether adult males given SL would show enhanced male sex behavior. Rats were castrated on day 60 and at the same time given SL, sham surgery(sham) or were unoperated(unop;anesthesia + incision). One month later they were tested for lordosis behavior with EB only(2ug x 3 days;test on day 4) and again 3 weeks later under the same hormonal regime except that .5mg Progesterone(P) was given 4-6 h prior to the test. Since these animals were not exposed to chronic EB, no facilitation of lordosis behavior occurred after either EB or EB + P($\bar{X}$ LQ<5 on all tests). Furthermore, SL rats did not show enhanced male sex behavior relative to that of sham or unop groups. This is in contrast to our recent report of enhanced male sex behavior in male rats given SL neonatally. We suggest that the SL itself does not induce neural plasticity with regard to male sex behavior except at a time when neural connections are still in an immature state. Once the connections for male sex behavior are established, they are apparently not altered by SL.
(Supported by HD 01182)

1110 REVERSIBLE DISRUPTION OF CENTRAL SEROTONERGIC SYSTEM POTENTIATES THE STIMULATION OF PROLACTIN SECRETION BY SEROTONIN AGONISTS IN MALE RATS. H.Y. Meltzer, M. Simonovic* and V.S. Fang*, Depts. of Psychiatry and Medicine, University of Chicago Pritzker School of Medicine, Chicago, Illinois, 60637.

Serotonin (5HT) neurons originating from raphe nuclei of the midbrain participate in the regulation of prolactin (PRL) secretion in the rat. We have previously reported that depletion of brain 5HT potentiates the stimulation of PRL release by 5HT agonists. In order to determine whether this phenomenon is due to supersensitivity of 5HT receptors we have measured dose-related increases in plasma PRL levels produced, under different conditions, by the following 5HT agonists: 5HT, 5HTP, quipazine, 5-methoxytryptamine (5MT), bufotenin (B), 5-methoxy-N,N-dimethyltryptamine (5DMT) and N,N-Dimethyltryptamine (DMT). The dose-response (D-R) curves for each compound were determined by measuring plasma PRL concentration in blood samples obtained from male rats 15 min after the intraperitoneal (ip) injection of an agonist. Our results indicate that 5HT, B and 5MT are more potent in stimulating PRL secretion than 5DMT and DMT. In order to test for a possible increase in sensitivity of 5HT receptors caused by relative absence of 5HT from the synapse, we have selected two pretreatment (PT) conditions aimed at producing reversible disruption of 5HT transmission. PT-I consisted of a single administration of a large dose of parachlorophenylalanine methylester (PCPA) (300 mg/kg, ip), which depletes brain 5HT by inhibiting its synthesis. PT-II consisted of 4 injections of 5DMT (5 mg/kg, ip, every 3 hr). Frequent administration of 5DMT, which inhibits firing of raphe neurons and decreases 5HT turnover should produce prolonged inhibition of 5HT neuronal activity. The D-R curves for each compound were redetermined in these animals 24 hr after the beginning of each PT. Both PTs shifted the D-R curves to the left. The two PTs produced shifts of comparable magnitudes for any one agonist. The period required for the appearance of greater PRL response was also similar for both PTs and no further potentiation was noted when the two PTs were combined. In view of these similarities, we believe that the development of supersensitivity of post-synaptic 5HT receptors is a common mechanism underlying the effects of these two PTs. Studies are in progress to determine the effect of these PTs on 5HT turnover and on receptor sensitivity in vitro, as well as the effects of either lesions of central 5HT system or spaced injections of other drugs known to inhibit firing of 5HT neurons, such as tricyclic antidepressants, on the 5HT agonist stimulated PRL secretion. (Supported in part by ADAMHA grants MH 30938 and 29206, RCSA MH 47808 to HYM and USPHS MH 07083 to MS.

1111 THE EFFECTS OF LOCOMOTOR ACTIVITY ON CEREBELLAR cGMP. James L. Meyerhoff, R.H. Lenox, G. Jean Kant, Edward H. Mougey*, Lee L. Pennington*, and G. Rufus Sessions, Depts. of Medical Neurosciences and Microwave Research, Walter Reed Army Inst. Rsch. Washington, DC 20012.

Stress is reported to elevate cyclic 3'5' guanosine monophosphate (cGMP) in cerebellum in the rat. Because we found that the stress of forced immobilization failed to produce this elevation (Lenox et al., Neurosci. Abstr., 1977), we decided to study the effect of locomotor activity on cerebellar cGMP.

Male albino rats were initially allowed access to activity wheels for 3 periods, each of 24 hr duration. The access was subsequently reduced over the course of a week to 120 minute, 60 minute, 30 minute and finally, to ten minute periods on each of two consecutive days. After the second day of 10-minute access to the wheel, the animals were divided into experimental and control groups. The two groups were balanced with respect to tendency to run in the wheel, based on averages of the preceding 2 day's running scores. The experimental group was allowed to enter the activity wheel for 5 minutes a day for 3 consecutive days. The control rats were also allowed to enter the activity wheel on these 3 days, but the wheel was not permitted to turn; with the access door shut they merely explored the immobile wheel for 5 minutes. On the fourth day, the animals were treated in a manner identical to the preceding 3 days except that immediately following 5 minutes in the moving or fixed wheel, the animals were placed in a lucite holder, and rapidly sacrificed by exposure to microwave irradiation. We used a 2.5 kilowatt, 2450 megahertz, microwave inactivation system, as modified in our laboratory. Following sacrifice and decapitation, trunk blood was collected for radioimmunoassay for corticosterone (CS) and prolactin (Prl), and brain tissue samples were obtained for radioimmunoassay for cGMP.

During five minutes in the activity wheel, the experimental group averaged 52 revolutions (range 30-76). This group had cerebellar cGMP levels more than twice as high as levels in control rats which merely explored the wheel while it was prevented from revolving (1.999 ± 0.103 vs 0.919 ± 0.095 picomoles per mg wet wt.). Both groups had relatively elevated plasma CS and Prl. These levels, however, were not statistically different between groups suggesting that locomotor activity, not environmental stress, was associated with the elevations in cerebellar cGMP. These data suggest that locomotor activity may be a major contributor to the elevation of cerebellar cGMP in rats exposed to environmental stress.

1112 SECONDARY SYNCHRONIZING STIMULI, THE SUPRACHIASMATIC NUCLEUS (SCN) AND THE ENTRAINMENT OF CIRCADIAN RHYTHMS IN THE RAT. R. Y. Moore and B. Ziegler*, U. Calif. at San Diego, La Jolla, CA. 92093

The purpose of this study was to determine the effectiveness of secondary synchronizing stimuli in entrainment of endogenous circadian oscillating mechanisms. Albino rats (n=12) were placed in individual cages illuminated on an LD, 12:12 schedule throughout the study. Activity was continuously monitored; drinking behavior was assessed by the interruption of a light beam each time the rat placed its head in a plastic cylinder surrounding the drinking tube attached to the water bottle. After a control period in which all animals showed normal rhythmicity in activity and drinking, half of the animals were subjected to a deprivation schedule, water available 0900-1000 hrs each day. These exhibited bursts of activity and drinking during the morning period but had normal activity rhythms and continued to place their heads in the plastic cylinder during the dark period even though no drinking tube was present. After two weeks, the animals again had free access to food and water and showed normal activity and drinking rhythms. All animals were then blinded and exhibited free-running rhythms. Introduction of a deprivation schedule as before again produced a burst of activity between 0900 and 1000 hrs but free-running activity and drinking behavior rhythms persisted with an unchanged τ. SCN lesions abolished the free-running rhythms but reintroduction of a deprivation schedule again produced the morning burst of activity and drinking which again disappeared when the deprivation schedule was stopped. These observations indicate that a deprivation schedule will result in a diurnal change in activity and drinking but does not affect any central circadian oscillating system in the presence or absence of the SCN. Supported by USPHS Grant NS-12267.

1113 EFFECT OF OVARIAN HORMONES ON SYNCHRONY OF HAMSTER CIRCADIAN RHYTHMS. Lawrence P. Morin. Dept. Psychol., Dartmouth College, Hanover, N.H. 03755.

Adult female hamsters were continuously housed under constant fluorescent light (LL) in translucent plastic cages each containing a 17 cm diameter running wheel. Light energy levels measured inside the cage end facing the light source were about 12.2 uwatts/cm^2 (also measured as a mean 48.3 lux). Freerunning locomotor rhythms were recorded on an event recorder. Splitting of the rhythm occurred during 108 days of LL in 7/12 intact control female hamsters within a range of 27-86 days. The phase relationship between the two oscillator peaks in intact animals was $157.5 \pm 4.0°$. Among animals which were ovariectomized after about 50 days of freerunning in LL 8/13 split into two peaks with a phase relation of $166.5 \pm 2.5°$. Ovariectomized animals entrained to LD 14:10 were given subcutaneous silastic capsules containing estradiol benzoate, progesterone or blanks and significant treatment differences appeared when the animals were given access to wheels in LL. Splitting occurred in only 1/7 animals given estradiol benzoate. In contrast, progesterone (4/7) or blank (9/11) capsules were associated with splitting and other rhythm anomalies such as desynchrony of endogenous oscillations. The results suggest a role for estradiol as a mediator of synchronous internal rhythmicity.

1114 NEONATAL SCN ABLATION: EFFECTS ON THE DEVELOPMENT OF THE PITUITARY-GONADAL AXIS IN THE FEMALE RAT. Sarah S. Mosko and Robert Y. Moore. Dept. Neurosciences, U. Calif., San Diego, La Jolla, Calif. 92093.

Lesions of the suprachiasmatic nuclei (SCN) in adult rodents are known to abolish a variety of behavioral and physiological circadian rhythms. Previously we reported that, like lesions performed in adulthood, ablation of the SCN in neonatal rats prior to the formation of the retinohypothalamic projection results in persistent vaginal cornification (PVC) in the postpubertal female. Long-term assessment indicates that this effect is permanent. In the present study, we have further characterized the nature of this neuroendocrine alteration resulting from destruction of the SCN in 2 day old female rats.

Extensive lesions of the SCN do not alter the onset of puberty, as indexed by the age of vaginal opening, in either sighted or neonatally blinded animals. Daily examination of vaginal smears over the first 3 weeks of puberty reveals a higher cornification index (proportion of days in vaginal estrus) in SCN rats, irrespective of whether or not they are also blinded, than observed in littermate controls. PVC, however, does not necessarily appear in SCN rats until after puberty: during puberty, irregular sequencing of smears or constant diestrus are also frequently observed; normal cycling is seen in only a few cases where damage to the SCN is only moderate. In addition, PVC in adulthood is accompanied invariably by abnormally small, polyfollicular ovaries and large pituitaries in both blinded and sighted rats with lesions. Furthermore, while sighted control females display a surge of circulating luteinizing hormone on the afternoon of proestrus, sampling of plasma in the morning and afternoon for 9 consecutive days in SCN rats reveals continuously low hormone levels. This constellation of endocrine alterations is absent in rats with lesions in nearby hypothalamic sites which spare the SCN. Since the organization of the rodent estrous cycle is circadian, these results emphasize further the central importance of the SCN in circadian rhythm generation and demonstrate the necessity of an intact SCN for the development of normal, cyclic reproductive function.

Supported by USPHS Grants NS-12267 and NS-05446-01.

1115 EFFECTS OF SUCKLING ON SERUM PROLACTIN LEVELS AND CATECHOLAMINE CONCENTRATIONS AND TURNOVER IN DISCRETE BRAIN REGIONS OF LACTATING RATS. John A. Moyer, Thomas L. O'Donohue*, Lorraine R.•Herrenkohl, Richard R. Gala*, and David M. Jacobowitz. Laboratory of Clinical Science, NIMH, Bethesda, MD. 20014

The effects of suckling on serum prolactin (PRL) levels and catecholamine concentrations and turnover were examined in eight discrete brain regions associated with norepinephrine (NE) and dopamine (DA) containing pathways and with brain regions associated with the regulation of PRL secretion. Turnover rates were assessed by using the synthesis inhibitor alpha-methyltyrosine (aMT) in combination with microdissection techniques for the removal of individual brain regions and sensitive radioenzymatic assays for NE and DA. PRL secretion was induced by mothers experiencing 6 hours of pup removal with subsequent pup replacement.

Suckling or the administration of aMT to mothers resulted in a marked increase in circulating titers of PRL in these mothers compared to saline treated mothers who were not allowed to suckle. A decrease in steady-state NE concentrations in the anterior hypothalamus and a decrease in steady-state DA concentrations in the ventromedial nucleus were noted in the suckling mothers. The comparison of relative rates of NE depletion after aMT treatment revealed a suckling-induced decrease in turnover in the anterior hypothalamus and a suckling-induced increase in turnover in the ventromedial nucleus.

These findings suggest that suckling-induced activation of PRL results in a stimulatory action on noradrenergic processes in the ventromedial nucleus. In addition, a decrease in NE turnover in the anterior hypothalamus of suckling mothers suggests the involvement of noradrenergic systems in suckling-induced PRL release.

The changes in turnover rates of NE in the ventromedial and anterior hypothalamic nuclei suggest that noradrenergic processes in these regions may participate in the suckling-induced alterations of endocrine processes. The determination of individual suckling related neuroendocrine and behavioral relationships requires further investigation.

1116 ACTH Release by Human Pituitary Cells in a Superfusion System G.H. Mulder*and D.T. Krieger. Mt. Sinai Sch. Med., Dept. of Med. Div. of Endocrinology, New York, New York 10029

In vivo studies on the regulation of ACTH release in man are difficult to perform. The present studies utilize an *in vitro* approach, using superfused columns of normal pituitary or tumor cells (Mulder et al. Endocrinology 100: 1143, 1977).

Cells were prepared by collagenase dissociation of tissue. Cell viability (trypan blue exclusion) was 80-90%; the degree of viability bore no relation to the postmortem time span (7-24 hours). Tissue obtained at surgery yielded cells with ≈ 95% viability. The number of cells obtained varied from 1.3 to 2.5 x 10^4 per mg tissue. From 0.5 to 2 x 10^6 freshly prepared cells were placed in a flow-through chamber and were superfused with M-199 at 0.5 ml/min. and 37°. 1 ml fraction of eluate were immediately acidified (IN HCl) and frozen for later ACTH determination by RIA (West antibody) or bioassay (dispersed adrenal cortex cells). 30-60 minutes after the start of a superfusion the cells had reached a stable level of basal ACTH release. By using four-way valves, the cell columns were exposed to discrete pulses of a crude NIH rat hypothalamus extract (NIH-HME). The response of the cells was immediate and transient. Linear log. dose response curves were constructed following 1 minute pulses of NIH-HME (doses: 0.1 to 0.8 rat hypothalamus). Up to 466 pg ACTH/10^5 cells was released, which is about 0.1% of the cellular content. Slopes obtained with different normal pituitary preparations were identical, but different cell preparations showed different sensitivities and released different amounts of ACTH. Repeated pulses (once every 30 min.) gave repeated peaks -- sustained perfusion with extract caused sustained periods of ACTH release. 0.5 µg/ml cortisol resulted in an inhibition of up to 80% after 1 minute pulses containing the extract of 0.5 hypothalamus and 20-30% with 1 hypothalamus. The inhibition is slow in developing (≈ 20 min) and disappearing, and is often followed by a rebound phenomenon. Dexamethasone at 0.2 µg/ml was also effective in inhibiting the cells' ACTH release. These results are very similar to what has been observed with rat cells. The 1 pituitary tumor (chromophobe) cell preparation assayed thus far showed a greatly exaggerated response to NIH-HME. In pilot experiments, MIF (10^{-6}M) and isoproterenol (5 x 10^{-6}M) had no effect on basal ACTH release. This system appears to be well-suited for analysing many aspects of ACTH synthesis and release in various human cells.

1117 THE EFFECT OF GONADAL STEROIDS ON ACTIVITY OF CHOLINE ACETYLTRANSFERASE IN DISCRETE BRAIN REGIONS OF GONADECTOMIZED RATS. E. A. Muth*, W. R. Crowley, and D. M. Jacobowitz. Dept. Pharmacol. George Washington Univ. and Lab. Clin. Sci., NIMH, Bethesda, Maryland 20014.

In order to assess the possible involvement of cholinergic mechanisms in the feedback actions of gonadal hormones, the activity of choline acetyltransferase (ChAT), the enzyme catalyzing acetylcholine synthesis, was measured in 25 microdissected brain nuclei of male and female rats after gonadectomy and subsequent treatment with gonadal steroids. Male rats were castrated or sham-operated at 60 days of age. One group of castrated rats received testosterone propionate (TP, 100 µg/day, s.c.) and another group sesame oil vehicle daily for 14 days. Sham controls also received oil. Castration significantly elevated ChAT activity in the medial preoptic nucleus and in the posterior medial amygdala, while TP lowered ChAT activity to sham levels in these areas. The TP treatment also significantly decreased ChAT activity in the nucleus tractus diagonalis.

Female rats, ovariectomized four weeks previously, received either estradiol benzoate (EB, 5 µg, s.c.) or oil at 0 hr, followed 48 hr later by either progesterone (P, 1.5 mg, s.c.) or oil, and were killed at 54 hr. EB + P reduced ChAT activity in the caudal nucleus tractus diagonalis and in the periventricular nucleus and increased ChAT activity in the supraoptic nucleus. P alone also reduced ChAT activity in the caudal nucleus tractus diagonalis. The present results demonstrate that cholinergic activity in certain discrete brain regions known to be targets for testicular and ovarian hormones is altered by gonadectomy and gonadal hormone treatment and suggest involvement of cholinergic systems in the central effects of gonadal hormones on gonadotropin secretion and mating behavior.

1118 OXYTOCIN-NEUROPHYSIN PATHWAYS TO THE LOWER BRAINSTEM AND SPINAL CORD OF THE RAT. Gajanan Nilaver*, Julie Wilkins*, Jennifer Michaels*, Donald L. Hoffman*, Ann-Judith Silverman, and Earl A. Zimmerman. Departments of Neurology and Anatomy, College of Physicians and Surgeons, Columbia University, New York City, N.Y. 10032.

In recent years projections from magnocellular paraventricular nucleus of the hypothalamus have been traced to a number of extrahypothalamic sites including lower brainstem and spinal cord. Several laboratories have reported that this pathway contains neurophysins (NPS) and may be oxytocin (OT)- and or vasopressin (VP)-ergic. Swanson showed that fibers to the nucleus solitarius in the ox contain OT-NP (Brain Res. 128:346, 1977). Studies of the relative contributions of fibers containing OT-NP and VP-NP in the rat have been obviated by lack of specific antisera to each rat NP. This problem was circumvented in two ways: (1) by studying homozygous Brattleboro rats with diabetes insipidus (DI) rats which have OT, OT-NP and lack VP, VP-NP; (2) by preabsorption of antiserum to rat NPS with DI rat NP (OT-NP) extracted from hypothalami and pituitaries of DI rats. Sections of hypothalamus, medulla and spinal cord of normal and DI rats were reacted by immuno-peroxidase technique using these antisera to NP and antisera to OT and VP. Use of unabsorbed antiserum to NPS revealed similar numbers of reactive fibers in both rat types which appeared to terminate in the solitary and dorsal motor vagal nuclei of the medulla. Positive fibers throughout the cord were found in the intermediate grey and the substania gelatinosa including sacral segments; they appeared to descend in the dorsolateral funiculus adjacent to the substania gelatinosa. Lesser numbers of OT, and only a few VP fibers were seen in medulla and cord. No reactive cell bodies were seen caudal to the hypothalamus. Absorption of antiserum to NPS with DI extract totally removed staining in DI rat and severely, but not totally, reduced it in normal rat medulla and cord; in normal rat hypothalamus it selectively abolished NP in OT but not VP cells. These findings indicate that hypothalamic projections to the medulla and spinal cord are predominately OT, OT-NP pathways. Like substance P and enkephalin, OT is found in the substania gelatinosa of the spinal cord where it may have a role in pain regulation.

Supported by USPHS, NIH Grant AM20337

1119 INVOLVEMENT OF THE ADRENAL MEDULLA IN THE MATURATION PROCESS OF THE ADRENAL CORTEX. Anthony D. Okonmah* and Karam F. A. Soliman. School of Pharmacy, FAMU, Tallahassee, Florida 32307.

The establishent of corticosterone circadian rhythm was studied and related to the response to some cholinergic and anticholinergic drugs in the immature rat. Three different age groups of Sprague-Dawley rats were placed on 14:10 light dark cycle at a controlled temperature of 23±1°C. Litter size was limited to 10 animals per litter. Animals were sacrificed by decapitation at the ages of 12-13, 18-19 and 21-22 days old. Plasma was obtained for corticosterone determination every 4 hours along the 24 hrs. period. The results from the different age groups indicate the absence of diurnal rhythm in this adrenocortical hormone. Following the absences of nycthothermal variation in glucocorticoid secretion, cholinergic study was designed. Different sets of rats in the age range of 12-14 and 29-30 days old rats were utilized. One group was subjected to cholinergic drug treatment while the other group also recieved the same treatment but was later exposed to ether stress. Physostigmine (0.20 mg/kg) and neostigmine (0.1 mg/kg) administered did not produce any significant changes in corticosterone level. Neither did 0.1 mg/kg of teteraethylammonium, hexamethonium nor atropine caused any change in the level of corticosterone. Since epinephrine was found to prolong the plasma half-life of corticosterone in this group of immature animals, it may be suggested that the cortex maturation is probably related and regulated by the adrenal medulla.

1120 EFFECTS OF ESTROGEN PRIMING ON SEXUAL BEHAVIOR AND ON STEROID RECEPTORS IN THE FEMALE RAT BRAIN. B. Parsons*, N.J. MacLusky*, M.S. Krieger*, B.S. McEwen and D.W. Pfaff. (SPON: C. Pfaffmann) Rockefeller University, New York, NY 10021.

Ovariectomized rats pretreated with estradiol benzoate (EB) display greater behavioral sensitivity to a subsequent dose of EB than do animals which did not receive pretreatment. This study investigated the effects of 17-β-estradiol (E_2) pretreatment on sexual behavior and steroid receptors in the rat brain.

Female rats ovariectomized for 21 days received a 5 mm silastic implant of E_2 or of cholesterol (C) for one week. Some animals were sacrificed for chemical analyses; the remainder had their implants removed. Five days later, the remaining animals were either sacrificed for chemical analyses, or were reimplanted with 5 mm E_2 for behavioral testing. Females were tested with an experienced male 44-46 hr after reimplantation. Then, females received 500 µg progesterone (P), and were retested 4 hr later. Animals pretreated with E_2 showed significantly higher lordosis quotients than animals pretreated with C, both when tested with E_2 alone and when tested with E_2 + P.

Cytosol estrogen receptors were measured by incubating pituitary (PIT) and hypothalamus, preoptic area and septal region (HPS) extracts pooled from 2 animals with 1 X 10^{-9}M ^{3}H-E_2 for 2.5 hr at 4°C. Bound ^{3}H-E_2 was measured by gel filtration on Sephadex LH-20. Corrections for non-specific ^{3}H-E_2 binding were made using parallel incubations containing 1 X 10^{-6}M unlabeled R2858 (17-α-ethynyl-11-β-methoxy E_2) in addition to 1 X 10^{-9}M ^{3}H-E_2. For progestin receptors measurements, cytosols were incubated with a synthetic progestin, ^{3}H-R5020 (17,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione) for 4 hr at 4°C. Bound ^{3}H-R5020 was measured by Sephadex LH-20 gel filtration. Corrections for non-specific binding were made using parallel incubations containing 2 X 10^{-8}M unlabeled R5020, in addition to 0.4 X 10^{-9}M ^{3}H-R5020.

Seven days after E_2 implantation, PIT and HPS cytosol estrogen receptor levels were significantly reduced, while progestin receptor levels were significantly increased, as compared to C controls. Five days after E_2 implant removal, PIT and HPS E_2 receptor levels, and HPS progestin receptor levels were indistinguishable from those seen five days after C removal. PIT progestin receptor levels also fell after E_2 implant removal, but remained slightly higher than in C pretreated animals.

Therefore, the 'long term' potentiation of lordosis by estradiol does not appear to be the result of a change in either estrogen or progestin receptor levels in the HPS of the female rat brain.

1121 DAILY RHYTHMS IN CSF MELATONIN AND BRAIN TEMPERATURE IN PRIMATES. Mark J. Perlow, Steven M. Reppert*, Lawrence Tamarkin*, Richard J. Wyatt and David C. Klein*, NIMH, St. Elizabeth's Hospital, Washington, DC 20032 and NICHD, Bethesda, MD 20014.

CSF melatonin and brain temperature have been studied using chronically restrained monkeys (Macaca mulatta; 5.5 to 6.5 kg). These animals were adpated to chronic restraint for 3 weeks and housed in sound-attenuated chambers. Lighting was automatically regulated (LD 12:12) so that the lights were on from 0600 to 1800 hrs EST. CSF was removed via an indwelling polyethylene catheter which terminated in the cisternal subarachnoid space; the rate of removal was 1 ml/hr. Melatonin in the CSF was measured by a RIA procedure which was validated for monkey CSF by GCMS. Brain temperature was measured continuously; the sensor was a YSI thermistor probe (No 44018) implanted on the dural surface overlying the frontal cortex.

Under these conditions daily rhythms in both CSF melatonin and brain temperature are apparent. The melatonin rhythm has a 3- to 15-fold amplitude with high values (12 to 40 pg/ml) occurring only at night. The daily changes in CSF melatonin closely reflect daily changes in plasma melatonin; plasma melatonin values are higher than CSF values. There is rapid transfer of melatonin from blood to CSF as indicated using tracer amounts of [^{3}H]melatonin; the discrepancy between the total amount of melatonin in CSF and plasma appears to reflect differences in binding proteins.

A rhythm in brain temperature also occurs in the restrained monkey, with high values of about 37.6°C occurring during the day; night values are about 36°C. Both the CSF melatonin rhythm and the brain temperature rhythm persist in constant darkness, suggesting that one or more endogenous oscillators drive these rhythms, and that they are truly circadian in nature. A difference, however, exists between the CSF melatonin and brain temperature rhythms as regards the effects of constant light. A 36-hr exposure to light blocks the melatonin rhythm resulting in continuously low daytime values. On the other hand, the rhythm in brain temperature persists in constant light in only a slightly attenuated manner.

1122 EFFECT OF KNIFE CUTS BETWEEN THE SUBFORNICAL ORGAN (SFO) AND ORGANUM VASCULOSUM LAMINAE TERMINALIS (OVLT) ON THE CENTRAL ACTIONS OF ANGIOTENSIN II (AII). M. Ian Phillips, J. Phipps and Steven Bealer, Department of Physiology and Biophysics, University of Iowa, Iowa City, IA 52242.

The SFO and the OVLT have both been proposed as receptor areas for the effects of AII. These effects include drinking behavior and blood pressure increases. Independent claims have been made that lesioning the SFO or the OVLT area abolishes or impairs the responsiveness of a rat to AII injected intraventricularly (IVT) or intravenously (iv). Since connections have been demonstrated between the SFO and the OVLT it is possible that OVLT lesions have destroyed the output pathway of the SFO. If the SFO is the only AII receptor area, cutting these tracts should abolish responsiveness to AII iv or IVT, but if the OVLT area is also a receptor, cutting the connections would not impair the action of AII.

To cut the SFO efferents a Halasz knife guide was lowered at +1mm/0/4.5mm stereotaxic coordinates, the knife protruded 2mm and turned 90° left and 90° right. Such a cut is shown in figure 1, which is redrawn from a histological section.

Twelve rats were prepared with implanted catheters and brain cannulas. In seven rats with a cut, drinking to 100ng AII IVT was 8.0 ± 3.0 ml and in uncut controls drinking was 7.2 ± 1.2 ml. There was no significant difference between the blood pressure response to AII IVT in controls and experimentals. AII iv given by infusion over 30 minutes also produced drinking in experimental subjects.

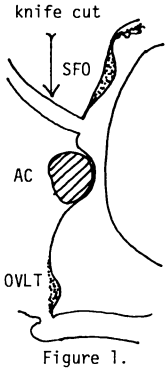


Figure 1.

These results indicate that descending fibers from the SFO are not necessary for the responses to central or peripheral AII. There may be dorsal efferents from the SFO. The results prove that the effects of lesions in the OVLT area ("AV3V") are not the result of interrupting efferents from the SFO. This adds to the evidence of low dose responses, ventricular plugging, lesioning and binding studies, that the OVLT is an independent receptor area for AII. (Supported by grants from NIMH and NSF.)

1123 SERUM LH AND PROLACTIN CONCENTRATIONS IN INTACT AND CASTRATED RATS TREATED WITH 5-HYDROXYTRYPTAMINE Nancy S. Pilotte and John C. Porter. Dept. OB-GYN and Physiology, Southwestern Med. Sch., Dallas, TX.

The effects of 5-hydroxytryptamine (5HT) on serum prolactin and LH levels in rats of both sexes has been investigated. The group of male rats included orchiectomized as well as intact animals. The group of female rats included acutely and chronically ovariectomized animals as well as ovariectomized rats bearing Silastic implants containing 17β-estradiol. All female rats were castrated on the morning of proestrus. All animals were given 5HT (20 mg/kg BW, sc) or 0.5 ml phosphate-buffered saline (PBS) (0.5 ml, sc) daily for 3 days. Thirty min after the last injection, each rat was decapitated and exsanguinated. The acutely castrated animals received the first injection immediately after castration, whereas the chronically castrated animals received the first injection 28 days after castration. 5HT treatment decreased serum LH levels in acutely-castrated males but increased serum LH concentrations in castrated females bearing estradiol implants (Table 1). Serum prolactin levels of all animals were elevated after 5HT administration.

Table 1. Mean Serum LH and Prolactin Levels

Group		LH (ng/ml)	p	Prolactin (ng/ml)	p
Male					
Intact:	PBS	43 ± 5.9*	NS	39 ± 6.4	<.001
	5HT	39 ± 5.8		278 ± 54.0	
Acutely castrated:	PBS	395 ± 38.9	<.01	41 ± 8.9	<.001
	5HT	230 ± 21.4		260 ± 31.8	
Chronically castrated	PBS	492 ± 36.0	NS	24 ± 5.7	<.05
	5HT	399 ±120.7		123 ± 37.1	
Female					
Acutely castrated + E_2	PBS	<30	<.01	76 ± 14.9	<.01
	5HT	65 ± 9.7		332 ± 84.3	
Acutely castrated	PBS	62 ± 10.9	NS	36 ± 9.2	<.05
	5HT	79 ± 20.0		77 ± 13.9	
Chronically castrated:	PBS	364 ± 30.9	NS	18 ± 1.8	<.01
	5HT	307 ± 46.0		53 ± 11.7	

*Mean and SE; N = 10

The results suggest that serum prolactin concentration can be increased by the systemic administration of 5HT.

1124 AFFERENT AND EFFERENT CONNECTIONS OF PUTATIVE PEPTIDERGIC NEURONS OF THE PARAVENTRICULAR NUCLEUS (PVN). Q.J.Pittman, H.W.Blume and L.P.Renaud. Div. of Neurology, Montreal General Hospital and McGill University, Montreal, Canada, H3G 1A4

Peptidergic neurons of the PVN project to the posterior pituitary (PP) where they release oxytocin, vasopressin and their neurophysins. There is now anatomical evidence for neurophysin-containing pathways to other sites, including median eminence (ME), brainstem and amygdala. We have carried out an electrophysiological study of PVN cells in order to further define their afferent and efferent connections.

Experiments were conducted on pentobarbital anaesthetized Sprague-Dawley rats implanted with bipolar stimulating electrodes in the amygdala and midbrain central gray. Bipolar electrodes were also placed on the surface of the ME and/or across the PP stalk. Micropipettes inserted by a ventral approach were used to record extracellular unit activity. A PDP 11/40 computer was utilized for analysis of spike discharge patterns.

Antidromic invasion techniques were used to identify efferent pathways from PVN. Antidromic invasion was recorded in 6 PVN cells after amygdala stimulation and in 2 PVN cells after midbrain stimulation, thus providing support for the existence of pathways to these two areas. The PVN-PP projection was evident in the observation of antidromic invasion from the PP in 93 PVN cells. A further 68 PVN cells, including 5 having phasic activity, displayed antidromic invasion from the ME, thus supporting the existence of a PVN-ME pathway. For the most part, this population of cells appeared to be separate from that which projected to the PP. However, 13 of 138 PVN cells tested with both PP and ME stimulation displayed antidromic invasion from both sites. This would indicate the presence of simultaneous axon projections to both areas.

Afferent influences from the amygdala were evident in neurons with projections to the PP and in those projecting to the ME. 32% (n=90) of PP projecting neurons and 19% (n=48) of ME projecting neurons responded in an orthodromic manner after amygdala stimulation; the predominant initial effect was a decrease in excitability. In contrast, none of the PVN cells with projections to either the PP (n=54) or the ME (n=29) showed orthodromic responses to midbrain stimulation. Thus the amygdala, but not the midbrain central gray can be shown to influence the excitability of these putative peptidergic neurons.

These observations provide electrophysiological evidence for the existence of projections from the PVN to not only the PP,but also to ME, amygdala and midbrain, and provide evidence for simultaneous projections of some PVN neurons to both ME and PP. (Supported by MRC)

1125 RELEASE OF LUTEINIZING HORMONE INDUCED BY N-METHYL ASPARTATE IS BLOCKED BY GABA OR TAURINE BUT NOT BY DOPAMINE ANTAGONISTS. M.T. Price, J.W. Olney and T.J. Cicero. Washington University School of Medicine, St. Louis, MO 63110

Glutamate (Glu) and certain acidic analogs excite central neurons when applied iontophoretically or destroy neurons of the arcuate nucleus of the hypothalamus (AH) when administered systemically. Since AH is an important neuroendocrine regulatory center, the selective action of these agents on AH neurons makes them potentially valuable systemic neuroendocrine probes. Their bimodal action (neuroexcitatory or neurotoxic) permits their use for either provocative or ablative experimental purposes - e.g., removal of AH neurons by neonatal treatment with high doses of Glu (ablative approach) results in markedly reduced AH concentrations of dopamine (DA) and choline acetyltransferase, which suggests there may be at least two subpopulations of AH neurons - dopaminergic and cholinergic. In provocative experiments, subtoxic doses of Glu, i.e., doses that do not destroy AH neurons, cause an acute elevation of serum luteinizing hormone (LH) in adult rats and subtoxic doses of the more potent excitotoxic analogs of Glu powerfully mimic this effect. One such analog, N-methyl aspartate(NMA), has proven particularly useful in studies of LH release.

In the present experiments we examined the ability of the neuroinhibitory amino acids, GABA and taurine, or the DA receptor blocking agents, pimozide and chlorpromazine, to influence NMA-induced LH release. All compounds were administered subcutaneously to 25 day old male rats. The four potential blocking agents were given either by themselves or in combination with a dose of NMA (25 mg/kg) which had previously been found highly reliable in inducing 5 to 10-fold elevations of LH within 7½ min. In drug combination experiments, GABA and taurine were injected immediately before NMA while pimozide and chlorpromazine were given 24, 14 and 2 hrs prior to NMA injection. We found: (1) Pimozide (0.6 mg/kg) and chlorpromazine (25 mg/kg) had no significant effect either on basal LH levels or on NMA-induced LH release. (2) Neither GABA nor taurine (up to 1000 mg/kg) had any effect on basal LH levels. (3) Both GABA and taurine (300-1000 mg/kg) completely blocked NMA-induced LH release.

It is known from microelectrophoretic experiments that GABA and taurine block excitation of central neurons by Glu and its analogs. The observation that GABA and taurine block the LH releasing action of NMA strengthens the hypothesis that NMA-induced LH release is triggered by depolarization of AH neurons. The failure of DA receptor blocking agents to block NMA-stimulated release of LH suggests that the subpopulation of AH neurons responsible for this effect is not dopaminergic. NIH grants NS-09156, DA-00259, MH-14677 and RSD Awards MH-38894 and MH-70180.

1126 VASOPRESSIN, CARDIOVASCULAR AND BEHAVIORAL RESPONSES TO NICOTINE IN THE CAT. T.A. Reaves, Jr., H-M. Liu*, M. Qasim* and J.N. Hayward. Dept. Neurology & Neurobiology Program, University of North Carolina, Chapel Hill, North Carolina, 27514.

The antidiuretic and cardiovascular effects of nicotine have been attributed to its action at several central neural sites such as the hypothalamus, the limbic system (Milton & Paterson, J. Physiol. 241: 607, 1974) and the brainstem (Porsius & Van Zwieten, Prog. Brain Res. 47: 131, 1977) as well as at peripheral neural sites in the cervical parasympathetic baroreceptors (Cadnapaphornchai et al., Am. J. Physiol. 227: 1216, 1974). Our studies in the unanesthetized monkey suggested a linkage between vasopressin (VP) release and nicotine-induced behavioral events (Hayward & Pavasuthipaisit, Neuroendocrin. 21: 120, 1976). The objective of this study was to examine the relationships between VP-release and nicotine-induced behavioral, cardiovascular and autonomic responses in the acute and chronic cat.

In the chronically prepared, chamber-isolated, adult cat we observed behavior and obtained blood samples from an indwelling cardiac cannula for measurement of plasma VP by RIA (Hayward et al., Endocrinol. 98: 965, 1976). During a 10-minute intravenous (i.v.) infusion of nicotine (25-50 ug/kg/min), plasma VP rises rapidly to 20-60 fold above control values in association with the behavioral responses of restlessness, ear twitching, salivation, chewing, retching and vomiting.

In the acutely prepared chloralose-anesthetized, paralyzed and artificially respired cat, we recorded mean femoral arterial blood pressure (MABP), heart rate (HR), pupil size, salivation and cortical EEG and obtained blood samples from a femoral venous cannula for plasma VP. During a 10-minute i.v. infusion of nicotine (25-50 ug/kg/min), plasma VP rose rapidly to 5-15 fold above basal values in association with an immediate and transient MABP rise of 50-100%, a biphasic HR response, pupillary dilatation, an increase in salivation and an unchanged EEG. Bilateral cervical vagotomy did not change these responses to nicotine. Hypophysectomy abolished the nicotine-induced rise in plasma VP but did not alter the cardiovascular and autonomic responses.

We conclude that nicotine releases VP from the neural lobe in the cat in association with behavioral, cardiovascular and autonomic responses. Nicotine-induced cardiovascular events are linked in some way with VP-release while behavioral responses and the cervical vagus are not essential.

(Supported, in part, by Grants No. NS-13411 and NS-05696 from USPHS and by No. 1977-78-A-3 from the North Carolina Heart Association)

1127 SIMILAR AND DIFFERENTIAL ROLES FOR THE SEPTUM AND MEDIAL PREOPTIC AREA ON ESTROGEN-DEPENDENT NEUROENDOCRINE PROCESSES. Jorge F. Rodriguez-Sierra and Ei Terasawa. Wisconsin Regional Primate Research Center, Madison, WI 53715. Lesion of either the septum (SEP) or the medial preoptic area (MPOA) in female rats enhances their sensitivity to estrogen which is responsible for the induction of sexual receptivity. It is also well demonstrated that lesion of the MPOA advances the onset of puberty. The present experiment was designed to test whether those estrogen-dependent phenomena are regulated by the same neural elements or not. Radiofrequency lesions were placed stereotaxically in the SEP of MPOA of prepubertal (25 days of age) rats. The condition of the vagina was inspected daily and vaginal smears were taken after its opening until the day of ovariectomy. The first and subsequent ovulations were determined by laparatomy. All animals were ovariectomized around day 50 of age. Three weeks after ovariectomy, animals were injected with estradiol benzoate (EB, 5 ug/Kg), followed by progesterone (0.5 mg/rat) 44 hours later. Animals were tested for lordosis responding with a male 4 hours after progesterone. The sham-operated (n=6) and control (n=6) animals showed comparable days of vaginal opening (39.7 $\pm$ 0.8 and 38.3 $\pm$ 0.8 days, respectively), which were followed by the first ovulation one day later. Animals bearing SEP lesions (n=8) exhibited a significant delay in vaginal opening (33.6 $\pm$ 1.3 days) and first ovulation. Half of the SEP-lesioned animals did not ovulate on the day following vaginal opening, although they showed normal estrous cycles subsequently. In contrast, most of the MPOA-lesioned animals ovulated after vaginal opening, but displayed continuous vaginal cornification thereafter. Both SEP- and MPOA-lesioned rats displayed significantly higher LQ scores than control animals (SEP = 76.3; MPOA = 60; Control = 10). Thus, it was suggested that the neural mechanisms involved in estrus behavior are different from those involved in the onset of puberty in the female rat, although both phenomena are estrogen dependent. The SEP and the MPOA exert a similar inhibitory effect on sexual receptivity in the adult, while these brain structures exert opposite effects (SEP-facilitatory; MPOA-inhibitory) on the onset of puberty. (Supported by NIH Grant RR00167)

1128 BRAINSTEM AND DIENCEPHALIC SINGLE-UNIT ACTIVITY CORRELATES OF SEXUAL BEHAVIOR RECORDED IN UNRESTRAINED FEMALE CATS. James D. Rose. Dept. Psychol., Univ. Wyoming, Laramie, WY 82071.

Sexual behavior in the cat entails a sequence of events in which the male initiates contact by grasping a fold of skin on the dorsum of the female's neck in his mouth, then mounts and begins pelvic thrusting, which continues until intromission occurs. After a few seconds of intromission, the female becomes hostile and the male releases her. The female then exhibits the afterreaction, a period of vigorous licking, rubbing, and rolling which was triggered by intromission. Genital stimulation does not elicit the afterreaction in anestrous cats. The present work was aimed at identifying the patterns of single-unit activity which accompany the display of the cat's estrogen-dependent behavioral responses to manually-applied genital and somatosensory stimulation. Single unit activity was recorded in 16 cats by means of a skull-mounted microdrive or with permanently-implanted fine wire microelectrodes. The majority of the midbrain and diencephalic units sampled displayed a characteristic pattern of unit activity in response to the test stimuli. Application of the neck grip induced a slowing of unit activity which was correlated with the inhibitory effect of this stimulus on the cat's locomotor behavior. Unit resposes to genital stimulation were typically accelerations of firing at the onset and offset of the stimulus. Upon release of the neck grip there was a surge of firing which preceded and continued into the onset phase of the afterreaction. Genital licking, usually the first event in the afterreaction, was associated with a decline in unit activity. The transition from licking to rolling was immediately preceded by accelerated unit firing which occurred before any change in behavior or neck electromyographic activity was apparent. Activity in anterior brainstem units was related to the overall level or general class of behavioral activity (licking, rolling) being displayed by the cat, but caudal brainstem cells showed firing patterns more closely associated with specific fragments of a motor pattern. On stimulation trials when genital stimulation did not elicit an afterreaction, as in anestrous cats or estrous cats which were sedated with ketamine HCl, there was no surge of midbrain activity following vaginal probing and release of the neck grip. Because the somatosensory responsiveness of the units varied greatly between cells, it is unlikely that sensory feedback from movements could account entirely for the consistent patterns of unit activity seen during the elicitation and display of the estrous behavioral responses.

Supported by N.I.H. Grants NS-12260 and NS-13748.

1129 ESTRADIOL-CONCENTRATING STRUCTURES AND AFFERENT CONNECTIONS TO ESTROGEN TARGET AREAS IN THE CAT BRAIN. Mark J. Rowinski, Howard D. Rees, and Richard P. Michael*. Dept. of Psychiat., Emory Univ. Sch. of Med. and Ga. Ment. Health Inst., Atlanta, GA 30322.

The distribution of estradiol-concentrating cells in the cat brain was determined by autoradiography in two ovariectomized adult females 1 hr after iv injection of 3 µg of ^{3}H-estradiol (NEN, 92 Ci/mmol). Heavily labelled cells were found in the bed nucleus (n.) of the stria terminalis, lateral septal n., periventricular and medial preoptic nuclei, anterior hypothalamic area, periventricular n., ventromedial n., tuberoinfundibular area, the cortical and basomedial nuclei of the amygdala, amygdalo-hippocampal area, periamygdaloid cortex, and ventral dentate gyrus. Less heavily labelled structures included the islands of Calleja, n. of the diagonal band, medial septal n., the lateral, paraventricular, dorsomedial, and posterior hypothalamic nuclei, periaqueductal gray, interpeduncular n., medial n. of the solitary tract, and spinal n. of V. Estrogen accumulation by many neurons in the interpeduncular n., which has not been observed in any other species to date, raises the question of the possible neuroendocrine significance of this structure in the cat.

To determine the sources of neuronal input to one of the most heavily estradiol-concentrating regions, namely, the medial preoptic-anterior hypothalamic area, horseradish peroxidase (HRP) studies were conducted in 3 adult male and 6 adult female cats. At 48-78 hours after the injection of 0.05-0.15 µl of 30% HRP in saline, neurons containing the granular reaction product were consistently found in subiculum, entorhinal cortex, retrosplenial gyrus, septum, and amygdala. Ventromedial, dorsomedial, tubero-infundibular, perimammillary and lateral regions of the hypothalamus were also found to contain neurons labelled by retrograde transport of the enzyme. Many of these were relatively close (<15µm) to blood vessels and had processes directed toward the vessels. HRP labelled neurons were also found in most cases in anterior thalamic nuclei, periaqueductal brain stem structures, and some cortical areas. Taken together, these results indicate that estradiol-concentrating cells in the cat are widely distributed with concentrations in the basal forebrain and hypothalamus and that these cells are likely to be influenced by non-estrogen sensitive limbic structures involved in the mediation of affective states.

Supported by NIMH Grant #MH19506. General support provided by the Georgia Department of Human Resources.

1130 FACTORS CONTROLLING PROGESTERONE-STIMULATED GONADOTROPIN RELEASE. K.B. Ruf, Dept. Ob/Gyn, Royal Victoria Hospital, McGill University, Montreal, Canada H3A 1A1.

Estrogen-primed ovariectomized rats exposed to standard lighting conditions and given progesterone (P) release large amounts of LH in the afternoon and evening, but not in the morning (Caligaris et al., Endocrinology 89: 331, 1971). The following hypotheses could explain the absence of the morning response: a) inactivity of central monoaminergic pathways mediating the P-effect, b) reduced pituitary sensitivity to LHRH, c) diurnal variation in the formation of obligatory P-metabolites. Adult ovariectomized rats were maintained under 14 h light/10 h dark for 1 month. They were primed with estradiol benzoate at either 0700 h or 1200 h colony time and challenged with P or P-metabolites (5α-dihydro-P, 20α-dihydro-P, 3α-hydroxy-5α-pregnan-20-one) or with D-Ala^6-des-Gly-NH$_2^{10}$)-LHRH-ethylamide exactly 72 h later. The monoamine precursors DL-threo-dihydroxyphenylserine or 5-hydroxy-L-tryptophan(5-HTP) were administered in conjunction with P in the morning. 5-HTP given at 0630 h significantly increased P-induced LH and FSH release in the morning ($p < 0.005$). LH and FSH release induced by the LHRH analog and monitored over 4 h was significantly greater in the afternoon (LH: $p = 0.028$; FSH: $p = 0.009$). All P-metabolites tested (as well as ACTH) led to significant LH surges in the afternoon, but not in the morning. The absence of the P-response in the morning may thus be caused, at least in part, by reduced activity of central serotoninergic neurons and reduced pituitary responsiveness to LHRH. In contrast, the formation of P-metabolites does not appear rate-limiting.

1131 FACILITATION OF LORDOSIS BEHAVIOR FROM HYPOTHALAMIC AND MESENCEPHALIC ELECTRICAL STIMULATION. Yasuo Sakuma* and D.W. Pfaff. The Rockefeller University, New York, NY 10021.

Estradiol-concentrating neurones are found in several cell groups thought to be involved in the regulation of female copulatory behavior. We now report that electrical stimulation of some of these cell groups can cause changes in the lordosis reflex. Monopolar electrical stimulation was applied through chronically implanted platinum-iridium electrodes to the ventromedial nucleus of the hypothalamus (VMN) (n=48); medial preoptic area (POA) (n=9), or mesencephalic central gray (CG) (n=49) of ovariectomized, estrogen-treated female rats. Lordosis reflex occurrence and strength were tested using cutaneous manual stimulation or male rat mounting.

Electrical stimulation of the VMN facilitated lordosis in response to manual stimulation ($P < .001$) and in response to male mounting ($P < .01$). Percent increases ranged between 53 and 150% of the prestimulation control. A gradual increase followed a relatively long period of stimulation of about 1 hr, and required low frequency, 10-30 Hz. Threshold for effective stimulation was, on the average, 12.5 μA. Adrenal progesterone release was not required for the VMN facilitation of lordosis, since stimulation was effective in adrenalectomized rats, in dexamethasone-primed animals, and in animals preloaded with exogenous progesterone. In contrast, POA stimulation suppressed lordosis performance both in response to manual stimulation ($P < .01$) and male mounting ($P < .01$). The time course of the inhibition was slow, similar to that seen in the facilitation by VMN stimulation. Electrical stimulation of the CG induced immediate and large increases in the lordosis reflex, both in response to manual stimulation ($P < .001$) and male mounting ($P < .001$). What distinguished the facilitatory response to CG stimulation from the effect of VMN stimulation was its fast time course. The increase was often obvious within 2 min from the start of CG stimulation. This facilitation from CG, which does not depend on the integrity of the hypothalamus, increases in a graded manner to increased stimulus intensity with average threshold of 10 μA, and is optimally induced by stimuli delivered at 50-150 Hz.

These results suggest that different cell groups with estradiol-concentrating neurones are not functionally uniform in their influence on a reflex such as lordosis. For instance, the difference in the time course of facilitation in response to VMN and CG stimulation indicates that CG could be in the direct reflex arc for lordosis, while the VMN is not. Instead, the VMN may exert a tonic hormone-sensitive bias on reflex arcs completed in the brainstem.

1132 EFFECTS OF AGENTS WHICH DEPLETE SEROTONIN ON ULTRASTRUCTURE OF THE MEDIAN EMINENCE AND PITUITARY PARS INTERMEDIA. Linda C. Saland* and William G. Dail, Jr. Department of Anatomy, University of New Mexico, School of Medicine, Albuquerque, New Mexico 87131.

The pituitary pars intermedia contains catecholamine nerve fibers which are believed to participate in inhibition of hormone release. Recently, Kraicer and Morris (1976, Neuroendocrinol., 21:275) showed serotonin-stimulated adrenocorticotropin (ACTH) release from intermedia cells in vitro. They suggested that serotonin-containing fibers may be present in the pars intermedia. To investigate this hypothesis, we have studied the effects of antiserotonergic drugs on the morphology of nerve fibers in the pars intermedia and in the median eminence. 5,7-dihydroxytryptamine (5,7 DHT) and p-chloroamphetamine (PCA) significantly lower brain serotonin levels, and produce morphologic evidence of toxic effects and/or degeneration of nerve fibers. Administration of a single 150 μg dose of 5,7 DHT to the lateral cerebral ventricle of adult male rats leads to the appearance of some degenerative changes in fibers of the median eminence, a brain area known to contain serotonin terminals. Similar changes are observed with one 10 mg/kg intraperitoneal dose of PCA. Three sequential injections of PCA (5 mg/kg or 10 mg/kg) result in clearcut areas of degenerating fibers in the median eminence, particularly in the zone near the portal vessels. In contrast, nerve terminals on pars intermedia cells appear normal after all drug treatments. After PCA injection, some secretory cells of the intermediate lobe contain numerous, tightly packed granules. We interpret the results to suggest that antiserotonergic agents affect nerve fibers in the median eminence, and that the morphologic effects are greater with multiple doses of PCA. Lack of morphologic alterations in intermediate lobe nerve fibers suggests that those fibers are not serotonergic. Alternatively, the purported serotonergic fibers of the pars intermedia may be unresponsive to drug treatment, as has been shown for pituitary catecholamine fibers after administration of 6-hydroxydopamine (Shoemaker et al., 1977, Soc. for Neurosci. Abstracts, #1149). Positive effects on secretory cells of the pars intermedia may reflect drug inhibition of granule release.

1133 RADIOIMMUNOLOGIC LOCALIZATION OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IN INTRA- AND EXTRA-HYPOTHALAMIC SITES OF THE RAT BRAIN. W. K. Samson*, S. I. Said* and S. M. McCann (SPON: A. Giachetti), Depts. of Physiology and Pharmacology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

The presence of significant quantities of VIP in whole hypothalamus and the demonstration that VIP can act at the level of the hypothalamus to modulate anterior pituitary hormone release led us to reinvestigate the exact localization of the peptide in structures which might be related to anterior pituitary function. Blood was taken from the external jugular vein of lightly etherized adult male Sprague-Dawley rats. They were subsequently decapitated and anterior and posterior lobes of the pituitary were removed. Brains were frozen on dry ice prior to sectioning in the frontal plane on a cryostat. Sections 100 μm in thickness were taken through the plane of the desired nuclei or areas and tissue was removed with flattened 15 gauge needles. Tissues from two rats were pooled, homogenized in 1 ml 0.2N acetic acid, and centrifuged. Supernatants were assayed for extracted protein and radioimmunoassayable VIP content at two dilutions. The following areas were found to contain VIP in quantities sufficient to be detected by RIA (expressed here as pg VIP/μg extracted protein): Suprachiasmatic Nucleus 262.3±35.3, Medial Amygdaloid Nucleus 106.7±9.5, Amygdaloid Cortex and Central Amygdaloid Nucleus 28.1±7.6, Medial Preoptic Area 16.9±5.9, Anterior Hypothalamic Area 20.7±1.5, Organum Vasculosum Lamina Terminalis 17.0±3.1, Median Eminence 8.5±1.7, Anterior Pituitary 5.6±1.2, Posterior Pituitary 6.1±0.7, Cerebellar Cortex 23.0±3.0. Plasma was found to contain 42.6±2.0 pg VIP/ml. Both Median Eminence and Cerebellar Cortex contained VIP levels that were near the lower limit of radioimmunoassay sensitivity (20 pg). Our present data using RIA agrees with immunohistochemical localization of VIP-positive staining elements in the rat brain. Thus, VIP appears to be localized in discrete hypothalamic and extra-hypothalamic sites which have been shown previously by lesion and stimulation studies to exert control over the release of anterior pituitary hormones. The lower levels of VIP found in anterior pituitaries and median eminence fragments, together with our earlier in vivo and in vitro studies, suggest that VIP acts at the level of the cell bodies of neurons which produce or modulate the production of hypothalamic releasing or release-inhibiting factors.
(Supported by NIH grants HD09988 and AM10073, as well as HL14187 and CA21570)

1134 SUPRASELLAR CONTROL OF LUTEINIZING HORMONE (LH) SECRETION BY N-METHYL ASPARTIC ACID (NMA). Bruce A. Schainker* and Theodore J. Cicero, (SPON: James S. Nelson) Depts. of Psychiatry and Anatomy & Neurobiology, Wash. U. Sch. Med., St. Louis, MO 63110.

Parenterally administered NMA, an acidic "excitotoxic" amino acid, acutely elevates serum LH levels in immature (25 day) male rats at subtoxic doses (Price et al., N.S. Abst. 3:335, 1977). Other analogues, such as glutamic acid and kainic acid, act similarly but their relative impotence and extreme neurotoxicity, respectively, limit their usefulness as neuroendocrine probes.

In order to confirm NMA's provocative effects upon serum LH levels we administered NMA s.c. to young adult (54 d, 177 g avg) male Sprague Dawley derived rats 7.5 min prior to sacrifice. Serum LH values (mean ± S.E.M., n=6), determined by radioimmunoassay after H_2O, 16.5 mg/kg, 33 mg/kg or 66 mg/kg BW were 113±54, 446±116, 609±108 and 461±96 ng/ml, respectively.

A suprasellar locus is the presumed site of action of subtoxic doses of NMA since circumventricular organs of the brain, especially the arcuate nucleus of the hypothalamus, are vulnerable to toxic doses. However, to demonstrate whether NMA acts at the level of the pituitary to induce LH secretion we incubated 16 vials, each containing 4 hemipituitaries from 61 day male Sprague Dawley rats, for 3 hrs. in Medium-199 in the presence of LH releasing hormone (LHRH), NMA, both or neither. Radioimmunoassayable LH levels secreted into the media are tabulated:

Compound in Medium	LH in Medium/2ml flask/3 hr S.E.M.	
Control	35 ± 6	
LHRH 10^{-8}M	150 ± 10*	*p < 0.001 vs cont.
NMA 10^{-5}M	19 ± 7†	†p > 0.10 vs cont.
LHRH 10^{-8}M + NMA 10^{-5}M	140 ± 8*	

These results demonstrate that NMA does not induce LH secretion by a direct action upon the pituitary, but rather presumably acts at a suprasellar locus.

Accordingly, NMA will undoubtedly provide a useful tool to the neuroendocrinologist in elucidating the central control over pituitary LH secretion since it: 1) acutely (within minutes) elevates serum LH in the young adult (54 days) as well as immature (25 day) male rat of different strains; 2) does not act at the level of the pituitary; 3) is easily administered to produce its effects - viz., parenterally; and 4) is the only known agent that acutely provokes marked LH secretion in the male rat when given parenterally at subtoxic doses. This research was supported in part by USPHS grants DA-01407 and DA-00259. T.J. Cicero is a recipient of Research Scientist Development Award AA-70180.

1135 THE DISTRIBUTION OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) IN THE FETAL GUINEA PIG BRAIN. M. Schwanzel-Fukuda* and A.J. Silverman. Dept. Anat., Columbia Univ., New York, NY 10032.

Brains from littermate male and female fetal guinea pigs were studied using an antiserum to LHRH ("F" rabbit, supplied by S. Sorrentino) and the unlabeled antibody enzyme technique. Time-mated guinea pigs (Hartley albino) were decapitated, the fetuses removed and their brains fixed by immersion in Bouin's solution. Tissue was embedded in paraffin and 6 µm sections cut and processed for immunocytochemistry. Immunoreactive LHRH neurons and processes were present on the 35th day of a 68-72 day gestation period. In the females, LHRH cells were found predominately in the arcuate nucleus (AN) but were also numerous in the suprachiasmatic nucleus (SCN), preoptic area (POA), olfactory tubercle (OT), septal region (S), and nucleus and tract of the diagonal band (DB). Fibers were observed in these regions and in both oral and caudal portions of the tuber cinereum. In the males, LHRH neurons were clustered in AN with a few scattered neurons in SCN, DB and S. LHRH fibers were observed in AN and in the caudal portion of the tuber cinereum, but not in the rostral regions of the brain. At 40 days of gestation, the total number of LHRH neurons in the female brain was twice that in the male brain. In both sexes cells were now present in AN, SCN, POA, OT, S, DB. LHRH positive fibers were found in all of these regions and in the median eminence. At 45 days of gestation the situation vis-à-vis the LHRH neurons was reversed; there were now twice as many positive cells in the male as compared to the female. The same regions contained positive cells and fibers as was noted for day 40. At 50 and 60 days of gestation, there was a decrease in the total number of LHRH neurons detected in both sexes. The frequency of occurance of immunoreactive neurons was approximately equal in both sexes by this period in development. These cells and their fibers were now found predominately in the rostral regions (SCN, POA, DB, OT, S); very few were noted in AN. Positive axons were first noted caudally at 50 days in the capsule of the mammillary body, fasciculus retroflexus, interpeduncular nucleus and midbrain tegmentum. From 35 days onward there was a continuous increase in the numbers of LHRH axons to the median eminence.

This study represents a description of a transitory sexual dimorphism in the LHRH neurosecretory system of a developing mammal. Supported by HD10665 and HD10533.

1136 LACK OF PARALLELISM BETWEEN EFFECT OF LESIONS OF THE CORTICO-MEDIAL AND BASO-LATERAL AMYGDALA ON PLASMA PROLACTIN AND CORTICOSTERONE RESPONSES TO STRESS. Jo. A. Seggie, Departments of Neurosciences and Psychiatry, McMaster University, 1200 Main St. Hamilton, Ontario L8S 4J9.

In 1977 it was reported at the Society for Neurosciences (abs #642) that cortico-medial amygdala lesions had no effect on the pattern of plasma corticosterone response to the stress of handling or exposure to a novel environment. However, lesions of the baso-lateral amygdala resulted in a corticosterone response to these stresses that was of shorter latency and greater magnitude in comparison with non-lesioned controls. The present study was undertaken to determine the effect of lesions of the cortico-medial or baso-lateral amygdala on plasma prolactin responses to the stress of 5 seconds of handling and three minutes exposure to a novel environment in comparison with corticosterone stress responses in the same experimental preparations.

Adult male rats were housed in individual cages under a light cycle of 12 hours light/12 hours dark. Separate groups of normal, sham-operated or amygdala-lesioned rats were subjected to one of two stressors at the same time of day and sacrificed by decapitation 0, 5, 10 or 15 minutes after the end of stress. Trunk blood was collected for hormone assay. A low level of stress was imposed by picking the animal up for 5 seconds while a higher level of stress was imposed by placing the animal into a novel environment for 3 minutes. In the first study, lesions were placed bilaterally in the baso-lateral amygdala while in the second study the lesion group sustained cortico-medial amygdala lesions.

Baso-lateral amygdala lesions had no effect on the pattern of plasma prolactin responses to either of the stressors. The cortico-medial amygdala lesion however, resulted in a prolactin response following exposure to a novel environment that was of shorter latency and greater magnitude than that seen in non-lesioned controls. The prolactin response to handling was unaffected by this lesion.

Thus the two types of amygdala lesions have different effects on the corticosterone and prolactin responses to stress in this study. Furthermore, success in visualizing this differential influence may well depend on the nature of the stress stimulus used to challenge the system under observation.

Supported by project grants from the Ontario Mental Health Foundation (O.M.H.F.). Dr. Jo Seggie is an O.M.H.F. Scholar.

1137 EFFECTS OF RESERPINE ON VASOPRESSIN AND NEUROPHYSINS IN THE EXTERNAL LAYER OF THE MEDIAN EMINENCE IN THE RAT. V. Seybold*, R. Elde and T. Hokfelt*. Dept. of Anatomy, Univ. of Minnesota Medical School, Minneapolis, MN 55455.

The indirect immunofluorescence technique was used to qualitatively assess the effect of reserpine on peptides in the median eminence (ME) of the rat. Changes in the distribution of neurophysins (NP) and vasopressin (VP) were of particular interest. These secretory products could be localized in both the internal layer of the ME in cross-sections of axons en route to the posterior pituitary, and in the external layer in nerve terminals adjacent to portal capillaries.

Acute reserpine treatment (10 mg/kg i.p. 24 hr prior to sacrifice) specifically depleted NP and VP in the external layer of the ME. Depletion was observable at 8 hr post-injection, but the effect was maximal at 24 hr. No qualitative changes in internal layer NP, VP or oxytocin were observed. Levels of other peptides (somatostatin, TRH, LHRH) localized by immunofluorescence in the external layer did not appear to be altered by 24 hr reserpine treatment. Chronic reserpine treatment (2 mg/kg/24 hr i.p. for 5 days, animals sacrificed 24 hr after the last injection) resulted in an apparent increase in external layer NP and VP.

The acute effect of reserpine is believed to be unrelated to the monoamine depleting effect of the drug. The disappearance of NP and VP from the external layer of the ME could not be duplicated with other depletors of monoamines. Treatment with α-methyl-p-tyrosine (250 mg/kg/8 hr i.p. for 24 hr) and p-chlorophenylalanine (300 mg/kg i.p. for 24 hr) did not produce observable decreases in NP and VP in the external layer by immunofluorescence. Furthermore, the monoamine oxidase inhibitor, phenipra-zine (10 mg/kg i.p. 14 hr prior to sacrifice) did not reverse the acute effect of reserpine on NP and VP.

The paraventricular nucleus (PVN) was determined as the source of NP and VP in the external layer. Bilateral lesion of the PVN eliminated all of the external layer of NP and VP. Bilateral lesion of the suprachiasmatic nucleus has no qualitative effect on NP and VP in either layer of the ME.

Therefore, the NP and VP fibers in the external layer of the ME can be distinguished from NP and VP fibers terminating in the posterior pituitary on an anatomical basis, as well as by a differential response to acute reserpine treatment. Furthermore, the effect of acute reserpine treatment on NP and VP in the external layer may be due to a direct effect of the drug on neurosecretory neurons and not reflect reserpine's action on the storage of monoamines.

Supported in part by a grant from the Graduate School of the University of Minnesota and a PMA Fellowship to V. Seybold.

1138 COMPARISON OF ANGIOTENSIN ACTIONS AT TWO SITES IN RAT BRAIN. John B. Simpson and Michael L. Mangiapane, Depts. of Psychol. and Physiol.-Biophysics, Univ. Washington, Seattle, WA 98195

There are at least two significant central sites of action of angiotensin II (A II) in rats for provoking water intake and hypertension. These loci are the subfornical organ (SFO; Mangiapane and Simpson, Neurosc. Abstr., 1977) and the tissue proximal to the optic recess of the third cerebral ventricle (OR), including the organum vasculosum (Hoffman and Phillips, Brain Res., 1976). These two loci were compared. One group of rats each received an intracranial cannula (26 ga) terminating in the SFO, in tissue proximal to SFO, or in the dorsal third ventricle (III V). SFO cannulae did not perforate ventricular ependyma. A second group each received a cannula terminating within the OR. For ventricular loci (OR or III V), ependyma was ruptured only at the classified site of injection. Measures of water intake and mean aortic pressure were made following intracranial injections of various doses of A II.

Injection of 10 ng or less of A II at the SFO provoked highly correlated dipsogenic and pressor effects. The pressor effect in every case preceded the onset of drinking and was found in rats not ingesting water. The pressor effect was not secondary to the elicited behavior since it occurred in anesthetized animals. Maximal efficacy for both A II effects was observed when the cannula terminated in the SFO and not in adjacent tissue or III V. Application to OR of those doses of A II also provoked dipsogenic and pressor effects. The sensitivity of this region to dipsogenic and pressor effects was less than the SFO and approximately equal to III V. Further, OR injection of these doses of A II provoked a pressor response which frequently commenced only after drinking began. The pressor effect of OR injections of A II may be secondary to the elicited behavior. This is suggested by the striking similarity of the time course and magnitude of the OR pressor effect to that seen in animals spontaneously ingesting water. In contrast, SFO-injected animals are unlike spontaneously drinking animals because the pressor response typically is near-maximal by the onset of elicited drinking.

Thus, SFO injections of low doses of A II cause a pressor effect which is not secondary to the elicited drinking, whereas OR injections of the same doses of A II can cause a pressor effect which is secondary to the elicited drinking. These data, then, suggest at least two different modes of and neural sites of dipsogenic action of A II.

Supported by HL 21799 and HL 21800.

1139 ROLE OF ACETYLCHOLINE AND ANGIOTENSIN IN THE OSMOTIC CONTROL OF VASOPRESSIN RELEASE BY THE ORGAN CULTURED RAT HYPOTHALAMO-NEUROHYPOPHYSEAL SYSTEM. Celia D. Sladek and Robert J. Joynt*. Depts. Neurology and Anatomy, Univ. Rochester School of Medicine, Rochester, N.Y. 14642

The organ cultured hypothalamo-neurohypophyseal system (HNS) has been used as an in vitro system for studying control of vasopressin (VP) release. Each of these organotypic explants includes the supraoptic nucleus, median eminence, and neural lobe. The HNS releases VP at a constant rate in vitro and releases VP in response to acetylcholine (ACH), angiotensin II (AII), and osmotic stimulation. VP release in response to ACH and osmotic stimulation is blocked by the addition of hexamethonium (10^{-5}M, Sladek and Joynt, Neurology 28:366, 1978), but not by atropine ($5x10^{-5}$M). Thus, a nicotinic cholinergic synapse appears to mediate osmotic stimulation of VP release. Furthermore, saralasin, an AII antagonist, blocks osmotically stimulated VP release by the HNS (Sladek and Joynt, Neurosci. Absts. 3:357, 1977). These data suggest that AII also modulates osmotic control of VP release. The relationship between AII and cholinergic modulation of VP release was examined by assessing the effect of hexamethonium on AII stimulation of VP release, and the effect of saralasin on cholinergic stimulation of VP release. Hexamethonium (10^{-5}, 10^{-4}, 10^{-3}M) was ineffective in blocking VP release by AII (10^{-5}M). Saralasin (10^{-4}M) did not block stimulation of VP release by either ACH (10^{-5}M) or nicotine (10^{-5}M). These findings suggest independent AII and cholinergic mechanisms controlling VP release, however the effectiveness of both saralasin and hexamethonium in blocking osmotically stimulated VP release indicates some interaction between these regulators of VP release. A hypothetical model which would fit these data consists of a VP producing cell possessing nicotinic-cholinergic receptors and a separate osmoreceptive cell which communicates with the VP cell by way of a cholinergic synapse which does not impinge directly on the VP cell. Information from the osmoreceptor is modulated by AII possibly by modulating release of or acting as a neurotransmitter from an interneuron.

(Supported by NIAMDD grant AM-19761 and Research Career Development Award NS-00259.)

1140 A ROLE OF THE SUPRACHIASMATIC PART OF THE MEDIAL PREOPTIC NUCLEUS ON THE AFTERNOON OF PROESTRUS IN FEMALE RATS. Ei Terasawa and Stanley J. Wiegand. Wis. Primate Res. Center, Madison, WI 53706.

Previously we have reported (Neuroscience Abstract 3: 1161, 1977) that the medial preoptic nucleus (MPN), a small periventricular nucleus caudal to the organum vasculosum of the lamina terminalis, is necessary for estrous cyclicity in rats. In the present experiment, function of the MPN, as well as adjacent structures, for the release of gonadotropins during the critical period on the day of proestrus was examined. In regular 4 or 5 day cyclic rats brain lesions and stimulations were performed on proestrous day, and ovulation was determined by direct observation of the ampulla on estrous morning. If the ampulla was dilated, it was dissected out and the number of ova counted under a microscope. If the ampulla was not dilated, the ovary was not removed and vaginal cyclicity was followed postoperatively.

Experiment I: Bilateral electrolytic lesions with a platinum electrode were made in the MPN, the periventricular part of the medial preoptic area (PMPO, just dorsal to the MPN), or the suprachiasmatic nucleus (SCN) under ether anesthesia on proestrous morning (900-1000). Lesions of either the MPN or the PMPO blocked ovulation (0 of 12, 0 of 11 rats ovulated, respectively), while lesions of the SCN failed to block (4 of 4 rats ovulated). However, in the animals with PMPO lesions, subsequent cyclic ovulation was indicated by resumption of vaginal cycles, while most of the animals with MPN lesions showed persistent vaginal estrus.

Experiment II: In order to overcome the ovulation blockade with MPN- or PMPO-lesions, electrochemical stimulation (ECS) was applied to the medial preoptic area (MPOA) under ether anesthesia on proestrous afternoon (1400-1500). Anodal DC of 75 μA to the MPOA through stainless steel electrodes for 60 sec consistently induced ovulation in the pentobarbital blocked proestrus rats (all 8 ovulated). ECS to the MPOA with the same current resulted in ovulation in only 1 of 10 MPN-lesioned rats. With higher current (200 μA for 30 sec), ECS of the MPOA induced ovulation in 3 of 11 MPN-lesioned animals and 6 of 7 PMPO-lesioned animals. Sham ECS in the MPN- or PMPO-lesioned animals were without effects.

It is concluded that 1) the major portion of the neurons necessary for the release of LH-RH on the afternoon of proestrus originate in the vicinity of the MPN, but not the SCN; 2) most, but not all, of the neurons in the MPOA responding to ECS may exert their influence indirectly via neural cells located in the MPN; and 3) neural fibers originating in the MPN may be distributed diffusely through the dorsal periventricular region as they pass caudally to the medial basal hypothalamus. (Supported by NIH grants RR00167 and 1 R01 HD11355-01.)

1141 BINDING OF CORTICOSTERONE TO SYNAPTIC PLASMA MEMBRANE FROM RAT BRAIN. A.C. Towle* and P.Y. Sze (SPON: B.E. Ginsburg). Dept. of Biobehavioral Sci., Univ. of Connecticut, Storrs, Ct. 06268

To investigate the possibility that glucocorticoids may act directly on nerve terminals in the brain, the binding of corticosterone to synaptic plasma membrane (SPM) was examined. Male rats were adrenalectomized for 3 days and perfused with saline prior to brain dissection. SPM and other subcellular fractions were prepared from the hippocampus, hypothalamus and cerebral cortex by a centrifugation procedure using discontinuous sucrose density gradients. After equilibration with 3H-corticosterone at 4° C, SPM was applied to DEAE-cellulose filters and washed free of unbound 3H-steroid. The binding of 3H-corticosterone to cell cytosol (105,000 g supernatant after 17,000 g) and to soluble content of synaptosomes (soluble fraction after osmotic shock) was determined by Sephadex G-25 gel filtration. In all cases, specific binding was distinguished from non-specific binding by competing the 3H-corticosterone with 1,000-fold unlabeled steroid.

From ligand bound/mg protein vs. log [ligand concentration] plots, specific binding of 3H-corticosterone to SPM obtained from all 3 brain regions shows a similar sigmoidal curve, reaching saturation of dissociable binding sites at 3×10^{-7}M steroid. Compared with 3H-corticosterone binding to cell cytosol (saturation at $2\text{-}6x10^{-8}$M), the affinity of the steroid binding sites in SPM appears to be lower than those in cytosol. From the 3 brain regions, SPM isolated from hypothalamus shows the highest binding capacity. This is in contrast to cytosol binding which is highest in hippocampus. Thus, SPM binding may represent a different case from the soluble corticoid binding protein(s) known in brain cytosol. The nature of this SPM binding with regard to kinetic characteristics, differential affinity for various glucocorticoids, and the physical properties of the binding sites will be discussed. It should be noted that synaptosomal soluble content shows only minimally detectable binding capacity, indicating that in brain neurons the cytosol binding protein(s) may be confined to the perikarya.

Specific binding of glucocorticoids to cell plasma membrane has been described in liver by other investigators. The demonstration here of glucocorticoid binding to brain SPM suggests that the steroid hormone may act on nerve terminals by possibly regulating membrane properties.

(Supported by MH-29237)

1142 REGIONAL PATTERNS OF ENDOGENOUS CORTICOSTERONE BINDING IN RAT BRAIN: BASAL VS. STRESSED STATES. Barbara B. Turner, Elaine Smith* and Bernard J. Carroll*. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

We wished to determine the physiological distribution of corticosterone binding (cytosol) in brains of resting, unstressed rats. The regional pattern of binding in basal animals could then be compared with that following acute stress. The observation of area differences would suggest specific anatomical sites as having significant roles in the stress response. The following parameters were measured in each of 8 regional cytosols: total corticosterone, bound steroid, and the number of unoccupied binding sites. Eighty adult male rats were killed between 0900-1000h. "Basal" animals were perfused within 3 min. of cage approach; "stressed" rats were subjected to 30 min prior shaker stress. Tissue from 4-5 rats was pooled for each region and homogenized (20 ml/g) in Tris-EDTA buffer, pH 7.4. Free steroid was removed by dextran-charcoal. All samples were extracted into CH_2Cl_2, chromatographed on LH-20, and corticosterone levels measured by radioimmunoassay. Unoccupied sites were estimated by the addition of 3H-corticosterone (3.5×10^{-8}M) to aliquots of the homogenate.

In the unstressed rat the highest levels of cytosol binding were observed in the preoptic and septal regions (POS), followed by the pituitary and hypothalamus. In general, only 50% of cytosol binding sites were occupied in the basal animal except for the POS which had 80% occupation. Total sites were also higher in the POS than in other brain regions with the exception of the pituitary. Stress produced an increase in endogenous binding only in the pituitary. Four regions including the HC showed virtually no change; however, a substantial decrease (40-50%) in binding was observed in the POS. All brain regions, but not the pituitary, showed a decrease in total cytosol sites following stress. An increase in the percentage occupancy of the remaining sites was observed in all regions with the exception of the POS.

These results suggest unique roles not only for the pituitary but also for the preoptic and septal regions in the mediation of the stress response. (Supported in part by NIMH training grant 07417 to BBT.)

1143 A STIMULATORY ROLE OF THE SEROTONERGIC DORSAL RAPHE-HYPOTHALAMIC PROJECTION IN LH SECRETION. L. van de Kar*, S.A. Lorens+, A. Vodraska*, G. Allers* and L.S. Van Orden III. Depts. Pharmacol. and Psychiatry, U. of Iowa, Iowa City, IA 52242 and +Loyola Univ., Stritch Sch. Med., Maywood, IL 60153.

Various studies have indicated that 5-hydroxytryptamine (5HT) is regulating both luteinizing hormone (LH) secretion as well as sexual behavior. The site of 5HT action was assumed to be in the hypothalamus but it is not known which of the hypothalamic nuclei are involved in these regulatory systems. Studies using autoradiographic as well as lesion techniques have shown that both the midbrain dorsal (DR) and the median (MR) raphe nuclei project to the hypothalamus. Since it was demonstrated that the MR and DR have differential projections to telencephalic regions, projections of the MR and DR to hypothalamic nuclei were investigated.

Descrete MR and DR lesions were produced electrolytically in adult male rats. The rats were decapitated 17-20 days after surgery and 5HT content in various hypothalamic nuclei was determined by an enzymatic radioisotopic method (Saavedra et al., JPET, 1973). MR lesions produced a significant decrease in 5HT content of the medial preoptic area (49%), suprachiasmatic (70%) and anterior hypothalamic (60%) nuclei, the anterolateral hypothalamus (46%), arcuate nucleus (58%) and the hippocampus (61%). In contrast DR lesions produced a significant decrease in 5HT content only in the anterolateral hypothalamus (45%), arcuate nucleus (48%) and caudate-putamen (66%). In a second experiment 5,7-dihydroxytryptamine (5,7-DHT 5 µg in 1 µl) was injected into either the MR or DR 45-60 min following desipramine administration (10 mg/kg i.p.). The rats were decapitated 20-27 days after surgery and serum LH was measured by a double antibody radioimmunoassay, using a kit provided by NIAMDD. DR lesions produced a 37% decrease in serum LH levels and a 46% reduction in the 5HT content of the caudate-putamen. No change was observed in the 5HT content of the hippocampus. In contrast MR lesions did not affect serum LH levels or the 5HT content of the caudate-putamen but produced a decrease in the 5HT content of the hippocampus (52%). These results suggest that, although the MR projects both to the medial preoptic area and arcuate nucleus, these projections are not involved in LH secretion. In contrast, the projection from the DR to the arcuate nucleus may have a stimulatory influence on LH secretion.

1144 A GOLGI AND EM STUDY OF THE RAT HYPOTHALAMIC MAGNOCELLULAR PARAVENTRICULAR NUCLEUS. Anthony N. van den Pol. Sect. Neurosurgery, Yale Univ. Sch. Med., New Haven, Conn. 06510.

Neurons of the paraventricular nucleus, pars magnocellularis, (PVN) synthesize and secrete oxytocin and antidiuretic hormone. Although numerous reports (e.g., Szentágothai et al., Hypoth. Cont. Ant. Pit., Akadémiai Kiadó, '72) have suggested that the neurosecretory cells of the PVN are relatively resistant to impregnation by Golgi methods, by systematically varying block size, animal age, chemical concentration, and times in solutions in 207 rats, a Golgi method was found which yielded excellent results with magnocellular PVN neurons of 29 rats. To verify the identity of Golgi impregnated PVN neurons, soma size, 1 µ plastic sections counterstained with osmium/toluidine blue, amount of vasculature, and local topography were examined. Cells containing silver chromate were compared with horseradish peroxidase filled neurons and with ultrathin sections investigated with EM.

Two or three primary dendrites issue from PVN somata. Some dendrites have no branches; others bifurcate once or twice. Spine-like protrusions, 2 µ long, either straight or with a bulbous ending, emerge from dendrites and less frequently from perikarya. While Golgi impregnated dendrites of identified PVN cells sometimes have smooth contours, dendrites were also seen with very irregular outer membranes, with dilations interspersed with narrow constrictions; since these were also visible with EM, it is unlikely these are Golgi artifact. Although exceptions were noted, dendrites tend to stay within PVN borders, commonly entering into the medial and ventromedial parvicellular region. Abundant Golgi apparatus was found not only in somata, but also in large proximal dendrites. Soma-soma appositions exceeding 15 μ^2 are common; sometimes, a single thin glial projection separates neighboring cell bodies. Of 54 cells in which axon origin was determined, the axons of 21 cells came off proximal dendrites; the remaining cells had axons leaving perikarya. The majority of axons leave PVN in a lateral or ventrolateral direction; rarely an axon was seen which left PVN, but after proceeding 50 to 80 µ turned around and headed towards PVN. Although magnocellular axons establish contact in passing PVN dendrites or somata, whether these are functional or chance contacts is unknown. No unambiguous collaterals were found arising from PVN axons within the nucleus. Profiles containing large dense core vesicles are situated near blood vessels; whether these represent areas of local release of nonapeptides within PVN or axons passing by has not yet been determined. Quantitative analysis of 1 µ Nissl sections revealed that 3 times the area is taken up by vasculature in PVN as compared to the hypothalamus immediately lateral to PVN.

Supported by USPHS Grant, NS10174.

1145 SEX DIFFERENCES IN THE RESPONSE OF HIPPOCAMPAL CA1 PYRAMIDS TO GONADAL STEROIDS: EFFECTS OF TESTOSTERONE AND ESTRADIOL ON THE IN VITRO SLICE PREPARATION. Richard M. Vardaris and Timothy J. Teyler. Kent State University, Kent, Ohio 44242 and Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44266.

Monosynaptic extracellular EPSPs and population spikes were recorded from hippocampal CA1 pyramidal cells following stimulation of the Schaffer collaterals in explants from rat hippocampus. The major criteria used to establish appropriate physiological status of the preparations were: (a) EPSP and spike thresholds equal to or less than 3 or 5 volts respectively; (b) waveforms similar to those observed in intact preparations; (c) presence of paired-pulse facilitation.

Testosterone (T) and 17-beta-estradiol (E_2) were added to the Ringer's solution bathing hippocampal slices from normal adult male and female rats. The steroids were administered at a 100pM concentration, in the sequences E_2-T-E_2 or T-E_2-T. Slices were obtained from estrous and diestrous females. Electrophysiological measures were taken pre-drug, and at 10 and 20 min intervals post-drug using a conditioning-test stimulation procedure.

In males, E_2 enhanced the amplitudes of CA1 field potentials, whereas T depressed these responses. The same pattern of effects was seen with estrous females. In diestrous females, however, the pattern was reversed, with E_2 depressing responses and T enhancing responses. There were indications that the two steroids were interacting with different receptors, as has been reported for other tissues.

1146 EFFECTS OF AMYGDALA LESIONS OR OLFACTORY BULBECTOMY ON LUTEINIZING HORMONE (LH) RESPONSE TO OVARIAN STEROIDS IN RATS. J. Vitale and R. A. Gorski. Depts.Psychol. and Anat., UCLA, Los Angeles,CA 90024

Amygdala lesions and olfactory bulbectomy have divergent effects on behavioral indices of estradiol benzoate (EB) sensitivity.Since neural mechanisms subserving behavioral and hormonal responses to steroids may differ, this study was conducted to determine the effects of these lesions on plasma LH levels in response to EB and progesterone (P). 63 rats were divided into 3 groups: those receiving sham operations (SHAM), bilateral bulbectomy by a cut & suction technique (BULBX),or small bilateral lesions of the corticomedial amygdala using a platinum electrode (AMYG). Vaginal cycles were unaffected. 1 to 2 mos. post-lesion rats were ovariectomized. Tests for LH response began 3 weeks later. Each rat received in ascending order each dose of EB: 7,35, & 70 ug/kg, at 4 week intervals. Plasma samples were drawn via jugular puncture at 1700-1745 hrs (lights on 0500-1900) at these times: 44 hrs prior to EB (Pre-EB), and 5,29,53, & 77 hrs after each dose of EB. Half of each group was always injected with 2mg P 5hrs before the 77hr sample and half received oil. Plasma LH levels were assayed by RIA and evaluated with ANOVA and Duncan's Range Tests. Comparisons presented were significant at $p < 0.05$ or less. All 3 doses of EB suppressed LH relative to Pre-EB levels to an equivalent degree (26-52%) at 5hrs post-EB, redardless of lesion. At 29 hrs, higher doses of EB were more effective than 7ug/kg in suppressing LH (58-61% vs 22-34%,respectively). At 53 hrs, LH levels following 7 or 35ug/kg were not different from Pre-EB levels. However, after 70ug/kg, LH values of both SHAM and BULBX were elevated: SHAM, 123% of Pre-EB, at 695ng/ml; BULBX, 144% of Pre-EB, at 806ng/ml. Values for AMYG were 95% of Pre-EB, at 515 ng/ml, significantly different from SHAM and BULBX. P after 35 or 70ug/ml was equally effective in elevating LH above Pre-EB and oil-treated control values at 77 hrs, regardless of lesion group.

Thus, BULBX do not deviate from the SHAM pattern of LH responses to EB, although in behavioral studies they show increased sensitivity to EB as measured by the lordosis quotient. AMYG, typically normal in behavioral responsiveness, do not show facilitated LH response to 70 ug/kg before P. While all doses of EB exerted negative feedback on LH, the higher doses were more effective, and were necessary to prime the system for a significant LH response to P. Only the 70ug/kg dose increased LH above Pre-EB values directly. These results suggest that the effects of EB on LH depression, priming, and facilitation are differentially dose-dependent and that AMYG lesions differentially affect these functions. (Supported by HD-01182.)

1147 EVIDENCE FOR A HYPOTHALAMIC SITE OF ACTION OF VASOACTIVE INTESTINAL PEPTIDE (VIP) TO MODULATE PITUITARY HORMONE RELEASE IN CONSCIOUS FEMALE RATS. E. Vijayan*, W. K. Samson*, S. I. Said* and S. M. McCann, Depts. of Physiology, Internal Medicine and Pharmacology, University of Texas Health Science Center at Dallas and V.A. Hospital, Dallas, Texas 75235.

Vasoactive intestinal peptide (VIP) has been recently shown to be present in the hypothalamus and other areas of rat brain. To evaluate the possible role of VIP in influencing pituitary hormone release, ovariectomized (OVX) conscious rats bearing chronically implanted 3rd ventricular cannula were injected with 2 µl saline containing varying doses of VIP and plasma LH, Prl, GH, TSH and FSH levels were measured by RIA in jugular blood samples drawn through an indwelling silastic cannula. Control injections of saline iv or into the 3rd ventricle did not modify plasma hormone levels. Third ventricular injection of 4, 40 and 100 ng VIP produced a significant elevation within 5 min in plasma LH, while Prl levels were elevated by 40 and 100 ng doses; however, the highest dose of 500 ng had no effect on plasma LH or Prl levels. Plasma GH titers increased significantly after 3rd ventricular injection of each dose of VIP at 15 min and remained elevated for the duration of the experiment. Intravenous injection of VIP at doses of 40 and 1000 ng had no effect on plasma LH, but Prl levels were significantly elevated by the 1000 ng dose. Plasma GH was not modified by iv injection of 40 ng, while the 1000 ng dose induced a significant reduction. No significant changes in FSH or TSH levels were induced by 3rd ventricular or iv injection of VIP. In vitro incubation of hemipituitaries in 2 ml of TC medium 199 (Difco Labs.) at 37°C under 95% O_2 and 5% CO_2 with doses of VIP ranging from 10 ng to 10 µg had no effect on pituitary hormone release into the medium. Third ventricular injection of 100 ng VIP had no effect on mean arterial blood pressure, while iv injection of 1000 ng significantly lowered blood pressure within 5-10 sec of injection. The results indicate that 3rd ventricular injection of VIP in unanesthetized OVX rats can alter pituitary hormone release presumably by a hypothalamic site of action and are consistent with the concept that the peptide may act as a transmitter or modulator of neuronal activity controlling pituitary hormone release. (Supported by Grants from NIH #AM10073, #HD09988 and from the Ford Foundation to SMM and #HL14187 and #CA21570 to SIS)

1148 CENTRAL EFFECTS OF ENDORPHIN ON THE HYPOTHALAMUS AND ANTERIOR PITUITARY OF THE RAT: IN VITRO STUDIES IN HYPOTHALAMIC ORGAN CULTURE AND DISPERSED ANTERIOR PITUITARY CELLS. K. Yamauchi*, C.S. Hollander*, Y. Hirooka*, S. Richardson*, P. Ferdinand*, and J. Prasad* (SPON: J. Ransohoff). Endocrine Division, Dept. of Med. NYU School of Medicine, N.Y., N.Y.

In the course of in vitro studies undertaken to elucidate the in vivo endocrine effects of βendorphin we examined its actions (a) on thyrotropin releasing factor (TRF) and somatostatin (SRIF) release in organ culture of a hypothalamic fragment essentially encompassing the medial basal hypothalamus and (b) upon basal release of thyrotropin (TSH) in primary culture of dispersed anterior pituitary cells. TRF, SRIF, and TSH were measured by radioimmunoassays which were specifically validated for the in vitro systems we employed. In hypothalamic organ culture, βendorphin at 10^{-7}M, 10^{-6}M and 10^{-5}M increased TRF release in a dose responsive fashion to 200 ± 12.4% ($p<0.001$), 247 ± 32% ($p<0.001$) and 430 ± 79% ($p<0.005$) of control. The effects of βendorphin at 10^{-6}M and 10^{-5}M were significantly inhibited by naloxone at 5 x 10^{-7}M. SRIF demonstrated a complex biphasic response to βendorphin with significant release at 10^{-9}M but inhibition at 10^{-8}M. Surprisingly we also noted a small but highly significant and consistent increment in basal TSH release with 10^{-10}M to 10^{-6}M βendorphin from 305 ± 6ng/dish (control) to 467 ± 29, 482 ± 30, 428 ± 28, 496 ± 47 and 568 ± 28 ng/dish. All of these observations were significant at the 0.001 level. In conclusion βendorphin has complex actions with discrete effects on the release of TRF and SRIF by the hypothalamus and upon the release of TSH by the anterior pituitary. The physiological relevance of these observations to the in vivo situation remains to be established. However the complexity and biphasic nature of our findings suggest that a definitive assessment of the in vivo effects of βendorphin on TRF, SRIF and TSH,and probably the other hypothalamic and pituitary hormones as well,will require multiple acute and chronic measurements in conjunction with a careful dose response curve in a variety of pathophysiological circumstances.

NEUROETHOLOGY

1149 AERIAL MANEUVERING IN ORTHOPTERAN JUMPING AND FLIGHT: SENSORY BASIS OF HINDLIMB MOVEMENTS. Edmund A. Arbas, Dept. of Biology, Univ. of Oregon, Eugene, OR 97403.

Flying locusts employ several mechanisms for stabilizing flight and initiating turns, including: 1) changes in timing of motor output to bilateral sets of wing muscles; 2) curling of the abdomen in a rudder-like fashion; 3) lateral excursions of meso- and metathoracic limbs. These are but a few elements of a system of complex exteroreceptive reflexes which serve to assure smooth, coordinated, oriented flight.

A similar set of reflexes exists in certain flightless grasshoppers. Barytettix sp., a brachypterous, subtropical grasshopper, possesses no hindwings, and only vestiges of forewings. Tethered individuals respond to initiation of a windstream over their bodies by throwing their legs up into a stereotypic flight-like posture, and under simulated yaw conditions, perform asymmetrical orientation movements of their hind limbs. Barytettix never fly, but they are powerful jumpers, able to traverse up to about 40 body lengths (1.2 M) in a single leap. These movements may be used to stabilize the aerial phase of the jump.

Sensory ablation experiments were performed on the powerful flyer, Schistocerca gregaria, and on the flightless Barytettix, to examine the roles of particular sensory structures, including cephalic mechanosensory hairplates, antennae and cerci, in generation of the hindlimb movements. Those animals whose receptors had been removed or reversibly occluded by painting, were tethered in front of a moveable wind tunnel and subjected to simulated yaw conditions by changing the angle of airflow over their bodies. Presence or absence of limb movement was monitored visually for some experiments, while in others, a continuous record of limb position was obtained using a movement transducer (Sandeman, 1968 Comp. Biochem. Phys., 119: 267-283).

Symmetrical flight posture and aerial posturing of the limbs in the jump is initiated by a generalized "wind sense" mediated by a distributed system of receptors including hairplates, antennae and the cerci, as well as general body hairs, and perhaps the tympani. Asymmetrical maneuvers, on the other hand are specifically mediated by the hairplates and the antennae.

Both sets of reflexes are strictly coupled to the condition of being airborne, and are inhibited by tarsal contact with any firm substrate.

Supported by P.H.S. 5-T-32 GM07257.

1150 THE JAMMING AVOIDANCE RESPONSE IN AN ELECTRIC FISH PULSE SPECIES: BEHAVIOR AND SENSORY PHYSIOLOGY. Curtis L. Baker, Walter F. Heiligenberg and Joseph Bastian. Dept. Neurosci.,UCSD,La Jolla, CA 92093.

Weakly electric fish produce an electric field in their near vicinity; objects in the water cause distortions of this field which are monitored by electroreceptors on the body surface. This electrolocation ability is jammed by signals, normally from another fish, which are nearly coincident with the animal's own electric organ discharges (EODs). To prevent such jamming,gymnotoid electric fish with pulsatile EODs shorten their EOD intervals in response to pulses that scan their EOD from negative to positive latencies; they lengthen intervals, or only slightly shorten them, in response to pulses that scan from pos. to neg. latencies. Thus this Jamming Avoidance Response (JAR) minimizes the chances of long series of pulse coincidences, which are most detrimental to electrolocation. The JAR in pulse species strongly resembles the JAR in wave species (Bullock et al,J.Comp.Physiol.77:1,1972).

Based on behavioral data, a theory is proposed to account for the JAR in pulse species. Pulse stimuli at negative latencies with respect to the EOD trigger an excitatory process, which accelerates the EOD, while stimuli at small positive latencies activate an inhibitory process. The process which is triggered first dominates the over-all response.

Studies in curarized preparations demonstrate that the JAR is controlled by electroreceptive input alone, without reference to an internal electric organ pacemaker-related signal. A sufficient stimulus input consists of a train of strong, EOD-like stimulus pulses (S_1), which mimic the animal's experience of its own EOD, and a train of small pulses (S_2) of a slightly different repetition rate, which mimic EODs of a neighbor. Correct behavioral responses require S_1 repetition rates comparable to normal EOD frequencies; also spatial and temporal patterns of the S_1 must closely resemble those of the animal's EOD. These features are of little significance for S_2 pulses which, while scanning S_1 pulses, only provide a small perturbation of electroreceptive feedback from S_1 pulses.

S_1 pulses must also be of sufficient intensity to recruit electroreceptors, of which there are two types. Pulse markers fire one spike at a constant latency after the S_1, and do not respond to scanning by S_2s. Burst duration coders fire a burst of spikes following each S_1; the burst pattern changes during S_2 scans.

The electric fish JAR provides an ideal preparation for the study of neural correlates of behavior in a vertebrate, since it is possible to monitor the on-going behavior while simultaneously recording from single units in the nervous system.

1151 ROLE OF THE ANTERIOR HYPOTHALAMUS-PREOPTIC AREA IN THE REGULATION OF MALE REPRODUCTIVE BEHAVIOR IN THE LIZARD, Anolis carolinensis. David Crews, James Wheeler*, and Abraham Morgentaler*. Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138.

Bilateral radiofrequency lesions ($\bar{x}$ current=6.5mA; $\bar{x}$ duration 75 sec using glass insulated platinum-iridium microelectrodes with exposed tips of 100 or 250μm courtesy of F. Haer Co.) of the anterior hypothalamus-preoptic area (AH-POA) abolish male courtship and agonistic behaviors in both intact and castrated/androgen treated Anolis carolinensis. Lesions caudal and dorsal to the AH-POA also cause no significant decline in the behavior of intact animals. Histological examination of testes showed that lesions including and rostral to the AH-POA cause testicular collapse and regression to initial stages of spermatogenesis, indicating interruption of gonadotropin secreiton. Castration and subcutaneous testosterone implants restores courtship and agonistic behaviors in rostral and caudal lesion groups to prelesion levels, while AH-POA lesioned animals remain at their low behavioral levels. Neither AH-POA or control lesions cause significant changes in animals' body weight.

Free testosterone (T) or testosterone propionate (TP) implants (ejected pellet technique using 33g hypodermic tubing) placed unilaterally in the AH-POA restores sexual behavior in castrated, behavioral inactive lizards. Cholesterol implants had no effect on behavior nor did T implants located dorsal and lateral to the AH-POA. The mean response latency differed for the two hormones used: T averaged 2.6 days (range 1-4 days) while TP imlants averaged 5.4 days (range 3-8 days). Examination of secondary sex structures (renal sex segments) gave no indication of steroid leakage into peripheral plasma.

Supported by NSF BNS 75-13796 and NIMH Research Scientist Development Award 1 K02 MH00135.

1152 THE MAUTHNER SYSTEM MEDIATES DIRECTIONAL RESPONSES TO LOCAL VIBRATIONAL STIMULI. Robert C. Eaton, Dept. E.P.O. Biology, Univ. Colorado, Boulder, CO, 80309, Charles B. Kimmel & Stanley K. Sessions, Dept. Biology, Univ. Oregon, Eugene, OR, 97403.

Electrophysiological experiments show that the Mauthner (M-) cells of zebrafish larvae initiate startle responses to local vibrational stimulation produced by low-frequency, axial excursions of a microcapillary positioned against the body (Eaton et al., 1977, J. Neurobiol. 8:151). In free swimming animals this stimulation results in startle behavior involving a contraction of the contralateral body musculature, such that the animal gives a directional avoidance response. Can the M-system initiate directional responses to local vibrational stimulation? Previous technology did not readily permit determination of which of the two M-cells fired in response to a stimulus. To solve this problem we analyzed larvae with radiation-induced M-cell deletions (Kimmel et al., 1978, Develop. Biol. 62:526) to determine the probability at which a single M-cell will fire in response to bilateral stimulation. In 10 larvae with only one M-cell, the probability of eliciting an M-spike by suprathreshold stimulation on the M-cell side was 0.58 in 86 attempts whereas there was a significantly lower probability (0.11) for firing to the non-M-cell side in 94 attempts. In 17 irradiated and non-irradiated control fish with two M-cells, M-spikes were elicited with a probability of 0.53 to 0.60 in 250 attempts when stimulating either side. Thus, the irradiation treatment does not affect the probability of obtaining an M-spike to ipsilateral stimulation and the results suggest that the M-system is capable of distinguishing the directionality such that the ipsilateral M-cell is normally activated to a local stimulus. What sensory systems mediate this directionality? To test whether the auditory system is necessary, we inactivated the auditory input by surgically removing the otoliths from 5 embryos, 30-50 hr after fertilization and found that absence of the otoliths made no difference in the threshold for activation of the M-system when these fish were compared with normal control fish of the same age (5-9 days). Although, as previously shown, the auditory system functions in normal larvae, the present results suggest that audition is not necessary for activation of the M-cell to such local stimuli. Other systems possibly important are the Rohon-Beard cell system and the lateral line. In contrast to control animals with otoliths, animals without otoliths failed to give a startle response to water-conducted vibrational stimulation, thus illustrating the importance of audition for some types of startle behavior.

Supported by NSF Grant BNS 77-08685 to CBK and an Intramural Grant to RCE from Univ. of Colorado.

1153 NEUROPHYSIOLOGICAL BASIS OF CONFIGURATIONAL PREY-SELECTION IN THE COMMON TOAD. Jörg-Peter Ewert, Neuro-ethology and Biocybernetic Laboratories, University of Kassel, FRG.

From quantitative dummy experiments with rectangular moving stripes we know that toads Bufo bufo prefer "worm-like" prey objects (stripe axis oriented parallel to the movement direction) and avoid "antiworm-like" objects (stripe axis oriented perpendicular to the movement direction). The ability of toads to distinguish worms from antiworms is invariant to (i) the stimulus movement direction in the x,y,z-coordinates, (ii) the direction of the stimulus background contrast, (iii) the velocity of motion (within visible ranges), (iv) the stimulus distance (within behaviorally relevant limits). Extracellular recordings from single axons of the three retinal ganglion cell classes demonstrate that the configurational area effects on prey-catching are not simply derived from the output of one of these neurons. Extracellular recordings from single visual "small-field" neurons of retinal central projection areas in the thalamic pretectal (TP) region and the optic tectum in response to configurational parameters of moving contrast stimuli indicate for possible neuronal "gestalt filters". The TP small-field neurons are sensitive to the whole stimulus area, mainly its expansion perpendicular to the direction of stimulus movement. Tectum neurons of type 1 are sensitive to the whole stimulus area but mainly to its expansion in the direction of movement. There is a statistically distinct second neuronal population, tectum type 2 neurons, with response characteristics to configurational parameters, which reflects to a good approximation the probability that the stimulus fits the category prey. Presumably, tectum 2 neurons receive excitatory inputs from tectum 1 neurons, and there is evidence that they receive inhibitory inputs from TP-neurons. It is thought that tectum 2 neurons are involved in a system which triggers the prey-catching orienting movement, once a particular level of neuronal activity has been reached. This is supported by the results from electrical point stimulation of the optic tectum in freely moving animals.

1154 CENTRAL GREY LESIONS DEPRESS ULTRASOUND PRODUCTION AND LORDOSIS BY FEMALE HAMSTERS. Owen R. Floody and Thomas L. O'Donohue. Bucknell Univ., Lewisburg, PA 17837.

Female hamsters produce 35kHz vocalizations (ultrasounds) as courtship behaviors in reproduction. Since the mesencephalic central grey (MCG) has been implicated in the control of both vocalization (e.g., Brown, Science, 149, 1002, 1965) and reproductive behaviors (e.g., Pfaff et al., Progr. Physiol. Psychol. 5, 253, 1973), we studied the effects of MCG lesions on hamster ultrasounds and lordosis.

Ultrasound rates by ovariectomized, hormone-primed, females were observed in tests with stimulus ultrasounds or males. The postoperative ultrasound rates of females receiving MCG lesions were compared with preop rates for the same females, and with call rates of sham controls. MCG lesions consistently caused decreases in rates of ultrasound production. These decrements were most pronounced in tests with stimulus males, and in females with maximal damage to the caudal MCG.

Lordosis responses were observed in tests with manual stimulation or stud males. The incidence of lordosis decreased significantly following MCG lesions. This change tended to be all-or-none in character, so that lesioned females showed either no lordosis or lordosis of normal latency and duration. However, this dichotomy in behavior did not correspond to clear differences in lesion placement.

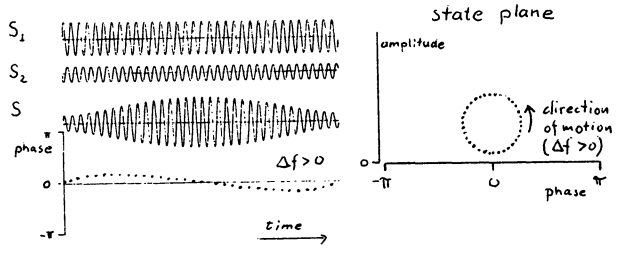
These findings support earlier descriptions of MCG mediation in vocal and sexual behaviors. In species for which these classes of behavior overlap, the MCG may function to coordinate different components of reproductive behavior with each other, or with other internal or external events.

(Supported by NIMH grant RO3MH29518).

1155 THE JAMMING AVOIDANCE RESPONSE (JAR) IN EIGENMANNIA: MOTION DETECTION IN A TWO-DIMENSIONAL STATE PLANE.
Walter Heiligenberg, Curtis L.Baker and Joanne Matsubara. Scripps Institution of Oceanography, UCSD, La Jolla, Cal. 92093

The JAR in the electric fish Eigenmannia is an attempt to move the electric organ pacemaker frequency away from similar frequencies of a jamming stimulus, normally the electric organ discharge (EOD) of a conspecific. The JAR requires simultaneous electroreception of the animal's own EOD and the jamming stimulus (Bullock et al. 1972, J.comp.Physiol. 77, 1-48). Correct JARs can be elicited if the EOD, silenced by curarization, is replaced by a sinewave stimulus, S_1, which sufficiently mimics the natural EOD field geometry. The EOD substitute, S_1, need not be locked to any particular phase of the pacemaker cycle and may freely run at frequencies tens of Hz different from that of the pacemaker. The JAR therefore appears to be driven by electroreceptive afferences alone, i.e. without an internal reference to the pacemaker cycle.

The addition of the EOD or its substitute, S_1, and a jamming sinewave stimulus, S_2, results in a sinusoidal signal, S, whose amplitude and phase with regard to that of S_1 are modulated at the beat frequency, Δf, the difference between the frequencies of S_2 and S_1. Whereas beat amplitude modulation is identical for positive and negative Δfs, phase modulations are opposites of one another. Amplitude and phase are coded by different types of electroreceptors, P- and T-units resp. (Scheich et al. 1973, J.Neurophysiol.36, 39-60), and simultaneous evaluation of their time courses of activity should yield the magnitude and the sign of the Δf. By plotting amplitude and phase as parameters in a two-dimensional state plane, closed graphs are obtained which are reproduced Δf times per second. The direction of motion of a point in this graph is determined by the sign of the Δf. Evidence is given that the animal detects motion in this state plane by a mechanism comparable to motion detection in the realm of vision.



1156 DOES THE MAUTHNER NEURON MEDIATE UNIQUE BEHAVIOR?
Charles B. Kimmel, Susan L. Powell*, Dept. Biology, Univ. Oregon, Eugene, OR 97403, & Robert C. Eaton, Dept. E.P.O. Biology, Univ. Colorado, Boulder, CO 80309.

On the basis of both electrophysiological and behavioral evidence we proposed that the Mauthner (M-) cells of larval zebra fish initiate fast start behavior in which the first movement is a short-latency (<10 msec) stereotyped contraction that causes the body to form the shape of a "C" (Eaton et al., J. Neurobiol. 8: 151, 1977). This initial phase is believed to represent the direct output of an M-cell and its motoneuron pool. We have studied larvae with deletions of the M-cells induced by irradiation at the gastrula stage (Kimmel et al., Develop. Biol. 62: 526, 1978) to learn whether presence of the M-cell is uniquely correlated with presence of this behavior.

High speed (400 f/sec) cine records of startle responses of 3 larvae missing both M-cells revealed starts to either side, with different combinations of latencies and strengths. The fastest starts were similar in these parameters, and in the detailed pattern of movement, to responses previously proposed as being M-initiated. To examine this critically we recorded 67 responses from 14 larvae (day 7-10), each possessing only 1 M-cell. Again, responses to either side were observed. There was a strong correlation, however, between the presence of short-latency C-type fast starts and presence of the M-cells.

A higher proportion of short-latency (<10 msec) responses were observed to the side possessing the M-axon than to the opposite side (18 of 37 vs. 4 of 30). All of the short-latency responses to the M-axon side were C-type fast starts (mean displacement: 0.61 body lengths in 10 msec), whereas those to the opposite side were heterogeneous and significantly less in mean strength (0.32 body lengths in 10 msec). Only 1 of these starts to the side missing the M-axon was as strong as the weakest short-latency response observed to the side where it was present.

We conclude that the presence of the M-cell greatly increases the probability that the short-latency C-type fast start will occur. On the other hand, other neural circuits are sometimes capable of producing apparently identical responses. It is not known whether these other circuits arise as a result of loss of the M-cells. However, even though such compensation may have happened, it was insufficient to match the performance advantage which occurred when the M-cell was present. (Supported by NSF, BNS 77-08685, and an Intramural Grant from Univ. Colorado)

1157 HOW WELL DO ELECTRIC FISH ELECTROLOCATE UNDER JAMMING? Joanne A. Matsubara* and Walter F. Heiligenberg (SPON: J. Bastian). Scripps Institution of Oceanography, UCSD, La Jolla, CA 92093.

Weakly electric fish can be classified on the basis of their electric organ discharges (EODs) into pulse and wave species. When certain wave species are submitted to electric stimuli, normally EODs of conspecifics, with frequencies sufficiently near their own EOD freq. they will shift their EOD freq. in order to maximize the freq. difference, ΔF. The significance of this jamming avoidance response (JAR) is one of maintaining a private freq. channel for unambiguous object detection. With the exception of Sternopygus, JARs have been observed in all South American wave species tested and even in the non-related African species Gymnarchus. The observation that Sternopygus does not exhibit a JAR suggests that it has an alternative mode of electric image processing which is less vulnerable to jamming. To test this hypothesis, comparative studies on the effects of jamming on the electrolocation performance (ELP) of three species were undertaken, providing the following results.

1) Sternopygus' lack of a JAR is adaptively correlated with an unusual immunity of its ELP to jamming signals. It is only with unnaturally strong sinusoidal stimuli, as great as 50 times stronger than the fish's own near field EOD intensity, that impairment of ELP begins in Sternopygus. In contrast, ELP in all other wave-emitting genera tested is greatly impaired by stimuli as weak as the animal's own near field EOD intensity. Hence, Sternopygus evidently possesses an alternative protection mechanism, other than a JAR, for electrolocation under natural jamming situations. It is suggested that a neuronal mechanism such as lateral inhibition between higher order representatives of neighboring electroreceptors could account for Sternopygus' exceptional immunity to electrical noise.

2) Under jamming regimes, Sternopygus (given stimuli of sufficient intensity), Adontosternarchus and Eigenmannia are most vulnerable to stimulus freqs. which differ slightly from their own EOD freqs.; i.e., when ΔF = 1 to 4 Hz. However, in all cases ELP improves for ΔFs sufficiently close to zero.

These behavioral data coincide with the prediction, based on theoretical considerations (Heiligenberg, 1977 Studies of Brain Function, Vol. 1, 1-85) and neurophysiological evidence (Behrend, 1977 J comp physiol 118, 357-371), that ELP improves as the ΔF either approaches zero or increases beyond critical values. As an alternative to the basic JAR, a fish could just as well phase lock its EODs to those of a conspecific (ΔF = 0) in order to protect its ELP.

1158 NEURONAL ACTIVITY AND AVOIDANCE BEHAVIOR IN GREEN LACEWINGS. Lee A. Miller and Jens Olesen*. Inst. of Biology, Odense Univ., Odense, Denmark, DK-5230.

Green lacewings are nocturnal insects that can evade bats primarily by folding the wings and passively falling to low cry repetition rates. Should a bat attempt to catch a falling green lacewing, the insect can momentarily extend the wings in a last chance maneuver. This switch in behavior from a passive fall to a momentary wing extension is provoked by an increase in cry repetition rate during an attempted catch. We mapped the dorsal longitudinal flight motor neurons (DL-MN) in the prothoracic ganglion with cobalt chloride applied to cut motor nerves. Up to 7 DL-MN's have been filled with positions similar to homologous neurons in locusts (M.Burrows,J.exp.Biol.62: 189,1975). We recorded intracellularly from neurons in the prothoracic ganglion using pulsed ultrasound as stimuli. Repeater neurons fire from 2 to 5 spikes for each 10 msec sound pulse. They have tuning and sensitivity characteristics similar to the sensory units. The activity of other neurons may be correlated with avoidance behavior. For example, DL-MN's, which were identified by simultaneous myographic recordings in the DL muscles, are often silent. Such DL-MN's can receive excitatory inputs and spike when stimulated. This activity may be correlated with wing extension during a fall. Some neurons have endogenous spike rhythms, which occur at rates slightly lower than the wing beat frequency and can be interrupted by a pulse train. The flight motor is quiescent during a fall (L.Miller,J.Insect Physiol.21:205, 1975). Other neurons show no activity until about 600 to 2000 msec after the pulse train ceases. Insects resume flight after a fall. We have not yet found neuronal correlates of switching as implied in the last chance behavioral response. Many epsp, ipsp and spike patterns show variability and are less obviously correlated with behavior. But, essentially all patterns of avoidance behavior in free flying green lacewings show some sort of variability, which may be neurally programed. Such variability could be of selective advantage by making the insect's behavior less predictable (K.D. Roeder,J.exp.Zool.194:75,1975).

These studies were initiated in the laboratory of Dr. Malcolm Burrows, Univ. of Cambridge. We acknowledge support by the Danish Research Council.

1159 FACTORS MODULATING THE OCCURENCE AND DIRECTION OF THE STARTLE RESPONSE IN GOLDFISH. Thomas J. Mueller. Dept. Biol. Sci., USC, Los Angeles, CA 90007.

The startle response was elicited in goldfish swimming freely in the center section of a large tank. The stimulus was an impact at a point on the surface of the water. The size of the tank was such that free field conditions existed for one to two milliseconds after stimulation. The resulting startle response was recorded using infrared serial repetitive photography.

Under these conditions the response was much more variable than previously reported. However, in a statistically significant number of trials the startle response, initiated within fifteen milliseconds or less of stimulation, resulted in the fish turning away from the sound source.

Circumstances prior to the moment of stimulation were found to affect the response. For example, fish given a flash of light one half second prior to stimulation habituated much more rapidly than control fish.

Work by others has shown that this startle response is initiated by the Mauthner cells in the hindbrain; presumably via the posterior branch of the VIIIth nerve which originates in the auditory portion of the labyrinth and synapses on the distal part of the Mauthner cell's lateral dendrite. It is generally accepted that with this apparatus, a loud noise will initiate a startle response. Based on the above data, I propose that the response is significantly modulated by a "set" imposed on the Mauthner cells by other sensory and CNS inputs prior to "release" of the response by auditory (or perhaps other) stimulation.

1160 SPECIES-TYPICAL BEHAVIOR OF HAMSTERS DEPRIVED FROM BIRTH OF NEOCORTEX. Michael R. Murphy, Paul D. MacLean, and Sue C. Hamilton*, Laboratory of Brain Evolution and Behavior, NIMH, Bethesda, MD 20014.

The purpose of this study is to test the hypothesis that the two older evolutionary formations of the forebrain--the striatal complex and the limbic system--are sufficient, along with the remaining neuraxis, for the expression of most forms of species-typical behavior.

Hamsters were used for this work because they have a short gestation period and display many natural forms of behavior in a laboratory setting. Experimental subjects were deprived of virtually all neocortex from the time of birth by thermal destruction of the developing layers on the first or second postnatal days. Supplemental observations were made on animals in which the neocortex was surgically removed by suction, or in which cortical neuroblasts were destroyed by prenatal administration of methylazoxymethanol acetate. Quantitative measures of mating and other behavior were made with the use of a computer-assisted event recorder and time-lapse television. Behavioral development was followed and recorded on a checklist derived from an extensive ethogram.

Hamsters deprived of neocortex grew and developed like normal animals. They showed the usual hamster-typical behaviors including nest and hoard building, tunnel blocking, use of a urination post, daily activity rhythm, play-fighting, territorial aggression, scent-marking, species preference and identification, and reproductive behavior. The experimental males had some motor difficulties during mating and required twice the normal number of intromissions to achieve ejaculation, but successfully impregnated females. A female with bilateral absence of neocortex mated, became pregnant and reared her young. Experimental animals displayed more stereotyped activity than the normal controls.

Some animals had varying amounts of damage to the midline limbic cortex. In those devoid of neocortex, there was corresponding reduction of the neothalamic nuclei.

The results of this study reveal that animals with intact striatal and limbic structures, but lacking neocortex, are capable of giving expression to a wide range of species-typical behavior.

1161 AFFERENT PROJECTIONS ASSOCIATED WITH ATTACK SITES IN THE VENTRAL TEGMENTAL AREA OF THE CAT. C. A. Opsahl* and W. M. Schoel* (SPON: J. P. Flynn). Dept. Psychiatry, Yale Med. Sch. New Haven, Ct. 06508.

Quiet biting attack on a deeply anesthetized rat was elicited by electrical stimulation of the ventral tegmental area in six cats. These sites were located in the region lateral and ventral to the red nucleus, from which quiet biting attack has previously been elicited (Chi, Bandler, and Flynn, Brain Behav. and Evol., 1976, 13, 91-110). The elicited behavior included pupillary dilation, approach from a distance, contralateral paw placing (in some cats), and a directed bite to the head and neck region of the rat. Hissing, paw striking, escape, back arching, and eating were never observed. After an attack site was located, the moveable, bipolar concentric electrode was cemented into place; and a number of trials were performed in order to obtain a complete description of the behavior and to ensure stability of the response. The cat was then anesthetized (Ketamine, 33 mg/Kg), and the inner shaft of the electrode was removed. A glass micropipette filled with a 30% solution of horseradish peroxidase (Sigma Type VI) was lowered to the attack site through the outer cannula of the electrode, and the HRP was iontophoresed into the tegmental site (4μA for 4 min). Between 24 and 30 hours later the cats were anesthetized and perfused with a solution containing 1% paraformaldehyde and 1% gluteraldehyde in 0.1M phosphate buffer (pH=7.4). The tissue was then processed according to a variant of the procedure first described by Graham and Karnovsky (J. Histochem. Cytochem., 1966, 14, 291-302).

Cells sending projections to the attack sites were frequently located in gyrus proreus, gyrus rectus, bed nucleus of stria terminalis, bed nucleus of anterior commissure, hypothalamus (anterior, lateral, perifornical, dorsal), zona incerta, amygdala (N. centralis, N. basalis), thalamus (N. reticularis, N. anteromedialis, N. centromedialis), substantia nigra, mesencephalic reticular formation, parabrachial nucleus, nucleus of the lateral lemniscus, superior colliculus, locus coeruleus, mesencephalic trigeminal nucleus, N. reticularis pontis oralis, and central grey. Additional sites of origin appeared with less regularity.

These results agree with previous findings of similar afferent projections associated with quiet attack sites in the lateral hypothalamus and pontine tegmentum (Smith and Flynn, submitted for publication). The projections to attack sites in the ventral tegmental area correspond in many cases with anatomical loci from which attack can be elicited or modulated. (Supported by NIMH grants MH-05507 and MH-08936).

1162 RHYTHMIC CUES FOR SONG RECOGNITION IN CRICKETS. Gerald S. Pollack and Ronald R. Hoy* Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853.

Female crickets respond to the calling song of a conspecific male by locomoting toward its source. Females offered a choice between conspecific and heterospecific songs locomote preferentially toward conspecific song. The present study centers on the role played by rhythmic song parameters in permitting female Teleogryllus oceanicus to recognize conspecific calling song.

Freely walking females were presented simultaneously with two electronically synthesized song models which differed only in the arrangement of sound pulses in time, played from two hidden speakers. The percentage of females that approached each speaker indicated the relative attractivenesses of the two songs.

T. oceanicus females preferred a model of T. oceanicus song to a model of the song of a related species, T. commodus. Thus, crickets can identify conspecific song using only rhythmic cues.

T. oceanicus song consists of sound pulses separated by three classes of intervals, arranged in a stereotyped sequence. A song in which the durations and relative proportions of these intervals were identical to T. oceanicus song, but in which the sequence of these intervals was random, was not distinguished from T. oceanicus song. This finding argues against a hypothesis for song recognition which asserts that females possess a copy of the central neuronal pattern generator used by males to produce calling song, and that females use this pattern generator as a template against which to compare auditory input.

Of the three intervals comprising T. oceanicus song only one is necessary and sufficient for song to be interpreted as conspecific. A song consisting only of this interval is not distinguished from T. oceanicus song, and is preferred to T. commodus song. A song containing only the other two intervals is treated as heterospecific, i.e., T. oceanicus song is preferred to such a song.

We have confirmed many of these findings using a different behavioral assay for song preference, in which tethered flying females were presented with two songs from speakers located to their right and left. In response to sound flying crickets perform rudder-like movements with their abdomens and hind legs in an attempt to turn. The direction of these movements indicates the relative attractivenesses of the two songs. This flight assay has so far yielded results similar to those obtained with walking crickets. Furthermore, the flight assay appears to be more sensitive and more reliable, and has the additional advantage of providing a simple, well-defined motor output which reflects song preference. This should facilitate physiological analyses of song recognition mechanisms.

1163 SEPTAL STIMULATION INHIBITS INTRASPECIFIC AGGRESSION IN HAMSTERS. Michael Potegal, Alan Blau*, and Murray Glusman. N.Y. State Psychiatric Institute, New York, NY 10032.

We have recently observed an apparently specific form of intraspecific aggression inhibition following septal stimulation in hamsters. Our subjects were 3-4 month old individually-housed male golden Syrian hamsters, selected for aggressiveness and implanted with a chronic, movable monopolar electrode. During testing sessions these subjects readily attacked non-aggressive target hamsters which were pretreated with the analgesic methotrimeprazine (2 mg/kg) and muzzled to prevent retaliatory biting. In between sessions the electrode was lowered in 300μ steps, one step at a time, along a 2-3 mm descending track. The subject's baseline attack latencies were reevaluated at every step of electrode descent and the effects of stimulating the septal region with 0.1 msec 100 pps biphasic pulses in the presence of a target were studied.

At current levels which did not produce any motor automatisms or stereotyped competing responses, septal stimulation prevented the initiation, and suppressed the ongoing performance, of subjects' attacks on the targets. At some sites in some animals we have also been able to inhibit mouse killing. At the completion of the track the electrode was raised to the site at which current levels for inhibition were at their minimum and a marking lesion was made. All the low threshold sites so marked lie within the septum. Stimulation at minimum current levels necessary for aggression inhibition (35-150μ amp) has had no effect on sunflower seed acceptance (a highly preferred food for hamsters) in any animals tested to date. In half the animals tested, this stimulation also has had no effect on copulatory behavior with a receptive female. Recordings from the stimulating electrode before and after stimulation have not yielded any indication of seizure activity. Preliminary tests of the reinforcing value of the stimulation in an operant chamber indicated that the stimulation may be moderately aversive. There is also some indication that this aversiveness may be minimized when aggression-suppression occurs at lower current levels. These results extend previous reports of septal inhibition of predatory (Siegel & Skog, Brain Res., 1970), and defensive (Brayley & Albert, J.C.P.P., 1977) aggression to include effects on intraspecific aggression. (Supported by N.I.M.H. Grant #R03 MH28836.)

1164 THE ROLE OF ENVIRONMENTAL CONCENTRATIONS OF MINERALS AND PITUITARY HORMONES IN REGULATING REPRODUCTION OF THE PARADISE FISH, MACROPODUS OPERCULARIS. Trudy Villars, Joseph Morris* and David Sever*. Saint Mary's College, Notre Dame, IND 46556.

Environmental cues such as temperature and photoperiod play a major role in teleost reproductive behavior. Changes in mineral content ('hardness') of the water following rainfall or drought might also be expected to affect reproductive readiness in some freshwater species. The present study examined 1) the effects of water 'hardness' on nestbuilding and spawning of the paradise fish and 2) the role of pituitary hormones in mediating such an effect.

The paradise fish is a fairly specialized species which has an elaborate reproductive sequence including a courtship of 1 to 4 days in which the male builds and defends a bubble-nest at the water surface. In the laboratory, pairs will spawn repeatedly and show no seasonal variation in reproductive readiness. Thus it is likely that environmental factors rather than internal rhythms determine spawning readiness in the natural environment.

Subjects were held and tested in 'hard', medium or 'soft' water. Local tap water with approximately 280 ppm calcium and magnesium was directly used in the 'hard' water condition and diluted with distilled water to 33 or 10 percent for the medium and 'soft' water conditions. Fish were paired and observed for their latency to nestbuild and spawn. A normal male introduced to a tank with a lone female will invariably build a nest by the second day after its introduction and three fourths of normal pairs will spawn by the fifth day (Villars and Davis, Phys. Beh. 19: 371, 1977). In the present experiment male nestbuilding was blocked 'hard' water and impaired in the intermediate concentration. Spawning was blocked in both 'hard' and medium conditions.

N	WATER CONDITION	PERCENT NESTING BY DAY 5	PERCENT SPAWNING BY DAY 5
10	'hard'	10%	0%
11	medium	90%	0%
15	'soft'	100%	66%

Preliminary histological analysis of the pituitary indicates a reduction of activity in the acidophilic cells of the pars distalis which are probably prolactin secreting. In other species such cells are inhibited by environmental salinity and play a role in osmoregulation (Schreibman et al. Am. Zool. 13: 719, 1973). In the male paradise fish prolactin is believed to stimulate nestbuilding behavior (Machemer, Z. Tierpsy. 28: 33, 1971). In the course of evolution pituitary hormones serving a role in osmoregulation could have acquired a secondary function in the induction of reproductive behavior in the paradise fish and related species.

NEUROMUSCULAR JUNCTION

1165 MODULATED RECEPTOR HYPOTHESIS FOR THE INCREASE IN DESENSITIZATION OF THE ACETYLCHOLINE RECEPTOR BY ANESTHETIC TYPE AGENTS. Roger Anwyl* and Toshio Narahashi. Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

The ability of a variety of agents to increase desensitization and to cause antagonism of extrajunctional acetylcholine (ACh) receptors of denervated rat muscle has been investigated. Desensitization was produced by evoking iontophoretic ACh potentials at a frequency of 10 Hz. Half-maximal increase in desensitization rate and half-maximal reduction in the amplitude of the ACh potential were caused respectively by: 1×10^{-7}M and 1×10^{-6}M histrionicotoxin; 1×10^{-6}M and 6×10^{-6}M chlorpromazine; 5×10^{-5}M cytochalasin B; 6×10^{-6}M and 6×10^{-5}M Triton X-100; 5×10^{-5}M and 5×10^{-5}M lidocaine and QX 314; 2×10^{-4}M and 1×10^{-4}M sodium pentobarbital; 8×10^{-4}M and 5×10^{-4}M heptanol; no effect and 5×10^{-4}M benzocaine; no effect and 5×10^{-4}M methylguanidine. Thus desensitizing agents are characterized by very hydrophobic groups and also by a charged atom (preferably nitrogen).

It is proposed that there are different binding sites on the ACh receptor or channel for the desensitizing and antagonistic action of these agents, although binding to both sites occurs only after the receptor has been activated by ACh. After binding of an agent to the desensitization site, the receptor channel closes at its normal rate but the receptor is converted to an inactive or desensitized state. Supported by NIH grant NS 14145.

1166 PRE- AND POSTJUNCTIONAL ACTIONS OF ERYTHROSIN B. George J. Augustine, Jr. and Herbert Levitan. Dept. of Zoology, University of Maryland, College Park, MD 20742.

The anionic dye Erythrosin B has both pre- and postsynaptic effects when applied to isolated frog neuromuscular junctions. Using standard electrophysiological techniques, we have attempted to quantify these effects in an effort to determine their cause. After exposure to Erythrosin B in concentrations ranging from 10 μM to 1 mM, the frequency of spontaneous miniature end-plate potentials (mepps) increases exponentially with time from control rates of less than 1/sec to greater than 100/sec. The period of elevated frequency is followed by an abrupt decline to a level below that of control. The time of onset of this decline is variable, but it occurs more rapidly with higher dye concentrations. 'Giant' mepps, with amplitudes as large as 25 mV, are observed in this period of depressed frequency. The rate of the dye-induced exponential increase in mepp frequency (α), determined from the slope of semi-logarithmic plots of mepp frequency vs. time, varies with the concentration of dye applied. Mean values of α range from .023 (± .004 S.E.M.)/min for 10 μM (corresponding to a 10-fold increase in mepp frequency every 43 minutes) to 0.44 (± .029)/min for 1 mM. These values are all significantly greater than the α of .004 (± .003)/min found for junctions which were not treated with dye. The mean α values are related to dye concentration by a power function, with an apparent K_m of approximately 0.5 mM determined from a double reciprocal plot. The increase in mepp frequency is apparently independent of external divalent cations, since it occurs in Ringer's free of Ca^{+2} and Mg^{+2}, with EDTA present (1 mM). Erythrosin B must therefore alter presynaptic function in some other manner. This dye also rapidly and reversibly increases the membrane potential and input resistance of muscle fibres. These postsynaptic effects are apparent at dye concentrations exceeding 0.2 mM and increase with concentration. An increase in the variation of muscle membrane potential with external K^+ concentration in the presence of the dye suggests an increase in K^+ permeability relative to other ions. This may be the result of both an increase in K^+ permeability and a decrease in membrane permeability to Cl^-. These postsynaptic alterations could account for the increase in mean mepp amplitude which accompanies dye-induced increases in mepp frequency.

1167 SUPPRESSION OF ORIGINAL NERVE INPUTS TO A MAMMALIAN SKELETAL MUSCLE BY A FOREIGN MOTOR NERVE. J. L. Bixby* and D. C. Van Essen* (SPON: J.-P. Revel). Division of Biology, California Institute of Technology, Pasadena CA 91125.

A foreign motor nerve placed over an extrasynaptic region of a mammalian skeletal muscle does not form synapses unless transmission from the original nerve is interrupted or the muscle is directly injured. We have found, however, that foreign synapses can be established in the presence of intact original innervation if care is taken to implant the foreign nerve directly over the original endplate region. A motor branch of the superficial peroneal nerve was placed over the soleus muscle near the site of original nerve entry in adult Sprague-Dawley rats. The foreign nerve remained in place and grew over the original endplate region in half (9 of 18) of the muscles examined 4-10 weeks after the operation. In most of these cases (7 of 9), stimulation of the foreign nerve elicited contractions in soleus muscle fibers directly under the nerve implant. The degree of cross-innervation ranged from only a few muscle fibers to several percent of the whole muscle, and was not obviously correlated with the time after the initial operation. Foreign and original nerve inputs co-existed on the same muscle fiber in many of the fibers examined at both short and long survival times. In substantial numbers of other fibers, however, the foreign nerve had completely suppressed transmission by the original nerve. Stimulation sometimes elicited sub-threshold responses via one or both nerves, suggesting that the suppression of original synapses and the establishment of foreign ones is a graded phenomenon. We believe, on the basis of cholinesterase staining and rise times of endplate potentials, that foreign synapses are established specifically at the original endplate sites in the great majority of cases. Control experiments suggest that the establishment of foreign synapses was not a result of temporary interruption or traumatization of original nerve inputs following the initial operation. These results indicate that an endplate in an adult muscle fiber can accept innervation from more than one source. At what we presume is a later stage, interactions can take place leading to the loss of one of these inputs, just as occurs during the maturation of neonatal muscles. The determination of which synapses are to be eliminated may be linked to a competitive advantage of terminals belonging to motor neurons having relatively few peripheral connections. Supported by NIH grant RR 07003

1168 NEWLY-FORMED NEUROMUSCULAR JUNCTIONS IN XENOPUS TISSUE CULTURE: FREEZE-FRACTURE AND THIN-SECTION ELECTRON MICROSCOPY. Paul C. Bridgman*, H. Benjamin Peng* and Yasuko Nakajima. Dept. of Biol. Sci., Purdue Univ., W. Lafayette, IN 47907.

Neuromuscular contacts were formed in vitro by adding dissociated neurotube cells to one-day-old myotome-cell cultures prepared from stage 20-21 Xenopus laevis embryos. Following one to three days of co-culture, preparations were processed either for freeze fracturing or for thin sectioning.

Previously we reported in our freeze-fracture work the existence of membrane particle clusters which are putative acetylcholine receptors (AChRs). In innervated cultures, these clusters were found in the muscle membrane along the nerve contact (PNAS 75: 500, 1978). In the present developmental study, most nerve-muscle contacts in one-day co-culture were marked by only sparsely populated small clusters. In two-day co-culture, however, very tight aggregations of particle clusters were observed along many nerve-muscle contacts. Most of these clusters were located within 1 μm from the nerve terminal. Each cluster was separated from its neighbor by a shallow groove free of membrane particles. During early stages of innervation, many caveolae (ranging from 50-100 nm in diameter) were observed in the muscle membrane along the path of the neurite. On the P-face replica, particles of the same size as that of putative AChR particles were often observed at the bottom of these caveolae. Thus, these caveolae might have a role in the incorporation of AChRs into the sarcolemma.

In thin sections, after one to two day co-culture, most muscle cells were well developed, showing regular arrays of myofibrils. At the junctional area nerve endings contained many synaptic vesicles, mitochondria and microtubules. The postjunctional membrane sometimes formed caveolae. Beneath the membrane there were occasional accumulations of dense material in small patches. The junctional cleft was narrow, about 10 ~ 20 nm wide. In several instances there were very close junctions between the pre- and post-junctional membranes. In addition, in one case, the presence of a small aggregate of gap junction-like particles was found in the post-junctional membrane in freeze-fractured material (1 1/2 day co-culture). We are now conducting a study in the same system, using α-bungarotoxin conjugated to horseradish peroxidase, to detect the localization of AChRs in thin-section electron microscopy.

(Supported by USPHS grants NS-10457, F32-NS05631, T32-GM07211).

1169 DISTRIBUTION OF ACETYLCHOLINE RECEPTORS IN THE MYOTOMES OF XENOPUS LAEVIS DURING NORMAL DEVELOPMENT. I. Chow* and M.W. Cohen. Dept. of Physiology, McGill University, Montreal, Canada.

Previous studies have indicated that innervation of the myotomes in Xenopus laevis begins at stage 21, when the embryo is 22-23 hr old, and that sensitivity to acetylcholine can be detected 1-2 hr earlier, at stage 19-20 (Blackshaw and Warner, Nature 262 217, 1976; Kullberg et al., Devel. Biol. 60 101, 1977). In the present study we have investigated how the number and distribution of acetylcholine receptors in the myotomes change during the course of development by using α-bungarotoxin, labelled with radioactive iodine or with fluorescent dye (tetramethylrhodamine). Specific uptake of radioactive toxin was demonstrated as early as stage 19. The number of toxin binding sites per myotome increased progressively with age throughout the period of development studied (up to 1 month). The distribution of these sites was examined in radioautographs of the same preparations embedded in Epon and cut longitudinally. At all stages of development grains were observed throughout the entire length of the myotomes. Away from the ends of the myotomes, the main site of innervation, the grains appeared to be randomly distributed and their density increased up to stage 36 (50 hr), when the embryo hatches and becomes a free-swimming tadpole. Subsequently the grain density along the myotomes declined but was still significant in 1-month-old animals. The density of grains at the ends of the myotomes was clearly greater than along the rest of their length at stage 24 (26 hr) and became more pronounced with age. Some examples of such a differential distribution were also seen at stage 22 but not at earlier stages. Fluorescent staining with rhodamine-toxin revealed the presence of discrete patches of high receptor density at the cell ends as early as stage 22. At this stage the patches were less than 1 μm in length and their occurrence was rare. Subsequently their size and number increased. Similar patches of stain were also observed randomly distributed along the myotomes but at much lower frequency. Experiments on 2-4 day old animals revealed that many of the fluorescent patches, at the cell ends as well as along the myotomes, are associated with cholinesterase activity, and therefore likely reflect sites of synaptic contact. Taken together these results indicate that the development of patches of high receptor density begins within about 1 hr after the onset of innervation. Such a temporal sequence is consistant with the notion that a local interaction between the axon and muscle cell induces the accumulation of acetylcholine receptors at developing synaptic sites in vivo. (Supported by MRC of Canada)

1170 PHARMACOLOGICAL EXPERIMENTS ON FROG MUSCLE USING AN ELECTROPHORETIC VOLTAGE CLAMP TECHNIQUE.* J. del Castillo and P. Specht. Lab. of Neurobiol. and Dept. of Pharmacol., Med. Sci. Campus, University of Puerto Rico, San Juan, P.R. 00936.

The conventional technique of electrophoretic drug application (Nastuk, Fed. Proc. 12: 102, 1953; del Castillo and Katz, J. physiol. 128: 157-181, 1955) has been employed successfully to map receptor distribution and to determine, both qualitatively and quantitatively, the chemical sensitivity of cell membranes. However, it has failed to generate significant information on the characteristics of drug-receptor reactions. Indeed, the electrophoretic "potentials" elicited by brief pulses of agonists can be made very similar in amplitude and time course by manipulating the position of the pipette and the braking and pulse currents. The actual drug concentration transients produced by those pulses are not known and therefore the different reaction kinetics cannot be analyzed.

To use the electrophoretic technique in the study of drug-receptor interactions at steady state, we have been experimenting with a system in which the agonist current is controlled, through a feed-back loop, by the membrane potential (del Castillo and Specht, Proc. Physiol. Soc., 1978). In this manner, the depolarization induced by the agonist can be fixed at any desired level for periods of up to several minutes while measuring the required drug current. The most obvious application of this technique is the measurement of the time course of desensitization, a phenomenon which is revealed as a steady increase in the drug current. For example, when acetylcholine is applied to maintain a depolarization of 5 mV, the current increases linearly with a slope of about 1 nA sec^{-1}. With some other drugs, such as tetramethylammonium and ethyltrimethylammonium, the current needed to maintain a steady depolarization decreases slowly. This technique has also been employed to study the interactions between agonists and antagonists.

*Supported by USPHS grants Nos. NS-14938, NS-07464 and RR01802. (Contribution No.84, Laboratory of Neurobiology).

1171 DELAY OF DENERVATION-DEGENERATIVE CHANGES IN RAT MOTOR NERVE TERMINALS FOLLOWING GLUCOCORTICOID TREATMENT. Anna B. Drakontides. Dept. of Anat., New York Medical College, Valhalla, NY 10595.

Glucocorticoids have been shown to directly affect mammalian motor nerve function and neuromuscular transmission. It has been demonstrated that these agents increase the excitability of mammalian motor nerves and the spontaneous release of transmitter. In view of these findings, studies were undertaken to determine whether these agents are effective in altering the time sequence of progressive degenerative changes or the characteristics of morphological alterations evident in motor nerve terminals following denervation. As these events have been well documented in the rat phrenic-nerve diaphragm, this preparation was chosen for study.

Triamcinolone 8mg/kg was injected intramuscularly in the thigh muscles of rats for 3-5 days. Either on the last day of drug treatment or 3-5 days following the last drug administration, under Nembutal anesthesia the left phrenic nerve was transected in the neck. At time intervals of 16, 18, 20 and 24 hrs after phrenicotomy the rats were sacrificed and segments of muscle containing intramuscular nerves were prepared for electron microscopy. In non-drug treated rats there is evidence of degenerative changes at the neuromuscular junction by 14-16 hrs after denervation. By contrast the majority of nerve terminals sampled from rats treated with triamcinolone 16 hrs after phrenicotomy appeared normal. In the non-treated rat 18-20 hrs after denervation there is unequivocal evidence of degeneration. Mitochondria are disrupted, synaptic vesicles are markedly reduced in numbers, lysosomal-like complexes are present, the nerve terminal becomes fragmented and Schwann cell processes invade the site of the terminal. In triamcinolone-treated rats, 18-20 hrs after phrenicotomy approximately 35% of terminals sampled were normal in appearance. The degenerative changes evident in other end-plates were primarily of the axonal terminal organelles; the terminal was not fragmented. Fragmentation of the nerve terminal was evident 24 hrs after denervation in triamcinolone-treated rats. At this time in non-treated rats, all end-plate sites are replaced by the Schwann cell.

Hall et al. (Ann. Neurol. 1; 263, 1977) have demonstrated in the 48 hr subacutely denervated cat soleus preparation that a short-term triamcinolone regimen significantly reduced the excitability loss that would normally appear in axons and nerve terminals. The present preliminary studies suggest that this glucocorticoid may delay the onset of degenerative changes associated with denervation. Supported in part by NIH Grant # RR5398.

1172 CELLULAR DISTRIBUTION OF 16S ACETYLCHOLINESTERASE IN MAMMALIAN TISSUES. Myron J. Duell*, Hugo L. Fernandez and Barry W. Festoff (SPON: P.A. Singer). Neurobiology Research Lab, VA Hospital, Kansas City, MO 64128 and Dept. of Neurology, Univ. of Kansas Med. Ctr.

Several molecular forms of acetylcholinesterase (AChE) distinguished by their sedimentation coefficients (16,10, and 4S) are present in endplate, but only two (10,4S) in non-endplate regions of skeletal muscle (Hall, J. Neurobiol. 4:343, 1973). The 16S form has been claimed to be endplate specific which decreases with denervation and may be produced by muscle cell cultures "induced" by neuronal elements. Several reports have indicated its absence from smooth muscle, brain, spinal cord and peripheral nerve (Vigny et al, J. Neurochem. 27:1347, 1976). Recently, however, small amounts of 16S AChE have been detected in peripheral nerve. Since cellular localization of 16S AChE may be important in understanding nerve-muscle trophic interactions, we re-evaluated the enzyme's cellular distribution and assessed its possible selectivity for a particular class of neural cell.

Tissue samples obtained from male Sprague-Dawley rats were processed for separation of AChE molecular forms (BW284C51 sensitive) on linear sucrose gradients (5-20%) containing 1% Lubrol-WX. All tissues examined, including whole blood, contained 4 and 10S AChE. The 16S form was detected in: diaphragm and anterior gracilis muscle endplate regions, spinal cord (L1-L6), phrenic, obturator, and sciatic nerves (peripheral, mixed motor-sensory), hypoglossal (predominantly motor, cranial XII), vagus (sympathetic efferent), and spinal ventral roots (L5-S1). 16S AChE was not detected in: diaphragm and anterior gracilis muscle non-endplate regions, smooth muscle (large intestine) and spinal dorsal roots (L5-S1). A 6.5S molecular form was found only in spinal cord and peripheral nerve. This may be a reflection of 6.5S AChE specificity for nervous tissue. Alternatively, this form could represent a state of aggregation or transformation of the other AChE species.

Results indicate that 16S AChE is not only present in muscle endplates, but is also found in spinal cord and peripheral nerve. In sciatic nerve, the 16S AChE activity may be totally attributed to motor neurons and not to sensory neurons or Schwann cells found in this peripheral nerve. This might be related to neurotrophic regulation of neuromuscular junction AChE, since neural 16S AChE might provide a source for the endplate enzyme. Current experiments are concerned with this possibility.

(Supported by the Muscular Dystrophy Assn. and the Medical Research Service of the Veterans Administration).

1173 DIFFERENT COMPONENTS OF BLACK WIDOW SPIDER VENOM MEDIATE TRANSMITTER RELEASE AT VERTEBRATE AND CRUSTACEAN NEUROMUSCULAR JUNCTIONS. Lawrence C. Fritz*, Mu-Chin Tzeng* and Alexander Mauro* (SPON: C. M. Connelly). Rockefeller Univ., New York, NY 10021

Crude black widow spider venom (BWSV) causes a massive increase in mepp frequency at vertebrate neuromuscular junctions, a similar increase in mepsp frequency at crustacean neuromuscular junctions, and can release a number of transmitters from mouse cerebral cortex slices. Using purified fractions of BWSV, it has previously been determined that (1) a 130,000 MW component, α-latrotoxin (formerly called B_5), accounts for all the known physiological effects at the vertebrate junctions and in mouse brain slices; and (2) the partially purified fraction E, whose major component has MW 65,000, depolarizes the crayfish stretch receptor.

The present study demonstrates that α-latrotoxin has no effect on the lobster neuromuscular junction, while fraction E can account for all of the physiological effects of the crude venom in that preparation. Application of 9-18 μg of fraction E to a small (~0.2 ml) vaseline enclosed pool surrounding exposed muscle fibers causes an increase in mepsp frequency, eventual block of epsp's and ipsp's, and the characteristic "giant mini" response, all of which are seen in response to crude venom. Application of α-latrotoxin (10 μg) however produces no effect. In contrast, at the frog neuromuscular junction, where 1-4 μg of α-latrotoxin in a 3 ml bath causes a rapid increase in mepp frequency, fraction E has no effect.

These results demonstrate that the effects of BWSV at vertebrate synapses and lobster neuromuscular junctions are mediated by different components. This suggests that the mechanism of BWSV action at vertebrate and at crustacean synapses may be different.

1174 SEROTONIN (5HT) MODULATORY ACTION AT THE LOBSTER NEUROMUSCULAR JUNCTION. MECHANISM OF FACILITATION OF TRANSMITTER RELEASE. Silvio Glusman and Edward A. Kravitz. Dept. Neurobiol., Harvard Med. Sch., Boston, MA 02115.

5HT facilitates synaptic transmission at the crustacean neuromuscular junction by increasing the probability that quanta of neurotransmitter will be released with nerve stimulation. We have examined this phenomenon in greater detail using the opener muscle of the dactyl of the lobster walking leg. The threshold for 5HT action in this preparation is approximately 5 x 10^{-9}M and a marked increase in excitatory junctional potential (ejp) size (2-3x) is seen at 10^{-7}M. In a typical experiment, the preparation was superfused for ten minutes with lobster saline containing 5-8 x 10^{-7}M 5HT. The amine was then washed out by continuous perfusion. Facilitation of the ejp can be seen for sixty min. after washout of 5HT from the bathing medium. The prolonged duration of 5HT action allows experimental manipulation to be performed during the period of amine treatment, followed by testing for facilitation after a return to control conditions. Using this approach we have observed that nerve activity during the amine treatment itself is not necessary to obtain facilitation; the time course of decline of facilitation, tested after removal of 5HT, is identical whether or not the excitatory axon is stimulated during exposure to 5HT. To examine whether facilitation results from movements of ions across the nerve terminal membrane during 5HT treatment, 5HT was superfused onto preparations in lobster saline of different ionic compositions: 1) calcium-free (calcium replaced by magnesium plus 1mM EGTA); 2) sodium free-calcium free (sodium replaced by Tris); 3) solutions with added $MnCl_2$ or Verapamil to block calcium channels. After return of the preparation to normal saline the degree of facilitation was examined; the extent and time course of facilitation after 5HT treatment in all of the test solutions was the same as in normal saline. To examine whether 5HT might cause a direct change in free calcium concentration within the nerve terminals, we analyzed the frequency and size of spontaneous miniature excitatory junctional potentials (smejps). In normal lobster saline 5HT increased the frequency of smejps with a time course similar to the increase in nerve-evoked release. There was no effect on smejp size. A similar increase in smejp frequency was seen in calcium free solution (high magnesium - 1mM EGTA), sodium free solution (replaced by lithium) with or without calcium, or in saline in which potassium conductance was blocked by TEA. Our results suggest that the long lasting facilitation produced by 5HT at lobster neuromuscular junctions is due to an increase in free calcium concentration within the nerve terminals, possibly caused by release of calcium from some intracellular storage sites. (Supported by NIH).

1175 SYNAPTIC EFFECTIVENESS IN FROG CUTANEOUS PECTORIS AND SARTORIUS MUSCLES: EVIDENCE FOR COMPETITIVE INTERACTION IN MULTIPLY INNERVATED FIBERS. Albert A. Herrera* and Alan D. Grinnell. Dept. Biol., UCLA, Los Angeles, CA 90024

Many neuromuscular junctions in the normal sartorius (sart.) muscle of Rana pipiens and R. catesbeiana are subthreshold to single stimulation in normal frog ringer (1.8 mM Ca^{++}). If the [Ca^{++}] is increased to 3 mM, twitch tension increases by 20% or more. If [Ca^{++}] is decreased to 1 mM, twitch tension falls by 50% or more. This is not true of the cutaneous pectoris (c.p.) muscle, which shows no change in tension in the same range of Ca^{++} concentrations. This difference has been verified by intracellular recording. In ringer containing 0.3 mM Ca^{++} and 1 mM Mg^{++}, the quantal content of c.p. neuromuscular junctions was an average 4 times greater than those in sart. fibers. Synaptic efficacy is known to depend on area of synaptic contact (Kuno et al. J. Physiol., 1971, 213:545-556) and on postsynaptic input resistance. In an attempt to explain the difference in safety factor of junctions in the two muscles, these variables have been examined in marked fibers where the morphology and physiology of identified junctions can be correlated. For a given fiber diameter, c.p. terminals are longer, but the difference is not adequate to explain the 4-fold difference in quantal content. Moreover, the terminal width and area of contact/unit length are significantly greater in sartorius fibers. Input resistance of c.p. fibers is, on the average, 1.5 to 2 times greater than sart. fibers, which could help explain the greater c.p. safety factor, but not the greater quantal content. Thus there remains a difference in synaptic effectiveness that cannot be explained simply in terms of terminal morphology. Sartorius fibers differ from c.p. fibers in that the former are multiply innervated. The functional ineffectiveness of the sart. junctions may be due to competitive interaction between these multiple junctions. We are examining the quantal content and morphology of junctions remaining at different times after partial denervation of sart. muscles, or after multiple innervation of c.p. muscles. The functional importance of low safety factor in sartorius junctions is unknown, but this property might constitute an independent mechanism for increasing the tension exerted by each motor unit as the repetition rate of nerve firing is increased, thereby increasing the smoothness with which contraction could be graded.

1176 SPINAL CORD AND BRAIN EXTRACTS INCREASE ACETYLCHOLINE RECEPTOR NUMBER ON CULTURED CHICK MYOTUBES. T.M. Jessell*, R.E. Siegel*, and G.D. Fischbach. Dept. Pharmacology, Harvard Medical School, Boston, MA 02115.

Motoneuron axons that extend from spinal cord explants form synapses with chick myotubes in culture. The synaptic region of the myotube is characterized by the presence of discrete clusters of acetylcholine receptors (AChR). In addition, extrasynaptic regions of myotubes located in the immediate vicinity of the explant exhibit an increased ACh sensitivity when compared to myotubes located far from the explant (Cohen and Fischbach, Dev. Biol. 59, 24, 1977). This general increase in ACh sensitivity may be mediated by a soluble factor released from spinal cord tissue. To investigate this possibility, membrane-free extracts were prepared from 14 day embryonic chick spinal cords and added, in buffered salt solution, to primary cultures of chick myotubes in 18mm wells (plating density: $6x10^4$ cells/well). Specific binding of ^{125}I-α bungarotoxin (^{125}I-αBTX) was used to quantify AChR number. In control cultures, grown for 8 days, ^{125}I-αBTX binding was 4.7±0.3 fmol/culture (mean±s.e.m.; 7 experiments). In 4 of these experiments, addition of spinal cord extracts to fused myotubes (75μg protein/day from days 4-7) resulted in an increase in ^{125}I-αBTX binding to 12.9±1.4 fmol/culture. The protein content of control and treated cultures differed by less than 20%.

Similar extracts prepared from the brains (with cerebellar cortex removed) of 14 day embryonic chicks produced a 1.9 fold increase in ^{125}I-αBTX binding. The effect of brain extract was dose-dependent over the range 15-300μg protein/day; at the highest concentration of brain extract added, a 4.5 fold increase in AChR was observed. In cultures treated with brain extract, increases in receptor density estimated by autoradiography and by electrophysiological determination of ACh sensitivity corresponded with increases determined by ^{125}I-αBTX binding. From autoradiographic studies, brain extract increased the total receptor density and the number of receptor clusters. Extracts prepared from the retina and cerebellar cortex produced smaller increases in ^{125}I-αBTX binding. Liver and heart extracts did not alter AChR number.

We are now examining whether this factor is located exclusively in cholinergic neurons. A reasonable correlation exists between receptor induction and the distribution of choline acetyl transferase activity in tissues examined. Furthermore a pure population of cholinergic nerves dissociated from chick ciliary ganglia increased AChR number 2-3 fold when cultured with chick myotubes, while chick dorsal root ganglia neurons did not alter AChR number. (Supported by NS11160; TMJ is a Harkness Fellow.)

1177 EFFECTS OF Δ^9-TETRAHYDROCANNABINOL ON EXCITABLE MEMBRANES AND NEUROMUSCULAR TRANSMISSION Nuran M. Kumbaraci*, W. L. Nastuk, Dept. of Physiology, Columbia Univ., N. Y., N. Y. 10032

Electrophysiological studies were conducted on the isolated sciatic nerve-sartorius muscle preparation of the frog Rana pipiens treated with the principal component of cannabis, Δ^9-tetrahydrocannabinol (THC). Bath application of 30 X 10^{-6} M THC for 3 hrs produced changes in the electrophysiological properties of muscle fibers and in neuromuscular transmission (NMT). Resting potentials of THC treated fibers did not differ from controls. Records taken at non-junctional regions showed that single supramaximal nerve stimulation failed to evoke a propagated action potential (AP) in 7 out of 16 fibers. In 60% of these non-neurally excitable fibers direct electrical stimulation initiated an AP. The critical membrane potential was in the control range. Analysis of the neurally initiated propagated APs recorded at non-junctional regions showed on the average a 17% decrease in rate of rise, a 20% decrease in rate of fall and a 12% decrease in overshoot. Thus THC 30 X 10^{-6} M depresses the ionic conductance mechanisms which underly propagation of APs in muscle fibers.

Recordings taken at surface neuromuscular junctions of THC treated muscle showed, in 87% of the fibers tested, only subliminal endplate potentials (EPPs) with amplitudes ranging from 5-30 mV. Occasionally a propagated AP was found superimposed on the falling phase of these subliminal EPPs. Apparently these APs were initiated from a second neuromuscular junction of the muscle fiber at which NMT had not been blocked. At those junctions where the EPPs were subliminal, the MEPP frequency was found to be reduced, but MEPP amplitude was either unchanged or greater than the control value. Occasionally, MEPPs 2-3 mV in amplitude were recorded. Thus it was concluded that THC at 30 X 10^{-6} M can block NMT.

To determine the manner by which THC blocks NMT, postjunctional membrane (PJM) sensitivity was tested by microperfusing carbamylcholine (CARB) onto the junctions of THC treated muscle. Application of 20 X 10^{-6} M CARB depolarized the PJM by 23.9 ± 3.7 mV in the THC treated preparations compared with 19.5 ± 2.2 mV in controls. Thus apparently THC does not block NMT by decreasing the sensitivity of the membrane to cholinergic agonists.

The effect of THC on quantal content was tested on the nerve-muscle preparation equilibrated in Ringer solution containing 0.9 mM Ca^{++} and 4.0 mM Mg^{++}. The failure rate in quantal release was determined using a low frequency train of nerve stimuli ("method of failures"). Perfusion of junctions of these preparations for 15 min with 30 X 10^{-6} M THC caused complete blockade of quantal release with 100% failure rate. Thus THC has a substantial effect in reducing the AP evoked release of acetylcholine.

1178 EFFECT OF α-BUNGAROTOXIN ON THE RISING PHASE OF THE MINIATURE ENDPLATE CURRENT. B. R. Land*, M. M. Salpeter, and E. E. Salpeter*. Section of Neurobiology & Behavior and Dept. of Physics, Cornell Univ., Ithaca, NY 14853.

Recent studies at various vertebrate neuromuscular junctions suggest that each quantal packet of nerve released acetylcholine (ACh) interacts with ACh receptors (AChR) over a small distinct post junctional area at very high ACh concentrations (Hartzell, Kuffler and Yoshikami, J. Physiol. 251: 427, 1975; Fertuck and Salpeter, J. Cell Biol. 69: 144, 1976; Matthews-Bellinger and Salpeter, J. Physiol., in press, 1978). According to such a model, the rise time of the miniature endplate current (mepc) reflects: a) the spreading rate of ACh in the cleft, b) the binding rate of ACh to receptor and esterase, and c) some constant time delays such as the conformational change to open the ion gate. Both (a) and (b) above are dependent on AChR and AChE site density. Furthermore, if (a) is a significant factor, then the time to peak of a mepc and its amplitude should be positvely correlated. To determine the relative importance of these parameters, we studied the rise time of the mepc in the lizard (Anolis Carolinensis) intercostal muscle, for normal AChR and AChE site densities and when these sites were reduced by α-Bungarotoxin (α-BTX) and diisopropyfluorophosphate (DFP) respectively.

Mepcs were acquired under voltage clamp (-100 mV at 23°C). Individual mepcs were then averaged into amplitude bins, 1 nA wide, after bringing them into register in time. Mean mepc rise time (from 10% to 90% of full amplitude) was 100±30 μsec in normal muscle, 220±40 μsec after incubation with BTX ($4x10^{-8}$M for 40 min) and 280±60 μsec with BTX plus DFP (10^{-3}M for 20 min). The mean mepc amplitude was 8±1 nA in normal muscle, 2±1 nA after BTX and 3±1 nA with BTX plus DFP. The mepc amplitude and rise time were positively correlated after BTX plus DFP, but not in normal muscle. From these results we conclude that a) a large fraction of the 100 μsec rise time of the unpoisoned endplate consists of a constant time delay, probably the conformational change of the receptor, and b) diffusion of ACh in the cleft becomes an appreciable factor in determining the mepc rise time of the BTX plus DFP poisoned preparation.

A determination of AChR site density by E-M autoradiography combined with mepc time-course data should therefore allow us to determine the actual ACh diffusion and binding rate constants in the cleft. (Supported by grant NS09315).

1179 ACh RECEPTOR-CHANNELS BEGIN TO OPEN WITHIN 30 μSEC AFTER AGONIST IS APPLIED. Henry A. Lester, Menasche M. Nass*, Mauri E. Krouse*, Norbert H. Wasserman* and Bernard F. Erlanger*. Division of Biology, California Institute of Technology, Pasadena, CA 91125, and College of Physicians and Surgeons, Columbia University, New York, New York 10032.

These experiments measure how soon ACh receptor-channels begin to open after agonist appears near receptors. Isolated Electrophorus electroplaques are arranged for transcellular recording. The innervated face is bathed in a 1 μM solution of cis-Bis-Q (3,3'-Bis-[α(trimethylammonium) methyl] azobenzene). This solution contains less than 1% trans isomer and has no effect on ACh receptors. Trans-Bis-Q, on the other hand, is a potent agonist; a concentration of 400 nM produces half-maximal receptor activation at the resting potential. A light flash increases the concentration of trans-Bis-Q in the solution to about 200 nM within 40 μsec (individual molecules of cis-Bis-Q are presumably photoisomerized to trans in less than 1 μsec after absorbing a photon). At 34°C the cell begins to depolarize detectably less than 30 μsec after the start of the light flash. More intense light flashes, we presume, will reduce the latency further. The minimum measurable latency increases at lower temperature ($Q_{10} \geq 2$), probably because the channel opening rate decreases. On the other hand, the growth phase of the miniature postsynaptic current (MPSC) has a much lower temperature dependence (Q_{10} of 1.2, Gage and McBurney, J. Physiol. 244, 385-407, 1975). Therefore the growth phase of the MPSC is probably not shaped primarily by the growth of ACh concentration; it may be limited by lateral diffusion of transmitter within the cleft. Our results indicate that channels begin to open rapidly when agonist is suddenly applied, and that only a small part of the synaptic delay is due to interaction of agonist and receptor. This work was supported by the NIH (grant NS-11756 and RCDA NS-242) and by the Muscular Dystrophy Association (grant and postdoctoral fellowship), and by NSF (grant PCM 74-02140).

1180 STRUCTURAL ORGANIZATION OF THE DEVELOPING AMPHIBIAN NEUROMUSCULAR JUNCTION. Michael S. Letinsky and Paige A. DeCino*. Dept. of Physiol. and Ahmanson Laboratory of BRI, Sch. Med., UCLA, Los Angeles, CA 90024.

The structural organization of the neuromuscular junction was analyzed at different stages of development. To accomplish this, we have developed a new, combined staining technique whereby pre- and postsynaptic endplate structures can be visualized simultaneously at the same junctional site. The basic procedure consists of first staining the presynaptic nerve using nitroblue tetrazolium (NBT), which upon reduction to its diformazan state colors the entire presynaptic terminal arborization intensely blue. The NBT nerve stain is compatible with common endplate acetylcholinesterase (AChE) stains such as the Karnovsky procedure (J. Cell Biol. 23:217-232, 1964). This staining combination vividly displays the blue colored nerve terminal processes outlined by the Hatchett's brown colored AChE reaction product. The technique independently stains pre- and postsynaptic elements of endplates successfully on developing, mature, and reinnervated frog muscle as well as on avian and mammalian skeletal muscle.

By using such a procedure, we have been able to characterize the developmental pattern of the growing nerve terminal processes relative to the distribution of the endplate AChE, a marker for postsynaptic specialization. The neuromuscular junctions in the cutaneous pectoris muscle from different stages of tadpoles and frogs (R. pipiens and R. catesbeiana) were examined. The earliest endplate specializations were observed on immature myotubes where NBT-stained unmyelinated axons formed small, unbranched contact points with weak subneural AChE activity. The initial contacts were formed by single axons, but with further development the endplates became multiply innervated. As tadpoles mature and go through metamorphosis, the multiply innervated terminals increase in size and become more complexly branched. Multiple innervation decreases with further development, but is still present in some terminals even in mature frogs. At all stages there were nerve terminal processes which appeared to be growing out of the established, AChE stained postsynaptic junctional arborization (escaped nerve fibers). The incidence of escaped fibers decreases with development, but is still present even at adult nerve muscle junctions. During the protracted course of development in frogs we have also observed endplates with portions of the AChE stained postsynaptic membrane devoid of any overlying nerve terminal processes. The decrease in multiple innervation coupled with the presence of escaped nerve terminal processes and AChE stained postsynaptic membrane without any overlying neural processes may reflect ongoing growth and reorganization of the neuromuscular junction.

1181 NEUROMUSCULAR TWITCH-TENSION STUDIES WITH C. SCULPTURATUS VENOM (CSV) AND PURIFIED FRACTIONS. Gesina L. Longenecker, Herbert E. Longenecker, Jr. and Barbara Beyers*. Depts. of Pharmacology and Physiology, Univ. of S. Ala. Coll. of Med., Mobile AL 36688.

Ours and other studies on neurotoxic scorpion venom have used electrophysiological techniques including voltage clamp and intracellular recording. These methods are adequate for examining mechanisms and are ultimately required, but they preclude simple statistical evaluation of vast numbers of fibers under many conditions and doses of reagents. Thus, using supermaximal indirectly evoked twitch-tension measurements of frog sciatic/sartorious preparations, we have conducted 40 experiments to restudy CSV and fractions (CSVF); both were furnished by D. Watt of Creighton University. Preparations were dissected and mounted in Ringer's containing 2.0 mM Ca++, 1.5 mM K+ and 114.5 mM Na+. Muscle length was adjusted for max twitch tension. Stimuli were presented at 0.1 per second. The preparations stabilized during 1-2 hours, during which tension decreased by about 50%. CSV and CSVF (0.5 to 10 ug/ml in Ringer's) were added for study after stabilization.

In standard Ringer's twitch tension was facilitated (2.36±.36) with max responses within 4 minutes. Indirectly increased twitch tension gradually subsided, reaching control levels in 14 minutes, with total and irreversible block by 140 minutes (control preparations with no venom were viable for at least 600 minutes). If the muscle chamber was flushed with Ringer's as venom response peaked, there was some reversibility of venom action, but twitch block still occurred. Duration of the facilitation period, time to peak facilitation and time to final block of twitch tension were increased with increased Ca++ concentration. Peak facilitation decreased with increased Ca++. These effects are consistent with repetitive neural firing caused by venom effects on the Ca++ dependent Na conductance system as described by others. Final twitch block and our inability to inhibit the occurrence of block with high Ca++ is not explained by this mechanism.

Preliminary studies with three highly purified CSVF (Toxins I, IV, and B140-1) showed that all toxins facilitated twitch; however, kinetics and appearance of records are different with each toxin. Toxin IV facilitates immediately and is a rapid and potent blocker of twitch tension. Toxin I shows delayed facilitation but eventually blocks twitch. B140-1 causes initial depression, then gradual increase in tension, then eventually blocks.

(This work was supported by NIH grant 5 R01 ES01321-02)

1182 ELECTROPHYSIOLOGICAL COMPARISON OF THREE PURIFIED NEUROTOXINS FROM C. SCULPTURATUS VENOM ON ISOLATED FROG NEUROMUSCULAR PREPARATIONS. Herbert E. Longenecker, Jr., Gesina L. Longenecker, Barbara Carter*, Depts. Physiology & Pharmacology, Univ. of S. Ala. College of Medicine, Mobile, AL 36688.

We, as well as other investigators, have demonstrated effects of crude Scorpion venom (CSV) on isolated frog sciatic nerve/sartorious muscle preparations. Following venom addition, repetitive firing of nerves is induced; also, prolonged nerve action potentials are elicited. These neural effects cause respectively repetitive stimulus-evoked endplate potentials (EPP) and prolonged EPPs. In this study we report results from 40 preparations using standard intracellular recording techniques on three highly purified venom fractions, Toxin I, Toxin IV and Toxin B-140-1. (Toxins were furnished by D. Watt of Creighton University.) All preparations were pretreated with glycerol to prevent muscle twitching. The Ringers' contained 1mM Ca++, 1mM Mg++, 2.5mM K+, 114.5 mM Na+ (pH 6.8).

Toxin I (.05-.8 ug/ml) caused facilitation (2.7±2.6) of the EPP, a prolonged period (82±24 mins.) of repetitive(R) EPPs (peaks 90±26) and eventual EPP block at 146±23 minutes. Miniature(M) EPP frequency(F) rose slightly from 0.6 to ~44/second. MEPP amplitude(A) was unaffected.

Toxin IV (.05-.8ug/ml) caused slight facilitation (.7±.21) of the EPP, then total EPP block at 64±20 minutes. REPPs (peak # 56±14) continued for 46±21 minutes. Before EPP block MEPPF rose from 0.8 to 24±15/sec, then decreased. Occasional MEPP (10-15/sec) bursts occurred following EPP block.

Toxin B-140-1 (.2-4.0ug/ml) caused prolonged EPPs (normal=2 msec, peak=204 ±105 msec). REPPs occurred (duration 140±24), but to a lesser degree (peak=5±1). The EPP was blocked (225 mins.) in 2 of 11 experiments. MEPPA was unchanged.

Each purified venom fraction effects prejunctional transmitter release phenomena distinctly, yet there are many similarities differing only perhaps by relative kinetic parameters. It seems reasonable to assume that facilitation of the EPP, the amount of REPPs, and increases in MEPPF relate directly to altered Na permeability of the nerve terminal. The aggregate effects can account for our prior observations with crude CSV. A new finding is that Toxins I and IV cause block of the EPP without apparent nerve terminal depletion. No obvious mechanism is apparent, although the response seems similar to that induced by Botulinum toxin. The EPP did not recover with increased Calcium (up to 2mM).

(This work was supported by NIH grant 5 R01 ES01321-02)

1183 THE AVIAN SYRINX: AN ANDROGEN SENSITIVE MOTOR ORGAN? V. Luine, I. Lieberburg*, F. Nottebohm, C. Harding*, and B. McEwen. Rockefeller University, New York, N.Y. 10021.

Song in male zebra finches is part of courtship and agonistic displays. The amount of singing is influenced by testosterone (T): castrate males sing little and T therapy reinstates normal amounts of singing. Castration (GDX) and T therapy also influence the volume of the syringeal muscle mass. The syrinx is the vocal organ of birds, innervated by hypoglossal motoneurons. Injections of ^{3}H-T lead to heavy concentrations of label over the nucleus of these motoneurons. The preceding observations, from the work of Arnold, Exp. Zool. 191 309 '75 and Arnold et al., J. Comp. Neurol. 165 487 '76, suggest that T may have an effect on the peripheral organs of song control. As a first step toward describing mechanisms responsible for these effects, we hypothesized that T affects neuromuscular transmission or even possibly syringeal muscle itself.

Adult, male zebra finches were Gdx and at various times after surgery were replaced with T or cholesterol. Levels of circulating androgens were verified by RIA. Two weeks following Gdx, syrinx wt.decreased by 24% (intact = 21.4mg). Replacement for 1 week returned weights to intact levels. Similar changes in larynx weight were not found. Activity of acetylcholinesterase (AChE) was measured in Gdx, intact and Gdx+T birds. Expressed as specific activity (nM/mg protein/hr) or as total activity (nM/muscle/hr), AChE activity decreased approximately 40% 2 weeks following Gdx. T replacement for 1 week restored AChE. AChE activity in hyoid or pectoralis muscle was not affected.

The possibility of T acting directly in the syrinx as in classic androgen target tissues was examined by measuring for high affinity T binding. Using standard cell fractionation techniques, ^{3}H-T binding was measured in cytosol prepared from syrinx, larynx, hyoid and pectoralis muscle and in serum and trachea. In the syrinx, high affinity T binding was detected (K_d=0.62nM) with a capacity of 14.8 fmoles/mg cytosol protein, values comparable to those in androgen sensitive tissues of the rooster. 83% of bound radioactivity recovered from syringeal cytosol was still in the form of ^{3}H-T. No high affinity binding was detected in the other tissues. The specificity of syringeal receptor binding is T = RU1881 = 5αDHT>estradiol>5 βDHT>progesterone.

The presence of high affinity T receptors and changes in AChE activity in syrinx after Gdx and concentration of label in hypoglossal motoneurons following ^{3}H-T systemic injections suggest T action via classic genomic mechanisms. However, the changes reported in syringeal muscle cannot at this time be attributed to direct neuronal or muscular effects. (Supported by NS07080 to BMc and MH18343 and 5S05-RR07065-12 to FN and RF70095.)

1184 SYNAPTIC TRANSMISSION IN THE RABBIT SUPERIOR CERVICAL SYMPATHETIC GANGLION: COMPARISON TO THE FROG NEUROMUSCULAR JUNCTION. K.L. Magleby,[1] J.E. Zengel*[1] J.P. Horn,[2] D.A. McAfee,[2] & P.J. Yarowsky.[2] [1]Dept. Physiol., Univ. Mia. Sch. Med., Miami, FL 33152 and [2]City of Hope Med. Center, Duarte, CA 91010

At least four processes can act to increase transmitter release during and following repetitive stimulation at the frog neuromuscular junction. These processes and their time constants of decay (20°C) are: 1st and 2nd components of facilitation, 50 and 300 msec; augumentation, 7 sec; and potentiation (PTP), 30 sec to min. In this study we investigated whether similar processes are present in the sympathetic ganglion.

Excitatory post-synaptic potentials (fast EPSPs) were recorded from rabbit sympathetic ganglia using the sucrose gap technique. The preparation was perfused with oxygenated Locke solution (25-27°C) with reduced Ca^{++} and elevated Mg^{++} to reduce quantal content.

The preganglionic nerve trunk was conditioned with 1-600 impulses at 5/sec. The EPSP amplitude typically increased during the conditioning trains. Testing impulses applied after the conditioning trains established that the EPSP amplitudes then returned to the control level with at least three apparent time constants of decay: 460±80 msec, 17±4 sec, and 140±40 sec (±S.D.). The addition of Ba^{++} (0.1-0.2 mM) to the Locke solution led to a greater increase in the magnitude of the EPSPs during and following the conditioning train. This increase was associated with an increase in the magnitude but not the time constant of the 17 sec process. This effect of Ba^{++} on the 17 sec process in the sympathetic ganglion is similar to the effect of Ba^{++} on augumentation at the frog neuromuscular junction. The addition of Sr^{++} (0.2-0.8 mM) to the Locke solution also led to a greater increase in the magnitude of the EPSPs during the conditioning train. Sr^{++} had little effect on the 17 or 140 sec processes, suggesting that it is acting on a shorter time course process, perhaps the 460 msec process. This effect of Sr^{++} is similar to the effect of Sr^{++} on facilitation in the frog.

If Ba^{++} and Sr^{++} act the same in the rabbit sympathetic ganglion as the frog neuromuscular junction and if the observed changes in EPSP amplitude reflect changes in transmitter release, then the results of this preliminary study suggest that in the sympathetic ganglion (25-27°C) the 460 msec process is facilitation, the 17 sec process is augmentation, and the 140 sec process is potentiation (PTP). Thus, at least three of the processes that act to increase transmitter release at the frog neuromuscular junction also appear to be present in the rabbit sympathetic ganglion. It is interesting to note that even though the data from the sympathetic ganglion were collected at a higher temperature than in the frog (25-27°C vs 20°C), the decays of the identified processes were slower. It is necessary, therefore, to identify the processes in different preparations by their pharmacological and kinetic properties in addition to their time constants of decay. Supported by NIH grants NS10277, NS07044, NS05820, NS05363, and a Scottish Rite Fellowship.

1185 A STATISTICAL MODEL INDICATES THAT MULTIPLE PEAKS IN MEPP AMPLITUDE HISTOGRAMS ARE SIGNIFICANT. D. R. Matteson* and M. E. Kriebel* (SPON: J. B. Preston). Dept. Physiol., Upstate Medical Center, Syracuse, NY 13210.

A statistical model describing the probability density function of MEPP amplitudes was derived from the following three assumptions. (1) Small mode MEPPs (Kriebel & Gross, 1974, J. Gen. Physiol.) result from the release of a subunit of transmitter. (2) Larger MEPPs result from the synchronous release of several subunits. The release of T subunits would produce a subpopulation of MEPP amplitudes with a mean equal to T times the mean of the small mode MEPPs. (3) The overall MEPP amplitude distribution is thus composed of the sum of several subpopulations, each subpopulation being capable of generating a peak in the distribution, with a mean amplitude at some integral multiple of the small mode mean.

In a qualitative way, MEPP amplitude distributions fit this model since they appear multimodal (Kriebel, Llados & Matteson, 1976, J. Physiol.). In order to obtain a quantitative estimate of the fit we tested the significance of the multiple peaks by testing the above multimodal model against a reduced bimodal model. The reduced model should give a significantly better fit if the larger MEPPs result from the release of a single quantum of transmitter and the small mode MEPPs result from the release of a constant fraction of a quantum (or a smaller quantum). The test of significance we utilized was the generalized likelihood ratio test.

Amplitude histograms obtained under a variety of experimental conditions are shown to be fit significantly better by the multimodal model. Furthermore, when mean MEPP amplitude is decreased by increasing temperature, extracellular Ca^{++}, or addition of colchicine, the small mode mean (and therefore the interval between successive peaks) does not change significantly. The model indicates that the decrease in MEPP amplitude occurs as a result of a decrease in the conditional probability associated with larger amplitude subpopulations and an increase in the conditional probability of intermediate amplitude subpopulations. This analysis provides further evidence for the hypothesis that MEPPs result from the synchronous release of transmitter subunits.

1186 INTERACTIONS BETWEEN STRONTIUM AND CALCIUM IN THE PROCESS OF EVOKED TRANSMITTER RELEASE AT THE FROG NEUROMUSCULAR JUNCTION. A.M. Mellow and E.M. Silinsky. Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

The evoked release of transmitter at the neuromuscular junction is manifested in two ways: (1) synchronous release, recorded postsynaptically as the end-plate potential (e.p.p.) and (2) asynchronous release, recorded as a delayed increase in miniature end-plate potential (m.e.p.p.) frequency following nerve stimulation. Both of these processes are dependent upon extracellular calcium ion (Ca^{2+}) and both can occur when Ca^{2+} has been substituted by strontium ion (Sr^{2+}). The orders of effectiveness, however, are $Ca^{2+} > Sr^{2+}$ for synchronous release and $Sr^{2+} > Ca^{2+}$ for asynchronous release. This study was undertaken to investigate the interactions between these ions in both forms of release in the isolated nerve - cutaneous pectoris preparation of the frog.

It has been shown previously that, under certain conditions, Sr^{2+} can antagonize Ca^{2+} - mediated synchronous release. Using the dose-ratio method from basic drug-receptor theory, we estimated the equilibrium dissociation constant (K_d) for Sr^{2+} to be 4 mM. This value indicates that Sr^{2+} has an affinity for the nerve terminal membrane that is one order of magnitude lower than has been calculated for Ca^{2+} by the same method. Under conditions in which Sr^{2+} antagonized Ca^{2+} - mediated synchronous release, it actually enhanced Ca^{2+} - mediated asynchronous release. Further, Ca^{2+} was able to effectively antagonize Sr^{2+} - mediated asynchronous release, in an apparently competitive fashion. These observations suggest that the two forms of evoked transmitter release are activated by different intraterminal mechanisms with separate divalent cation sensitivities. The interactions between Sr^{2+} and Ca^{2+} may provide a tool for further understanding the basic mechanisms of depolarization-secretion coupling at presynaptic nerve terminals. (Supported by NIH grant NS 12782 and NIH training grant GM 07263. AMM is a NIH Predoctoral Fellow.)

1187 CALCIUM-LIKE ACTION OF COLCHICINE ON FROG SKELETAL MUSCLE MEMBRANE. Terry M. Mikiten and Nikki Martin*, Dept. Physiology, The Univ. of Texas Health Science Center, San Antonio, TX 78284.

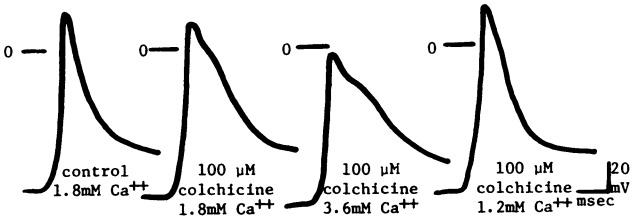
Because microtubules and tubulin have been implicated in the functioning of the postsynaptic membrane, we investigated the action of colchicine at the frog nerve-muscle junction. In these experiments, membrane potential is measured using conventional microelectrode technique. When applied in vitro in concentrations from 10-100 μM, colchicine increases the rate of desensitization of the postjunctional membrane (pjm) in the presence of 100 μM carbamylcholine (carb).

To test whether this is due to some action at the ACh receptor, colchicine was added to preparations bathed in 2-4 μM d-tubocurarine. No change ($p > 0.1$) in endplate potential amplitude is observed 30-60 min. after addition of the alkaloid.

Further, the final steady-state membrane potential during desensitization (control = 75.5 ± 1.4 S.E. mV) is shifted by colchicine in the direction of E_k (88.2 ± 1.7 S.E. mV; $p < .001$). This effect, as well as the increase in the rate of desensitization, can be duplicated by simply raising the extracellular calcium concentration.

Since calcium also alters some action potential parameters in frog skeletal muscle fibers, we measured these in the presence of 100 μM colchicine in different calcium concentrations. In these experiments, 100 μM colchicine increases (by 50%; $p < .001$) the duration of action potentials recorded at the pjm, and this is reflected by a decrease (by approx. 50%; $p < .005$) in the rate of membrane repolarization. Although it has no effect on resting membrane potential or rate of rise of the action potential, colchicine decreases the amplitude of the overshoot. Doubling the extracellular calcium alone has similar effects.

Because increasing or decreasing extracellular calcium alters the effects of colchicine, we propose that colchicine acts on both junctional and nonjunctional membrane by some mechanism which is calcium-like or calcium dependent (tracings below).



CALCIUM EFFECTS ON ACTION POTENTIALS IN COLCHICINE

1188 EFFECTS OF HISTRIONICOTOXIN ANALOGS ON THE IONIC CHANNEL OF THE CHOLINERGIC RECEPTOR. A.C. Oliveira*[1], E.X. Albuquerque[1], J.W. Daly[2], M.E. Eldefrawi*[1] and Amira T. Eldefrawi*[1]. Dept.Pharm. Exp. Ther., Sch. Med., Univ. MD., Baltimore, MD. 21201 and Lab. Chemistry,[2] NIH, Bethesda, MD. 20014.

Histrionicotoxin (HTX), a neurotoxin isolated from the skin secretion of the frog Dendrobates histrionicus, has been shown to block neuromuscular transmission by reacting rather specifically with the ionic channel of the cholinergic receptor. At present we report on some electrophysiological and biochemical studies performed with 3 HTX analogs: perhydrohistrionicotoxin (H_{12}-HTX), octahydrohistrionicotoxin (H_8-HTX) and isotetrahydrohistrionicotoxin (Iso-H_4-HTX). The effects of these analogs on endplate currents (EPCs) were studied in frog sartorius muscle under voltage clamp. All compounds (4-25 μM) decreased the EPCs peak amplitudes (70-85%) and half-decay times (25-30%) significantly. The decay of EPCs remained a single exponential function of time but its voltage dependence was decreased by the analogs. In presence of those neurotoxins, the current voltage relationship of the EPCs became highly nonlinear and very frequently showed hysteresis in the region of hyperpolarized potentials (-100 to -180 mV). Equilibrium potentials were unaffected. Effects of the analogs on ionic channel conductance and lifetime were studied by acetylcholine (ACh) noise analysis. They mainly decreased single channel conductance, but at hyperpolarized potentials (-120 mV) there was, in addition, shortening of channel lifetime. The effects of these analogs were also studied on the binding of [^{3}H] acetylcholine and [^{3}H] H_{12}-HTX to the ACh receptor and its ionic channel, respectively, in Torpedo ocellata electroplax membranes, using a centrifugal assay. At concentrations ranging from 0.1 to 30 μM, none of the HTX analogs displaced [^{3}H] ACh from its receptor sites. On the other hand, all three analogs were very effective in displacing [^{3}H] H_{12}-HTX from its binding sites in Torpedo membranes with I_{50} values ranging from 0.2 to 0.6 μM. There was good correlation between the electrophysiological and biochemical data. It is concluded that these analogs and the parent compound HTX, react mainly with the ionic channel of the ACh-receptor, but not with the ACh recognition site. (Supported by NIH grant NS-12063, NSF grant BNS 76-21683 and FAPESP, Sao Paulo, Brazil.)

1189 NEWLY FORMED NEUROMUSCULAR JUNCTION IN TISSUE CULTURE HAS A LOW SENSITIVITY TO LANTHANUM. H. Benjamin Peng*, Shigehiro Nakajima Allan Greenberg*. Dept. of Biol. Sci., Purdue Univ., W. Lafayette IN 47907

Lanthanum (La^{3+}) is one of the most potent agents in enhancing the spontaneous transmitter discharge at the adult amphibian neuromuscular junction. At millimolar concentration it increases MEPP frequency more than 10,000 fold (Heuser et al., Proc. R. Soc. Lond. B. 179: 247, 1971). However, in certain developing junctions, lanthanum has been shown to have little or no effect in transmission (Harris et al., Nature 268: 265, 1977; Kidokoro et al., C.S.H.S. 40: 373, 1976).

We have investigated the effects of lanthanum on newly formed neuromuscular junctions (NMJ's) in tissue culture. Neurons and muscle cells from Xenopus embryos were isolated and put into culture. One day after nerve-muscle co-culture, MEPPS could be recorded from muscle cells contacted by neurites. The MEPP amplitude showed a skewed distribution, ranging from a few millivolts to more than 25 millivolts. The input impedance of these cells was on the average 109 MΩ.

Since we were unable to maintain the co-cultures for more than a week, we used the tail muscle from mature tadpoles as a control in our studies. The tail muscle is the in-vivo counterpart of our cultured muscle cells. The MEPP amplitude in the mature tadpole muscle showed a normal distribution. Lanthanum, 100 μM in unbuffered Ringer solution, caused roughly a 16-fold increase in MEPP frequency over the Ringer control.

To study the effects of lanthanum on newly formed junctions, we recorded MEPPs from junctions 1 to 6 day after nerve-muscle co-culture. MEPPs were continuously recorded in unbuffered Ringer solution, followed by solution containing La^{3+}. Of the 14 junctions examined, only one showed a sensitivity to lanthanum approaching that of the mature junction, viz., it showed a 16-fold increase in MEPP frequency in solution containing 100 μM lanthanum. The majority of junctions (11) showed either no increase or less than 5-fold increase in MEPP frequency in 100 μM lanthanum solution. The remaining junctions showed an intermediate increase, between 6 to 10-fold. This indicates that the newly formed junction has a low sensitivity to lanthanum relative to the mature junction.

Our result suggests that the sensitivity to La^{3+} develops quickly in Xenopus NMJ cultures compared with rat (Kidokoro et al., op cit). However, the developing junction still has a low sensitivity to La^{3+} compared with the mature junction. Assuming that La^{3+} interferes with the Ca^{2+}-transmitter secretion coupling, our finding suggests that the maturation of NMJ involves a development of Ca^{2+}-dependent transmitter release mechanism. (Supported by USPHS grants NS-10457, NS-08601, F32-NS05631, T32-GM07211).

1190 DIVERSE MECHANISMS OF POSTSYNAPTIC RECEPTOR BLOCKADE AT THE MOUSE NEUROMUSCULAR JUNCTION. P. Pennefather* and D. M. J. Quastel. Dept. of Pharmacology, Faculty of Medicine, The Univ. of British Columbia, Vancouver, B. C., V6T 1W5, Canada.

A variety of drugs act to depress the amplitude of end-plate potentials by interference with receptor function. Examination of amplitude and time course of miniature end-plate currents (m.e.p.c.s) and of response to locally applied cholinergic agonists permits identification of at least three different kinds of inhibitory postsynaptic action. In the case of bungarotoxin and curare, m.e.p.c.s are reduced in amplitude and decay somewhat faster than normal. The time course of m.e.p.c.s becomes much closer to a pure exponential decay, as would be expected from reduction of the probability of "reverberatory" ACh action. In the presence of curare, or other agents that act similarly, the response of the end-plate to exogenously applied ACh or carbachol is depressed much more than the m.e.p.c.; this can be expected from simple models of ACh interaction with receptor, when binding of ACh to receptor is inhibited.

Local anaesthetics and some other agents (e.g., pentobarbital, diphenylhydantoin) characteristically cause the m.e.p.c. decay phase to be "split" into two or more components; there is little change of peak amplitude and the early (fast) phase of decay is little affected by ethanol or inhibition of AChE.

A third mode of postsynaptic "blockade" is exhibited by a variety of agents with general anaesthetic properties, including at least paraldehyde, pentane and some alcohols (e.g., butanol, pentanol). The action resembles that of curare in that (a) m.e.p.c.s are reduced in amplitude; (b) the time course of m.e.p.c.s becomes somewhat briefer than normal (with the exception of the alcohols, which also have an ethanol-like action to prolong m.e.p.c.s); (c) ethanol and AChE inhibition continue to prolong m.e.p.c.s and (d) there is no "split" such as is produced by local anaesthetics. However, these agents contrast with curare in that they inhibit the response of end-plates to exogenously applied cholinergic agonists much less than they depress me.p.c.s. Moreover, the time course of m.e.p.c. decay is not affected in the same way as with curare or bungarotoxin. The effects of these agents are difficult to explain, except in terms of modification of rate constants in rather complicated schemes of interaction of ACh with receptor.

Supported by grants from the Medical Research Council of Canada and the Muscular Dystrophy Association of Canada.

1191 RUETHENIUM RED BLOCKS SPONTANEOUS, EVOKED, AND IONOPHORE-INDUCED RELEASE OF TRANSMITTER AT THE NEUROMUSCULAR JUNCTION. R.J. Person and G.A. McLean, Dept. of Physiology and Biophysics, O.U. Health Sciences Center, Oklahoma City, OK 73190

Ruthenium Red (RuR), a heavy metal, histochemical stain for mucopolysaccharides, also blocks calcium (Ca) binding sites thereby inhibiting the translocation of Ca across membranes and probably also inhibiting Ca-dependent processes such as excitation-secretion coupling. We observed changes in evoked and spontaneous transmitter release, measured as endplate (EPPs) and miniature endplate potentials (MEPPs), at the frog neuromuscular junction using standard intracellular recording techniques while junctions were exposed to 1-5 μM concentrations of crude RuR (Sigma). Junctions were additionally exposed at various times to 100 μM concentrations of the cation ionophore X537A.

RuR at 5 μM blocked both evoked and spontaneous transmitter release within 2 min of initial exposure. At 1 μM, the dye produced a similar blockade with a monotonic decline in MEPP frequency, or, with an early acceleration of MEPP frequency. Evoked transmitter release was blocked by a reduction in quantal content. Although there was a 50% reduction in MEPP amplitude at 1 μM RuR, this reduction was not sufficient to cause the disappearance of recordable MEPPs. Simultaneous exposure of junctions to 1 μM RuR and 100 μM X537A resulted in the typical ionophore-induced catastrophic reaction: acceleration of MEPP frequency and subsequent block of MEPPs within 1-2 min of ionophore exposure with simultaneous muscle fibrillation and a decline of membrane potential in the region of the endplate. If X537A exposure was delayed until the RuR-induced blockade of MEPPswas nearly complete no response to the ionophore is observed except for resting potential decline.

These results demonstrate that RuR acts to block both extra- and intracellular Ca-binding sites. Intracellular blockade of Ca transport sites on organelles which modulate terminal [Ca] would explain an increase in MEPP frequency as suggested by Alnaes and Rahamimoff (J. Physiol., 248:265) but it does not explain the subsequent block of spontaneous release unless RuR also blocks an intracellular Ca-binding site required for transmitter release. Extracellular blockade of voltage dependent Ca channels is expected from the reported specific actions of the dye with a consequent reduction in evoked release. However, blockade of the effects of X537A action by RuR suggests that either the extracelluar membrane site of action of the dye is sufficiently nonspecific to prevent interaction of the Ca-ionophore complex with the membrane or, that one or more of the sites to which RuR binds are required for Ca translocation across the membrane via the ionophore. (Supported by Biochemistry Section, Office of Naval Research, N 00014-77-C-0630, NR 202-091.)

1192 δ-AMINOLEVULINIC ACID INHIBITS EVOKED RELEASE BY A PRESYNAPTIC MECHANISM IN RAT NEUROMUSCULAR JUNCTIONS. Jackson B. Pickett, Joel C. Bornstein* and Ivan Diamond Dept. Neurology, Sch. Med., U.C.S.F., San Francisco, CA 94143.

Acute attacks of weakness are a prominent feature of the hepatic porphyrias. The hepatic porphyrias are caused by a defect in heme synthesis leading to increased excretion of δ-aminolevulinic acid (ALA) and porphobilinogen. During acute attacks, serum levels of ALA may rise to 10^{-6} -- 10^{-5} M. It has been suggested that the symptoms of porphyria result from elevated serum levels of ALA. We investigated this possibility using conventional microelectrode techniques in a rat phrenic nerve-hemidiaphragm preparation.

End-plate potential (EPP) amplitude in curare-treated preparations was reduced 90% by 12 mM ALA. 0.2 mM ALA did not change EPP amplitude. EPP amplitude could be reduced by reducing the number of quanta released by a nerve stimulus, or by reducing the postsynaptic response to a single quanta. To distinguish between these two possibilities we increased the magnesium/calcium ratio in the bathing solution and found that the number of quanta released by 0.5 Hz stimulation was reduced by ALA without changing quantal size as determined by measuring miniature end-plate potential amplitude. The mechanism of this presynaptic inhibition of evoked release is unknown. ALA mimics the action of γ-aminobutyric acid (GABA) in frog spinal cord and at crayfish stretch receptors. However, picrotoxin (0.1 mM), a GABA antagonist, did not prevent the usual decline in EPP amplitude seen with ALA.

The concentration of ALA required to reduce EPP amplitude in rat neuromuscular junctions is more than that seen in the serum of patients with acute porphyria. This suggests that circulating ALA is not the sole cause of weakness seen in patients with acute porphyria.

(Supported by U.C.S.F. funds and N.I.H. grant P17 AM18520)

1193 THE APPEARANCE OF ACETYLCHOLINESTERASE AT NEWLY FORMED NEUROMUSCULAR JUNCTIONS IS REGULATED BY NERVE-MUSCLE ACTIVITY. L.L. Rubin, S.M. Schuetze*, C.L. Weill and G.D. Fischbach. Dept. Pharmacol., Harvard Med. Sch., Boston, MA 02115.

Acetylcholinesterase (AChE) accumulates at 70% of nerve-muscle synapses that form in spinal cord explant-muscle cocultures. Enzyme activity was detected histochemically and also by measuring the rate of decay (τ) of extracellularly recorded synaptic potentials (ExSPs). The two measures were closely correlated: 76% of synapses with τ<1.8msec (30°C) stained for AChE, whereas none with τ>2.6msec stained. In addition, the end-plate specific (19S) form of the enzyme was found by sucrose gradient centrifugation of extracts of dissociated spinal cord cell-muscle cocultures.

The appearance of synaptic AChE, in contrast to synapse formation itself and to the clustering of acetylcholine receptors (AChR), is dependent upon some aspect of nerve-muscle activity. When cultures were grown in tetrodotoxin ($3x10^{-7}$M), or the AChR antagonists curare (50-250μM) or α-bungarotoxin ($2x10^{-8}$M), the mean rates of ExSP decay were prolonged--the percentage of synapses with τ<1.8msec decreased by a factor of 5. Only 2% of the synapses in these cultures stained for AChE. Further, although total AChE activity did not decrease, the 19S form of the enzyme was not detected in cocultures grown in curare. Thus, nerve-muscle activity is apparently required for the assembly, as well as for the accumulation at synapses, of this unique form of AChE. Activity of muscle cells seems to be a crucial factor. Synaptic AChE did not appear when cultures were grown in 3μm curare, which reduced synaptic potential size by only 50%, but which nearly abolished muscle twitching. Moreover, muscle activity alone was sufficient for the appearance of AChE at synapses. Myotubes in cocultures grown in 50μm curare were stimulated directly (in the presence of curare) through an extracellular electrode at a rate of 2-3/sec for 8-12 hours. ExSPs at 5 of 5 previously identified synapses decayed more rapidly (mean τ at 30°C decreased, from 2.76msec to 1.74msec). This change, in which each synapse served as its own control, was quite significant.

We are now investigating factors which can replace synaptic transmission and/or muscle activity in inducing synaptic AChE. In cultures grown in 50μm curare and 10-100μm dibutyryl cyclic GMP, the majority of synapses exhibited rapidly decaying ExSPs, and 70% stained for AChE. Other factors tested thus far--including dibutyryl cyclic AMP (100μm), the calcium ionophore A23187 (0.5μm) and veratridine (1.5μm)-were ineffective.

1194 MATHEMATICAL MODEL OF THE DYNAMICS OF SYNAPTIC TRANSMISSION AT THE NEUROMUSCULAR JUNCTION. Claude Sarrazin* and Patrick Cavanagh* (SPON: Franco Lepore). Dept. Psychol., Univ. de Montréal, Montréal, Québec, Canada H3C 3J7.

Two different hypotheses are normally suggested to explain the phenomena of facilitation (F), depression (D) and post-tetanic potentiation (PTP) which characterize synaptic transmission (ST): the first attributes these phenomena to variations in release probability resulting from the action of calcium at the presynaptic level; the second attributes the phenomena to variations in the immediately available transmitter store. Our approach has been 1) to simulate with first degree linear equations the possible interactions of different physiological mechanisms (transmitter synthesis and vesicle recharging movement and mobilisation of vesicles within the synapse, exocytosis at release sites vesicle membrane recycling) that may influence ST during and following stimulation and 2) to correlate the postsynaptic potentials (PSPs) predicted by these mechanisms with the experimental PSPs obtained with different frequencies and durations of stimulation in a variety of studies of the neuromuscular junction. Using a modified Marquardt minimum sum of squares procedure for adjusting the values of the model's parameters, it was possible to account for all the observed phenomena (F, D and PTP; minimum correlation obtained was $r = 0.92$, $p < 0.01$) in the six groups of data analysed.

The values of model's parameters and their variations as a function of the components of synaptic preparations (Ca and Mg concentrations, temperature) revealed that the two hypotheses given above to explain F, D and PTP are not necessarily mutually exclusive but rather complementary. The main conclusions of the study are 1) that F and D are mainly imputable to the variations of the immediately available transmitter store and its blockage by "empty" vesicles rather than the modification of release probability, 2) that vesicular movement under the control of intracellular calcium is largely responsible for the variations of the available store during stimulation, and 3) that, following a long stimulation, the production of the PTP results from a residue of active calcium that acts both on the amount of available transmitters and on the probability of release.

1195 CHANNEL OPEN TIME DECREASES POSTNATALLY IN RAT SYNAPTIC ACETYLCHOLINE RECEPTORS. S.M. Schuetze* and G.D. Fischbach. (SPON: S.W. Kuffler). Dept. Pharm., Harvard Med. Sch., Boston, MA 02115.

The mean channel open time (τ) of acetylcholine receptors (AChR's) was studied in skeletal muscle fibers of the chick and rat at different stages of development. The average τ of AChR's in small (ca. 15μm^2) discrete membrane patches was estimated by spectral analysis of ACh-induced membrane current fluctuations recorded with an extracellular pipette filled with 40μM ACh. Previously (Schuetze et al., PNAS 75:520, 1978) no difference in τ was detected between receptors clustered at synapses and more diffusely distributed AChR's on cultured embryonic chick myotubes innervated by spinal cord explants. For both receptor populations, τ was rather long (4 ms at 23°) with a Q_{10} of 3.1. Apparently, τ is independent of innervation and receptor density. We now report that synaptic AChR's in anterior latissimus dorsi and intercostal muscle fibers of 4- to 18-week posthatched chicks have the same, relatively long open times as found in embryonic muscle grown in vitro. The decay of extracellularly-recorded synaptic currents, an independent estimate of τ, was prolonged in junctions of both muscles and was in good quantitative agreement with the results of fluctuation analysis. Thus the mean channel open time of AChR's clustered in adult chick junctions is longer than that recorded in other species at the same temperature. The metabolic half-life of chick synaptic AChR's increases from 30 hr to more than 5 d during the third week after hatching (S. Burden, Devel. Biol. 61:79, 1977). We conclude that τ is independent of metabolic stability as well as receptor clustering.

We have confirmed earlier reports that synaptic AChR's in adult rat diaphragm fibers have short τ's (1.4 ms at 21°). However, the open time of AChR's in junctions of neonatal (0-4 d post-partum) rat diaphragm fibers was much longer (4.5 ms at 21°) than in adult junctions. Synaptic current decay constants were 3 times longer in neonatal than adult fibers. The slow decay in neonates was not due to lack of acetylcholinesterase: the enzyme was demonstrated histochemically and methane sulfonyl fluoride (an AChE inhibitor) prolonged the decay two-fold. Studies of 1-15 d rats have shown that fibers with short τ's first appear 6 d after birth and are predominant by 13 d. Between 6-11 d, some junctions exhibited complex ACh power spectra and biphasic synaptic currents. These junctions may contain a mixture of AChR's with fast and slow channels. Experiments are in progress to determine what (if any) other changes in synaptic structure and function occur during this interval. In any case, it is clear that a change in AChR channel open time occurs long after functional transmission is established.

1196 MOLECULAR FORMS OF CHOLINESTERASES IN CHICKEN MUSCLES. Janez Sketelj*, Mark G. McNamee* and Barry W. Wilson. Depts. of Avian Sci. and Biochem., UCD, Davis, CA 95616

AChE activity varies during development, behaves differently in fast and slow chicken muscles and is defective in its regulation in dystrophic and denervated muscles. The relationship between the molecular species of AChE and BChE in these muscles and in plasma was studied by velocity sedimentation in 5-20% linear sucrose density gradients.

A 7s form of AChE predominated and 11s and 20s forms were found in normal and dystrophic embryo pectoral muscle. Muscle cultures did not contain the 20s form present in their tissue-of-origin.

A 20s AChE form predominated in normal fast PLD muscle of 10-13 week old chickens. 7s and 11s forms were especially reduced. Total AChE activity was much lower than in embryo muscle. The high AChE activity of dystrophic muscle was accompanied by increases in 7s, 11s, and to a lesser extent in 20s AChE forms.

AChE activity increased after denervation of normal and dystrophic muscles. In both cases activity of the 20s form disappeared and the 7s form increased. Twelve days after denervation AChE patterns were the same in normal and dystrophic muscle. Patterns from dystrophic muscle resembled those of embryo more than those of denervated muscle.

The AChE pattern of slow ALD muscle (a muscle little affected by dystrophy) was the same in both lines of chickens and differed from that of the fast PLD. Activity of the 7s form was high and 20s form low.

BChE activity was high in dystrophic PLD muscles. Most sedimented as a 5s form; 3 other forms (7s, 11s, 20s) sedimented with the corresponding AChE forms. The 20s form disappeared and 7s and 11s forms increased following denervation of dystrophic muscles. The final pattern was the same as that found in denervated normal muscle.

The relationships between these sedimentable forms and those revealed by acrylamide gels and the AChE forms in plasma of embryo and dystrophic muscle will be shown.

The results reconfirm that AChE rises after denervation of the chicken; they add to the evidence that AChE and BChE are regulated by neural activity, and that this regulation is interrupted with denervation and defective in dystrophy.

(Supported by the Fulbright-Hays Act, NIH and the MDA)

1197 QUINACRINE ON NEUROMUSCULAR TRANSMISSION. M-C. Tsai*, A.C. Oliveira*, A.T. Eldefrawi*, M.E. Eldefrawi* and E.X. Albuquerque. (SPON: J.E. Warnick). Dept. Pharmacol. & Exp. Ther., Univ. Maryland, Sch. Med., Baltimore, MD 21201.

The effect of quinacrine on neuromuscular transmission was studied on the rat soleus and frog sartorius muscles. At 10 μM and 30 μM quinacrine blocked the indirect stimulated muscle twitch by 40% and 80%, respectively. At 200 μM, neuromuscular transmission was completely blocked in 10 min and membrane depolarization induced by carbamylcholine (0.7 mM) was simultaneously reduced. At 50 μM, the drug reduced the carbamylcholine-induced membrane depolarization by 80%. However, it had no significant effects on either resting membrane potential or quantal size. Quinacrine (30 μM) markedly decreased the amplitude and time constant of the decay phase of the endplate current (EPC) recorded at -90 mV in glycerol-treated frog sartorius muscles by 78 and 62%, respectively. The voltage-current relationship of the EPC became nonlinear in the presence of quinacrine and the time constant of decay was less dependent on membrane potential. There was no effect on either the EPC reversal potential or the single exponential function of the falling phase. Quinacrine inhibited acetylcholinesterase (K_i=0.3 μM) and the binding of ^{3}H-perhydrohistrionicotoxin and ^{3}H-acetylcholine to acetylcholine receptor of the electric organ of Torpedo ocellata (K_i=17 μM and 60 μM respectively). Since the blockade of acetylcholinesterase occurs simultaneously with the partial block of the acetylcholine receptor-ionic channel complex, it was difficult to record a typical prolongation of the EPC as seen with other anticholinesterase compounds such as neostigmine. The data suggest that quinacrine alters neuromuscular transmission in a rather complex manner, that is, by altering the kinetics and voltage sensitivity of the ionic channel, partially blocking the receptor recognition sites for acetylcholine, and blocking acetylcholinesterase. (Supported, in part, by USPHS Grant NS-12063, NSF Grant BNS 76-21683 and FAPESP, Sao Paulo, Brazil.)

1198 END-PLATE BLOCK BY GUANIDINE DERIVATIVES. Shigenori Watanabe*, Jerry Farley* and Toshio Narahashi (SPON: S.C. Cheng). Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

The effects of methylguanidine (MG), 1,1-dimethylguanidine (DMG) and amylguanidine (AG) on the neuromuscular transmission in the frog were studied. MG, DMG and AG reduced iontophoretically induced ACh potentials with ED_{50} of approximately 0.5 mM, 5 mM and 0.2 mM, respectively. They had little effect on the input resistance of the muscle in these doses. The end-plate potential was reduced, but the end-plate blocking actions of low doses of MG and DMG was obscured by the stimulation of transmitter release in these doses. The I-V relation of the peak amplitude of end-plate current (EPC) and membrane potential (E_m) as determined by voltage clamp is normally linear. However, in the presence of DMG, MG or AG there was a dramatic rectification of the currents at E_m more negative than the reversal potential (E_r). E_r was unaffected by these compounds. At E_m more negative than E_r the EPCs were greatly reduced. At E_m more positive than E_r the I-V relation showed no rectification. Shifting E_r by approximately 20 mV to E_r' by replacing Na^+ with sucrose did not alter this pattern of rectification, that is rectification occurred only at E_m more negative than E_r'. The suppression of the outward EPC was removed by repetitive stimuli. We conclude that these guanidine derivatives block the end-plate channels. This block seems to be current dependent and reversed by outward current flow through the channels. Supported by NIH grant NS 14145.

1199 NERVE MUSCLE SYNAPSE FORMATION: FIRST ATTEMPTS TO BLOCK WITH EMBRYONIC MUSCLE MEMBRANES AND ANTI-MEMBRANE ANTIBODIES. C.L. Weill, C.J. Loria*, G.D. Fischbach. Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115.

Membranes were partially purified from homogenates of cultured chick myotubes and from freshly dissected 14 day embryonic muscles by differential centrifugation followed by separation on a discontinuous sucrose density gradient. Five fractions were collected at the following sucrose interfaces: I=8-20%, II=20-27%, III=27-32%, IV=32-40%, and V=40-55%. As previously reported (Schimmel et al. PNAS 70, 3195, 1973), the highest specific activity of ^{125}I-α bungarotoxin binding sites (ACh receptors) were found in fractions I and II. Typically, these fractions contained 9-18 times as many toxin sites/mg protein as the homogenate. Acetylcholinesterase activity was more uniformly distributed over the gradient but the specific activity of the relatively large (19S), end plate form was highest in fraction III. The 19S form was found in innervated (14-day) embryonic muscle but not in uninnervated cultured myotubes. Antisera were raised in rabbits and guinea pigs against the crude (40,000g) membrane pellet and also against each of the first three fractions. Anti-muscle antibodies were detected on uninnervated and innervated myotubes in vitro by indirect immunofluorescence. In all cases the fluorescence was apparently distributed uniformly over the myotube surface.

Muscle membranes (pooled fractions I-III) (but not anti-membrane antibodies) bind to nerve processes that grow out of cultured spinal cord explants. This was detected by indirect immunofluorescence and by scintillation counting after chemically iodinating the membranes. Preliminary experiments indicate that muscle membranes bind more avidly to a monolayer of spinal cord cells than to a monolayer of fibroblasts.

Our first attempts to block nerve-muscle synapse formation in spinal cord explant-muscle cocultures, with material from fractions I-III, have been negative, even when 100μg of protein (>1000 x the estimated amount of membrane in the target muscle cells) was added. Attempts to block synapse formation with anti-muscle antibodies have been complicated by the fact that at high concentrations the immune sera and purified IgG were invariably cytotoxic in the long run. Although no acute effects have been observed on membrane potential, ACh sensitivity, or synaptic transmission, most of the myotubes were lysed after 12-18 hours in the presence of 1:10 dilutions of the sera or 100μg/ml of IgG. The toxicity was selective in that nerve processes and mononucleated cells in the same cultures and liver cells, heart cells, and fibroblasts in other cultures were spared.

Supported by grants from the NIH (NS11160) and MDAA. C.L.W. is a MDAA postdoctoral fellow.

1200 NEUROMUSCULAR FUNCTION RELATED TO HISTOCHEMICALLY DETECTABLE ACETYLCHOLINESTERASE ACTIVITY AT THE ENDPLATE. Otto L. Wolthuis and Larry A. Kepner. Med. Biol. Lab. TNO, P.O. Box 45, 2280 AA Rijswijk Z.H., The Netherlands.

During studies of oxime therapy following intoxication with organophosphorous cholinesterase inhibitors (OP's), the question arose as to what relationship exists between histochemically detectable acetylcholinesterase activity at the endplate and the degree of neuromuscular function (NMF).

NMF of isolated phrenic nerve diaphragm preparations from rats was determined from recordings of tetanic contractions following indirect stimulation with four 3 sec trains of 25, 50, 100 and 200 stim/sec, respectively. Each curve was graded on a scale from 0 (full block), 1/3, 2/3 to 1 (fully sustained tetanus). After full blockage by the OP tabun, the reactivator obidoxime was administered in varying doses and recovery of NMF was determined. Immediately thereafter the diaphragms were stained histochemically for AChE-activity and the staining intensities were graded on a scale from 0 to 4. All gradings were done 'blind', by two investigators and the results were averaged.

A good correlation was found between AChE-activity and NMF (r=0.93; conf. limits 0.97-0.87). Furthermore, 1) AChE-activity fell below detection level and must be very low when considerable NMF still existed and 2) at approximately grade 3 of the AChE staining levels all tetanic contractions were fully sustained. The former finding is in accordance with earlier results obtained with other methods but the latter finding seems not to be. The degree of staining found seems to indicate a higher amount of endplate AChE-activity than the 50% required for normal neuromuscular function suggested by earlier studies. Possible explanations for this discrepancy are discussed.

It is concluded that a clearcut relation exists between endplate AChE-activity and NMF. The results suggest that the use of histochemical staining may provide more valid results than does measurement of hydrolysis of exogenous acetylcholine.

NEURONAL CIRCUITS AND PATTERN GENERATION

1201 SYNAPTIC PERTURBATION AND ENTRAINMENT OF THE GASTRIC MILL RHYTHM OF THE SPINY LOBSTER. Joseph Ayers, Dept. of Biology, B-022, U.C.S.D., La Jolla, CA. 92093.

The control of the gastric mill network of the spiny lobster by the IVN through fibers has been studied as a model system for the control of neuronal oscillators by synaptic input. The gastric mill rhythm is generated by a network of ten motor neurons and four interneurons resident in both the stomatogastric and commissural ganglia (Prog. Neurobiol. 7:215-290). The gastric rhythm basically consists of synergistic bursts in the E, LG and MG neurons which alternate with the discharge of Int 1. The effect of IVN input trains (500 msec duration, 20/sec), depends on where they occur in the ongoing gastric cycle. Inputs which occur during or near the end of the LG-MG burst delay subsequent bursts by prolonging the duration of the E-LG-MG burst and delaying the subsequent LG-MG burst by a proportional amount. Inputs which occur at the termination of an ongoing LG-MG burst may have one of two effects. If they occur before the end of the E neuron burst, they prolong the E cell burst and may trigger a short LG-MG burst. If the E neuron burst has terminated, they may trigger a longer burst which is of the same duration as a normal LG-MG burst, but intercalcated between the two ongoing LG-MG bursts. One may conclude therefore that the characteristics of the responses depend on the detailed activity status of several elements of the network. This complexity of organization contrasts with the response of the pyloric network to similar input, where the responses depend only on the activity state of one set of elements.

Repetitive IVN trains which occur at frequencies near that of the free run gastric rhythm can entrain the rhythm. If the repetitive stimulus is slightly faster than the free run gastric rhythm, it tends to occur after the termination of the ongoing LG-MG burst in the interburst interval. In such situations all entrained bursts have the characteristics of intercalcated bursts. If the entraining cycle is slightly slower than the free run gastric rhythm, the stimulus tends to occur near the end of the LG-MG burst where they cause a prolongation of the E neuron burst. Thus the phase relationships which obtain during entrainment of the gastric oscillator depend on the ratio of frequencies of the cyclic stimulus and the free run gastric rhythm.

Supported by: USPHS Postdoctoral Fellowship F32 NS0530 to JA and by NSF and NIH grants to A. I. Selverston.

1202 CROSS-CORRELATION OF EEG SIGNALS FROM OLFACTORY BULB AND CORTEX. Steven L. Bressler. Dept. Physio-Anat. UCB, Berkeley, CA 94720.

Incoming bursts of impulses along primary olfactory nerve fibers set the cellular circuitry in the olfactory bulb into oscillation at a characteristic frequency of 40 to 80 c/s. Bulbar activation is manifested by oscillation of the bulbar EEG (generated by the granule cell population) at this frequency. Mitral cells form the excitatory limb of the feedback circuit which generates the oscillation, so volleys of spikes propagate on mitral axons in the lateral olfactory tract at a density that oscillates with bulbar EEG activity. These spikes activate the prepyriform cortex to produce EEG activity at the same frequency as that of the bulb.

The hypothesis was proposed that during normal physiological activity the prepyriform cortex may be treated as a linear oscillator being driven by the oscillatory input from the bulb. This was tested by measuring the frequency of oscillation and the phase difference between bulbar and cortical signals.

Records were taken from alert, responsive rabbits in which indwelling electrodes had been implanted in the olfactory bulb and an array of electrodes had been placed on the cortical surface. The signals from these electrodes were amplified, digitized at 1 msec intervals, and stored on magnetic tape in 4 sec blocks, ten channels maximum. For a selected bulbar and cortical pair, segments containing bursts of oscillation in both were edited from the 4 sec block. These burst segments typically ranged from 100 to 300 msec in duration. A cross-correlation function was formed between bulbar and cortical signals for each segment after appropriate normalization. The function gave a value for the cross-correlation coefficient each time the signals were shifted with respect to one another by one digitizing interval(1 msec). The maximum coefficient value occurred at a shift which brought the signals into phase. This shift to "in phase" was that fraction of a cycle which supplied the phase angle between bulbar and cortical signals. Since the cross-correlation function of two sinusoids was sinusoidal at the same frequency, its zero-crossings provided a measure of frequency. When phase was plotted as a function of frequency and a linear regression was performed on the data, it was found that the cortex typically lagged the bulb and that the phase lag increased as the frequency increased. The slope and abscissa intercept of the experimental curve were found to conform well to those predicted on the basis of theoretical considerations.

The prepyriform cortex thus can be described by a linear oscillator that is driven by oscillatory input from the bulb. Variations in this mechanism that occur during olfactory information processing will be discussed. Supported by MH 06686.

1203 PREMOTOR NEURONS OF A GASTROPOD BUCCAL GANGLION. Andrew G. M. Bulloch* and Derek A. Dorsett* (SPON: R. M. Pinter). Dept. of Mar. Biol., Mar. Sci. Labs, U.C.N.W., Menai Bridge, N. Wales.

Studies of feeding behaviour in the Atlantic nudibranch mollusc Tritonia hombergi have shown the cycle of intrinsic buccal mass movements to be divided into three phases: Phase I ("Protraction"), Phase II ("Retraction") and Phase III ("Flattening"). Populations of motoneurons which control these phases of movements have been identified and designated as P, R and F cells respectively.

Correctly patterned motor output is determined by a number of common synaptic inputs of different sign to the three motoneuron populations. Three inputs have been specified: Input 1 (active before and during Phase I), Input 1a (active during Phase I) and Input 2 (active during Phase II). These inputs are thought to be derived from buccal interneurons called 1, 1a and 2 respectively.

Searches for the buccal interneurons have revealed several types of premotor neurons: (a) A multiaction neuron which synapses on motoneurons in a manner consistent with Interneuron 1. (b) A group of three or four cells which generate synaptic potentials on motoneurons resembling Input 1, but via polysynaptic pathways. (c) A dorsal white cell which can generate feeding output in a quiescent ganglion and is often active during Phase II. Although the neuron synapses directly on some motoneurons, the synaptic potentials are usually very small (<1mV) and are apparently superimposed upon Input 2. (d) Another neuron capable of initiating motor output, but not exhibiting spontaneous activity. In common with the cells described in (b) and (c), this cell receives inhibitory input from Interneuron 1a.

It is thought that some of these premotor neurons are part of a network which can initiate and sustain feeding, but their functional roles are not yet fully understood. The existence of interactions not predicted by motoneuron recordings indicates the complexity of the neural circuit which underlies feeding.

1204 THE NEURONAL BASIS OF THE HEARTBEAT RHYTHM IN THE LEECH, HIRUDO MEDICINALIS. Ronald L. Calabrese. Dept. of Biology, UCSD, La Jolla, CA 92093

The paired heart tubes of the leech, Hirudo medicinalis are controlled, via excitatory synapses, by a set of bilaterally paired segmental motor neurons, the HE cells. In turn, the HE cells are controlled, via inhibitory synapses, by a set of bilaterally paired segmental interneurons, the HN cells. The HN cells interact with one another both by inhibitory synapses and electrical coupling. In a totally isolated ventral nerve cord preparation, the HE and HN cells display a coordinated activity pattern, during which they produce rhythmic impulse bursts separated by IPSP's from HN cells. This coordinated activity pattern constitutes the central motor pattern for heartbeat in the leech.

To determine whether the heartbeat rhythm results from the synaptic interactions among the HE and HN cells or whether endogenous membrane properties cause certain elements to produce rhythmic impulse bursts, isolated nerve cord preparations were bathed in "low Cl^-" physiological saline. This saline reversibly blocks IPSP's from HN cells onto both HE cells and other HN cells but leaves electrical transmission among HN cells unaffected.

1)The HE cells normally produce rhythmic impulse bursts because their inherent steady discharge is periodically inhibited by the HN cells; in low Cl^- saline the HE cells produce tonic impulse trains.

2)The HN cells (except for the HN(5) cell pair) produce rhythmic impulse bursts endogeneously: (a)low Cl^- saline does not interrupt the impulse burst rhythm of HN cells (except the HN(5) cell pair); (b)in low Cl^- saline brief intracellularly injected hyperpolarizing current pulses can prematurely terminate an HN cell's impulse burst, resetting its impulse burst rhythm; and (c) in low Cl^- saline brief intracellularly injected depolarizing current pulses can prematurely initiate an impulse burst in an HN cell which outlasts the current pulse and resets the cell's impulse burst rhythm.

3)The inhibitory synaptic interactions among the HN cells serve to coordinate their independent activity cycles into their defined pattern; in low Cl^- saline the HN cells loose their fixed activity phase relations to one another.

These results indicate that the HN cells produce rhythmic impulse bursts endogeneously, that their inhibitory synaptic interactions serve to coordinate their independent activity cycles into a functional pattern and that this pattern is imposed the HE cells via inhibitory synapses.

1205 PATTERN DETECTION IN SPIKE TRAINS. Judith Dayhoff*and George Gerstein. Dept. Physiology, University of Pennsylvania, Philadelphia, Pa. 19104

Traditional spike train analysis methods measure time relations among only a few spikes or between a stimulus and spikes. Such methods cannot be used to identify patterns of firing which occur frequently but at arbitrary times. It is appropriate to search for repeating patterns because such patterns could be used for information transfer. We have developed a new method for identifying repeating temporal patterns in a single spike train.

The pattern detection method consists of a statistical test of whether a given temporal pattern occurs more frequently than expected on a random model. In the random model, each interspike interval is independent of all previous intervals. The patterns detected consist of an arbitrary number of sequential interspike intervals, forming a specific "word." The patterns can occur at arbitrary times in the spike train. The calculation does not require a priori specification of a template word. The pattern does not have to be exactly the same each time it occurs. A pattern which varies within certain limits can still be detectable if it occurs sufficiently often. The sensitivity for pattern detection of the new method has been measured by detecting known patterns which were inserted into random spike trains.

The method described here identifies patterns that recur excessively in the data. Once the patterns are identified, one can explore the relation of pattern occurrences to stimulus presentation or to motor function. If each occurrence of the pattern is treated as a point event, the resulting point process can be studied by traditional spike train analysis methods such as poststimulus time histograms, autocorrelograms, or cross-correlograms.

We have analyzed spike trains from experiments with crayfish claw motor and proprioceptive neurons. Certain patterns occur excessively in these data.

Supported by grants NS05606,5T32GM07229, BRSG#RR-05415-16

1206 A NEURAL CIRCUIT INVOLVED IN THE CONTROL OF LOCOMOTION IN APLYSIA. Steven M. Fredman and Behrus Jahan-Parwar. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

Aplysia californica is capable of two mutually exclusive forms of pedal locomotion. During normal locomotion (crawling) the foot is extended and attached to the substrate both anteriorly and posteriorly. A posterior moving wave of contraction pulls the animal forward. In rapid locomotion (galloping) the anterior part of the foot is extended and attached, but the tail is released and the entire body contracts off the substrate moving the body forward in a large step. Except for its greater magnitude, it resembles the final stage of crawling when the pedal wave reaches the tail. Crawling can be elicited by such stimuli as food at a distance and can be maintained for long periods. Galloping is elicited by noxious stimuli and is of short duration, lasting only a few cycles. It requires intact cerebro-pleural (C-PL) connectives. We have examined synaptic connections between neurons in the cerebral and pleural ganglia which appear to be involved in mediating part of galloping.

The initiation of crawling requires input from the cerebral ganglion descending to the pedal ganglia. The cerebral B cluster neurons which appear to be pedal and parapodial motoneurons (J. Neurophysiol., 1978) may be involved in the initiation process. They are tonicly excited by food chemicals. The pleural ganglia contain a group of neurons which appear to be involved in the tail retraction phase of galloping. When stimulated intracellularly they evoke a tail contraction which is followed by a contraction of the middle of the foot. These neurons are strongly excited by noxious stimuli, particularly to the tail and indirectly inhibited by stimuli which excite the B neurons. The pleural neurons in turn monosynapticly inhibit the B neurons. These connections, along with those made by other pleural neurons may serve as part of the control system for galloping. Each part inhibits the conflicting behavior. Stimuli which excite the B neurons and elicit crawling which requires both head and tail attachment, inhibit the pleural neurons which contribute to tail retraction. Noxious stimuli to the tail excite the pleural neurons, inhibiting the B neurons and crawling. The effect of removing this mutual inhibition can be seen when a noxious stimulus is presented during ongoing crawling to animals with C-PL lesions. The resulting tail and posterior body contractions totally disrupt crawling for up to several minutes. This can be interpreted as the result of a conflict between commands for mutually exclusive behaviors. Since crawling and galloping have common elements the neurons in this circuit may contribute to specific phases of crawling as well. This work was supported by PHS grant NS 12483 to BJP.

1207 DETECTION OF LOW-AMPLITUDE WATER MOVEMENTS: A NEW SENSORY MODALITY IN THE MEDICINAL LEECH. W. Otto Friesen and Rosier D. Dedwylder II*, Department of Biology, University of Virginia, Charlottesville, VA 22901.

The ability of the leech Hirudo medicinalis to detect water movements associated with water waves was investigated by observing the effects of waves on the behavior of intact leeches and on the activity of identified neurons in segmental ganglia of the leech nerve cord. For behavioral experiments, leeches were placed lengthwise at the bottom of a narrow trough, 10 cm wide and 80 cm long, which was filled to a depth of 2 cm with pond water. Drops of pond water were then released from a pipette, held at a height of about 6 cm, either 10 cm in front of or 10 cm behind the leech to produce a series of small water waves. Previously quiescent leeches responded to these waves by initiating directed swimming movements; namely, they swam straight ahead if the waves were initiated from in front or they turned and swam towards the rear if the waves were initiated from behind. These experiments demonstrate that water waves can provide leeches with directional cues as well as provide a stimulus to initiate swimming movements.

That the cells tranducing the stimulus provided by the water waves are not the previously identified mechanoreceptor neurons, T, P and N (Nicholls and Baylor. J. Neurophysiol. 31: 740-756, 1968; D. Mistick. Thesis. University of California, Berkeley, 1975), was demonstrated by the following experiments. A nearly isolated preparation, consisting of the leech ventral nerve cord attached to a small flap of body wall by the nerve roots of one ganglion, was placed in a dish and covered to a depth of 0.5 cm with saline. Waves were created by saline drops falling into the dish from height of about 6 cm. Intracellular recordings from identified mechanoreceptor neurons (including all of the touch cells) in the ganglion innervating the body wall flap failed to detect any wave-evoked impulse activity, even though simultaneous extracellular recordings from connectives and segmental nerves showed bursts of activity associated with the waves. In addition, intracellular recordings from AE cells under similar conditions showed that waves evoked 5 mv hyperpolarizations in these cells, whereas previous researchers (Jansen et al. J. Physiol. 242: 289-305, 1974) have shown that stimulation of T, P and N cells evokes excitatory potentials in AE cells. Thus leeches have sensory receptors, not previously identified, which are sensitive to low-amplitude water movement and whose activity can initiate swimming behavior. (Supported by a subgrant from the UVA-NIH Biomedical Sciences Support Program.)

1208 AUTORADIOGRAPHIC PROJECTIONS OF THE ANTERIOR MEDIAL CORTEX OF THE RAT. D.C. German, M. Dalsass*, M. Mendershausen* and R.S. Kiser. Depts. of Physiol. and Psychiat., U. of Texas Health Sci. Ctr., Dallas, TX 75235.

The anterior medial cortex of the rat is an area which supports intracranial self-stimulation behavior, and this behavior is thought to be related to its midbrain dopaminergic input (Myers & Mora, 1977). The connections of the anterior medial cortex, in the region of the anterior cingulate-prefrontal cortex, have been studied with silver degeneration (Leonard, 1969; Domesick, 1969), and horseradish peroxidase (Beckstead, 1976) methods. In order to further map the projections of this area, iontophoretic ejections of S^{35}-methionine or H^3-adenosine were made and the tissue was prepared for autoradiography. The rats treated with S^{35}-methionine (567-1065 Ci/mmole) survived from 1 to 4 days, and the rats treated with H^3-adenosine (35 Ci/mmole) survived 3 days. Following a 7-day (methionine) or 21-day (adenosine) exposure period, the tissue was developed, stained for Nissl, and examined microscopically. In agreement with previous silver degeneration studies, S^{35}-methionine-labelled fibers were observed running caudally in the cingulum and in the internal capsule through and in the dorsomedial caudate nucleus. Fibers left the internal capsule to end in several thalamic nuclei (e.g., dorsal medial, central medial, ventral medial, parataenial). Further caudally, labelled fibers were found in the medial forebrain bundle, the medial tip of the internal capsule, the central gray area, the pretectal area, and the ventral superior colliculus. Labelled fibers also ended in the region of the medial substantia nigra and nucleus A10 dopamine-containing neurons. Unlike the previous work, we also found label in the brainstem raphé nuclei, in a region medial to the locus coeruleus, and further caudally in the brainstem pyramidal tract. Also, we found bilateral projections to the claustrum, lateral amygdaloid nucleus, and extensive contralateral projections often similar to the above-mentioned ipsilateral projections. In the H^3-adenosine tissue, besides many of the above projections, heavily-labelled clusters of cell bodies were found in the anterior medial cortex contralateral to the injection site. Labelled cell bodies were also found in the dorsal medial thalamus. Adenosine has been reported to travel both retrograde and orthograde-transynaptic, and the present results may represent either transport method. These autoradiographic data further our knowledge on the interconnections of the anterior medial cortex in the rat. (Supported by USPHS grant MH-27574.)

1209 CYCLIC AMP MAY MODULATE PROLONGED ENDOGENOUS BURSTING AND SPIKE BROADENING IN THE VENTRAL WHITE CELL OF Pleurobranchaea californica. Martha U. Gillette*, Rhanor Gillette and William J. Davis. Thimann Labs, UCSC, Santa Cruz, CA 95064.

Intracellular injection of cyclic AMP as well as bath application of cyclic AMP analogues or isobutylmethylxanthine (IBMX, which augments native cyclic AMP) alter the parameters of the prolonged endogenous bursts of the Ventral White Cell (VWC), a neuron which can drive the neural output of feeding behavior of Pleurobranchaea. Such treatments increase burst duration and decrease interburst interval. They also accelerate the progressive broadening of the action potential waveform during the prolonged burst. The effects of these treatments may last until drug washout at 4 hr.

In the accompanying paper we present evidence that altering a Ca^{++}-activated K^+ conductance has similar modulatory effects to those reported here for cyclic AMP. Therefore, the relationship of cyclic AMP to this conductance was investigated. The waveforms of undershoots (after-hyperpolarizations) of action potentials in VWC's treated with intracellular cyclic AMP injections, IBMX or cyclic AMP analogues were found to be attenuated in identical fashion to those of VWC's treated with blockers of Ca^{++}-activated K^+ conductance (0-Ca^{++}, 30 mM Co^{++}, substitution of Ba^{++} for Ca^{++} or intracellular injection of EGTA.) Intracellular injection of high Ca^{++} buffer (5×10^{-7} M free Ca^{++}) reverses the effects of cyclic AMP on spike undershoot, as well as on spike broadening and bursting.

These findings suggest that cyclic AMP is altering either the ability or the availability of Ca^{++} to activate the Ca^{++}-activated K^+ conductance and that this is the action by which cyclic AMP modulates bursting and spike broadening. We speculate that cyclic AMP increases burst duration and frequency by enhancing the rate of removal of free cytoplasmic Ca^{++}. Since the VWC is still responsive to added IBMX after a 3 hr incubation in 2 mM CN^-, a potent blocker of mitochondrial Ca^{++} sequestration, the ultimate site of action of cyclic AMP in the control of intracellular Ca^{++} levels may be at a membrane Ca^{++} pump.

1210 PROLONGED ENDOGENOUS BURSTING AND SPIKE BROADENING ARE SUBSTRATES OF COMMAND FUNCTION IN THE FEEDING NETWORK OF Pleurobranchaea californica. Rhanor Gillette, Martha U. Gillette* and William J. Davis. Thimann Labs, UCSC, Santa Cruz, CA 95064.

A symmetrical pair of neurons in the buccal ganglion of Pleurobranchaea, the Ventral White Cells (VWC's), drives the cyclic neural output of feeding behavior when tonically stimulated with depolarizing currents. The VWC's are endogenously bursting neurons which display unusually long burst durations (1-4 minutes) and long interburst intervals (3-15 minutes). The long enduring bursts of a VWC may be triggered by short depolarizing current pulses or by excitatory synaptic inputs, which thus initiate vigorous feeding output. During repetitive activity, VWC spike duration progressively increases by 6-10 X. Inhibition of spike broadening by hyperpolarization during antidromically-driven trains shows that broadened VWC action potentials are much more effective than unbroadened spikes in driving motor output. This provides functional significance for a commonly observed property of neuronal plasticity, spike broadening during repetitive activity, in commanding motor network output.

Ion substitutions and the use of blocking agents show that Ca^{++} is the major charge carrier in the late phase of the broadened spike. The temporal, ionic and voltage parameters governing the VWC spike broadening fit a model for progressive spike broadening based on progressive depolarization-induced inactivation of a voltage-dependent K^+ conductance, which was proposed by Thompson and Getting (Neurosci. Abs. 3: 594, 1977.)

Further experiments indicate that a distinct Ca^{++}-activated K^+ conductance, while not causal, is a powerful modulator of spike broadening which determines rate and extent of broadening. Treatments which reduce Ca^{++}-activated K^+ conductance by lowering $(Ca^{++})_i$ enhance rate and extent of spike broadening; conversely, increasing $(Ca^{++})_i$ suppresses broadening. Since broadened spikes are more effective at driving network output than unbroadened spikes, $(Ca^{++})_i$ may determine the ability of the VWC's to drive the feeding rhythm.

1211 CRAYFISH MOTORNEURONES ARE AN INTEGRAL PART OF THE SWIMMERET CENTRAL OSCILLATOR. W.J. Heitler and B. Mulloney. Dept. of Zoology, University of California at Davis, Davis, CA 95616.

Experiments performed on the isolated abdominal nerve cord of Pacifastacus leniusculus indicate that the swimmeret motorneurones are not simply output elements which passively integrate and relay the activity of pre-motor interneurones and sensory neurones. Recordings from the integrative segments of swimmeret motorneurones show that individual motorneurones are coupled to populations of other motorneurones. The coupling typically takes a form such that depolarising current injected into one motorneurone excites its synergists and inhibits its antagonists, while hyperpolarising current excites its antagonists and inhibits its synergists. Reciprocal interactions occur between power- and return-stroke motorneurones. The interactions can result from subthreshold changes in membrane potential; they could be mediated either by electrical or graded chemical synapses. Subthreshold interactions have been demonstrated between motorneurones in adjacent ganglia, and it is likely that these at least are mediated by a polysynaptic pathway involving spiking interneurones.

When the C.N.S. is producing the rhythmic motor output associated with swimmeret beating in the intact animal, motorneurone membrane potentials undergo rhythmic oscillations of about 10 mV amplitude, which, in the isolated preparation, can be subthreshold in some motorneurones. Under these circumstances injection of current into a single motorneurone can affect the intensity, period, and phasing of the oscillator. Thus, continuous hyperpolarising current (1-2 nA) injected into a power-stroke excitor motorneurone (which was not itself spiking) increased the period of the rhythm, decreased the intensity of the power stroke activity, and increased the intensity of the return-stroke activity. Continuous depolarising current (1-2 nA) had the opposite effect on the relative intensity of the antagonist motor output, but had no effect on period. Evidently, it is easier for motorneurones to slow the oscillator down than for them to speed it up. However, short pulses of depolarising current dramatically reset the oscillator. Thus both depolarising and hyperpolarising current injected into a single motorneurone can directly alter the fundamental oscillator properties of period and phase.

The levels of current required to demonstrate these effects are low (< 2 nA), and thus it is likely that the changes in motorneurone membrane potential occurring in the absence of experimental interference are effective in producing these central interactions. The parameters of the motor pattern produced within the C.N.S. which controls swimmeret beating in crayfish must thus be an emergent property of a system of neurones which includes the motorneurones. Supported by U.S. P.H.S. Grant NS 12295.

1212 MEMBRANE PROPERTIES SHAPE SYNAPTICALLY DRIVEN BURSTS IN TRITONIA FLEXION NEURONS. Richard I. Hume and Peter A. Getting. Dept. of Biological Sciences, Stanford University, Stanford, CA 94305

The neuronal correlate of swimming recorded from the isolated brain of Tritonia diomedea is a series of alternating bursts in two relatively large pools of pedal ganglion neurons, the dorsal flexion neurons (DFN) and the ventral flexion neurons (VFN). We have examined how the pools of flexion neurons (FN) are organized, and some of the mechanisms that may be responsible for that organization.

Flexion neurons were sampled in a large number of preparations to assess the variability within the entire pool of flexion neuons. Studies of the organization of spikes within single bursts revealed that bursts can be classified into one of five general burst structures. To test the constancy of burst structure, five DFNs that could be identified by criteria other than their activity during swimming were studied in a number of preparations. Each identified cell had its own characteristic burst structure which was consistent from swim to swim and from animal to animal.

Variations in burst structure between neurons may have two sources: 1) intrinsic membrane properties and/or 2) synaptic drive. To test the contribution of intrinsic membrane properties, identified cells were driven with similar current stimulus waveforms and their resultant activity compared. The pattern of spikes generated was distinct for each cell, and the response correlated well with the structure of that cell's bursts during swimming. Cells may have dramatically different burst structures and response patterns even when they are closely matched in input resistance. Other experiments also support the idea that intrinsic cell properties contribute to burst structure.

We have also found that not all DFNs receive the same synaptic input and that synaptic interactions also contribute to burst structure. Our results suggest that the burst structure of a given neuron arises from the filtering of specific synaptic drive through the intrinsic properties of that cell's membrane.

1213 NEURAL CONTROL OF LOCOMOTION IN APLYSIA. Behrus Jahan-Parwar and Steven M. Fredman. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Cinematographic analysis of pedal locomotion in Aplysia revealed four major phases in each locomotor step: 1) detachment and lifting the anterior foot from the substrate; 2) extension of the detached body region; 3) attachment of the anterior edge of the foot to the substrate; 4) generation of a series of bilaterally coordinated waves of contraction in the resulting arch which pull the animal forward. The arch moves backward until the tail is released and retracted behind the rest of the body, completing the step. In recent work we have demonstrated that the neural program for pedal locomotion resides in the central ganglia and that the peripheral nervous system is not necessary for the spread of pedal waves (J. Neurophysiol. 1978a,b; Comp. Biochem. Physiol. 1978). In the present study we have investigated the role of the individual central ganglia in pedal locomotion using a series of connective lesions.

Coordination of both halves of the foot is maintained by the pedal commissure. When it was sectioned, contractile waves generated in each half of the foot became desynchronized. Subjects with bilateral sections of both cerebro-pedal (C-P) and cerebropleural (C-PL) connectives were unable to locomote normally. Although slow continuously running ripples were present in the foot, head extension and normal waves were absent. The same lesion performed unilaterally resulted in diagonal waves in the foot, with the lesioned side always trailing the intact side. Bilateral sections of the C-P also abolished normal locomotion. These animals were no longer capable of extending and attaching the anterior foot. When the C-PL were cut bilaterally goal directed locomotion remained essentially normal. However, noxious stimuli to the tail were no longer capable of eliciting rapid aversive locomotion (galloping) and disrupted any ongoing locomotion. Bilateral sections of the pleuropedal connectives impaired normal locomotion and eliminated rapid locomotion. Although capable of generating waves in the foot, the rate of these waves and locomotion remained very slow. A unilaterally isolated pedal ganglion failed to generate normal pedal waves on that side.

From these experiments we have drawn the following conclusions: The motor program for locomotion appears to reside in the pedal ganglia but requires the cerebral and pleural ganglia for full expression. The cerebral ganglion via the C-P is necessary for the initiation of normal locomotion. The pleural ganglia appear necessary for controlling the rate of locomotion. They also appear to provide a relay for ascending input needed for rapid locomotion. We are in the process of investigating the neuronal substrates of these relationships. This work was supported by a PHS grant NS 12483 to BJP.

1214 RECIPROCAL CONNECTION BETWEEN DOPAMINE CONTAINING PREFRONTAL CORTEX AND DEEP MESENCEPHALIC REGION. H. Kimura* and T. Maeda* (SPON: W.K. Ovalle). Dept. Anatomy, Shiga Univ. Med. Sci., Shiga, Japan.

Reciprocal neuronal connection between dopamine containing prefrontal cortex and deep mesencephalic region has been studied by means of glyoxylic acid fluorescence histochemistry after the destruction of the dopaminergic ascending pathway in combination with light and electron-microscopic examination of anterograde and retrograde transport of horseradish peroxidase. The dopaminergic cortical innervation is restricted mostly to the frontal region which corresponds to the prefrontal cortex receiving direct projection from the mediodorsal nucleus of the thalamus. Dopaminergic terminals are localized in two main areas. The area of the highest density is the pregenual field of the anteromedial cortex and the axons are distributed mainly in the intermediate cortical layers (III-V) except in the frontal pole where the laminer pattern of the cerebral cortex is not clear. A moderate fiber density is found in the sulcal field, forming the dorsal part of the rhinal sulcus, in which the dopaminergic fibers are also scattered in the molecular layer as well as in the intermediate cortical layers. No dopaminergic innervation is observed in the dorsolateral part of the frontal cortex. Two mesencephalic cell groups, which project fibers to the frontal cortex, were demonstrated by retrograde as well as anterograde transport of horseradish peroxidase. The dopaminergic fibers of the anteromedial cortex originate from the cell group situated in the field between the lateral part of the ventral tegmental area and the mediodorsal part of the pars compacta of the SN. On the other hand, the anterolateral cortex comprised of the dopamine containing dorsal bank of the rhinal sulcus and the adjacent area devoid of dopamine, namely the dorsolateral cortex, receives projection not only from a dopamine cell group in the lateral part of the pars compacta of the SN, but also from a nonaminergic cell group in the pars reticulata. These mesencephalic dopamine neurons receive direct afferents ipsilaterally from the pyramidal cells of layer V in the anteromedial cortex. Cortico-nigral fibers form axodendritic synapses, while reciprocal mesocortical dopaminergic projections form axosomatic synapses.

1215 EFFECTS OF LINGUAL NERVE SECTION OR NEOCORTICAL ABLATIONS ON RAT'S LICKING RESPONSE. Jon D. Kirwin*, Sarah P. Parkerson*, and David W. Watkins*. (SPON: J. A. Jane). Departments of Neurosurgery and Anatomy, University of Virginia School of Medicine, Charlottesville, VA 22901

Long-Evans hooded rats were deprived of water and then given access to a recessed drinking spout daily for 8 minutes. Their licking responses were recorded electronically during bouts of uninterrupted licking. Preoperatively, all rats licked the spout rhythmically at mean rates of 5.2-5.9 licks per second. There were differences among individual rats' mean lick rates, but for any individual, these rates were affected little by deprivation state. After bilateral lingual/chorda tympani nerve sectioning, we found: 1) initially, the rats failed to lick continuously, 2) within 2 day post-op, they licked rhythmically, but at less than normal rates (77-89% of pre-op rate), and 3) at one month post-op, their lick rates evidenced partial recovery (89-96% of pre-op rate). Following large, bilateral cerebral neocortical ablations, including all of the tongue motor and sensory cortex and parts of the prefrontal cortex rostral to the tongue motor cortex and situated just dorsal to the rhinal sulcus, we found: 1) the rats were motivated to drink, 2) their tongues were not paralysed, 3) they chewed on food pellets with repetitive jaw and tongue movements, 4) they swallowed food or water put in their mouths, but 5) they did not lick in our experimental situation which requires tongue protusion to contact the spout. With partial lesions of the face motor cortex, the rats licked rhythmically, but at significantly slower rates (90-97% of pre-op rate). With the spout positioned to require less tongue protusion, these rats' lick rates were normal. The neocortex plays a role in the rat's licking response, and appears to be related to tongue protusion and/or the initiation of licking. The sensory inflow from the anterior two-thirds of the tongue (i.e. lingual/chorda input) is not essential to rhythmic licking, but appears to exert a facilitatory influence on lick rate.

1216 THE NEURONAL BASIS OF THE SHORTENING RESPONSE IN LEECHES. William B. Kristan, Jr., Dept. Biology, University of California, San Diego, La Jolla, CA 92093.

Mild tactile stimulation of a leech produces a localized shortening response which consists of longitudinal muscle contractions limited to a single segment. At more intense levels of stimulation, the shortening response spreads to the entire body. The localized shortening response results entirely from the activation of previously identified tactile sensory neurons (Nicholls & Baylor, J. Neurophysiol. 31:740-756, 1968), particularly the cell responsive to pressure, i.e., the P cell. The evidence for this conclusion is threefold: 1. The stimulus threshold for activation of these tactile sensory neurons is indistinguishable from the threshold for the shortening response. 2. Hyperpolarization of these cells suppresses the shortening response. 3. Stimulation of these cells by passing intracellular current produces a normal shortening response. The motor neurons involved have also been identified previously (Stuart, J. Physiol. 209:627-646, 1970; Ort, Kristan & Stent, J. Comp. Physiol. 94:121-154, 1974). The motor neuron innervating all longitudinal muscles on one side of each segment (the L cell) produces a brief burst of impulses at the onset of the response whereas other motor neurons, with more localized motor units, produce more prolonged impulse bursts. These bursts are appropriate for their motor effects: the L cell produces a large, fast contraction whereas the other motor neurons produce much smaller, slower contractions. The duration of the motor neuron bursts results from the nature of the pathway between sensory and motor neurons: the excitation of the L cell is predominantly via monosynaptic connections from the tactile sensory neurons (Nicholls & Purves, J. Physiol. 209:647-667, 1970), whereas the excitation of other motor neurons is exclusively polysynaptic. The interneurons responsible for the prolongation of the local contraction are also involved in the generalization of the response to other body segments.

This work was supported by an NSF research grant BN575-23567.

1217 CENTRAL INTERACTIONS UNDERLYING PATTERN GENERATION FOR ESCAPE SWIMMING IN TRITONIA. Paul R. Lennard, Peter A. Getting and Jacqueline M. Grebmeier*. Dept. of Biological Sciences, Stanford Univ., Stanford, CA 94305.

Escape swimming in Tritonia consists of a preliminary reflexive withdrawal of the stimulated area of the body followed by a series of alternating dorsal and ventral flexions. A series of phase shift experiments have been employed to verify that two classes of interneurons, which have been previously identified (Getting, J. comp. Physiol. 121: 325-342, 1977), are directly involved in the pattern generation of the cyclical motor program for escape swimming. The two classes of interneurons include: 1) a population designated as dorsal swim interneurons (DSI), which is composed of three cells in each cerebral ganglion; and 2) a bilaterally symmetric pair of cerebral neurons termed the left and right cerebral cell 2 (C-2). The C-2's and DSI's have reciprocal excitatory connections and fire during the dorsal flexion phase of swimming. Both sets of interneurons receive input from afferents and drive pedal flexion neurons. The C-2's have also been shown to mediate the switch from reflexive withdrawal to the central swimming program and to be both necessary and sufficient for the maintenance of swimming.

Phase shift experiments were conducted in isolated brain preparations. Double-barrelled electrodes were used to impale both C-2's and as many as four DSI's. Depolarizing or hyperpolarizing current was injected into the C-2's or DSI's in order to change the timing and/or duration of activity in these cells during one of the cycles of a swim sequence. Changes in timing of the cycles subsequent to the perturbation were compared to sandwiched control swims during which no current was injected. Temporal manipulations of the burst in either population of interneurons produced permanent phase shifts in both the C-2's and DSI's as well as in pedal flexion neurons. The phase shift data indicates that both classes of interneurons contribute to the patterning of the locomotor output.

A quantitative analysis of the temporal relationships between the activity in the C-2's and DSI's was performed to clarify the role that these cells played in patterning the locomotor output. Both constant latency and constant phase relationships were found in the structuring of burst patterns in these two populations of interneurons. These parameters remain constant even though period increases for each subsequent cycle within a swim sequence. A model for central pattern generation of swimming in Tritonia, which integrates the known functional properties, synaptic connections, and temporal relationships of the DSI and C-2 interneurons, will be proposed.

1218 ASSOCIATIVE-CONTINGENCY LEARNING CAN BE MEDIATED BY A FAMILIAR NEURONAL NETWORK. William B Levy. Dept. of Psychology, Univ. Calif., Riverside, CA 92521.

Models of neural circuits which can accomplish associative forms of learning often require anatomy and physiology which are yet to be discovered in brain. An exception is the model presented by Gardner-Medwin (Nature 223, 916, 1969). This model utilizes a homosynaptic form of plasticity (a well-documented synaptic property) and is characterized by its capability to classically condition while resisting pseudoconditioning and sensitization. Unfortunately this model is not capable of learning or even appreciating CS-UCS contingencies and, in fact, will inappropriately show full conditioning when presented with a random CS-UCS paradigm.

Since animals learn in a manner related to contingencies, we have devised a circuit which would accomplish this form of associative learning while still retaining anatomy and physiology which are well documented in brain.

The circuit uses frequency coding as suggested by Gardner-Medwin. Continuity and noncontinuity of the CS and UCS are recognized and distinguished using spatial summation and lateral inhibition. Memory is stored as a form of post-tetanic potentiation. Contingency based responses are elicited by integrating the relative degrees and amounts of memorized continuity and noncontinuity. This integration is accomplished simply as a convergence of an inhibitory and excitatory input upon the final response output neuron.

In its simpler form the circuit uses five neurons and is strikingly similar to some of the known circuitry of the hippocampus.

1219 TESTING A MODEL FOR THE SPINAL LOCOMOTION GENERATOR. John E. Menzies*, Carol P. Albert* and Larry M. Jordan. Department of Physiology, University of Manitoba, Winnipeg, Manitoba, R3E 0W3.

Although the concept of a mammalian spinal locomotion generator is well established, its precise neuronal organization remains unclear. It has been shown that both Renshaw cells (RC) (McCrea and Jordan, Can. Fed. Biol. Soc., 146, 1976) and Ia inhibitory interneurones (IaIN's) (Feldman and Orlovskii, Brain Res. 84, 181, 1975) are phasically active during locomotion. Furthermore, these cells comprise a neural circuit such that activation of Renshaw cells via recurrent alpha motor axon collaterals would result in inhibition of agonist-coupled IaIN's and subsequent disinhibition of antagonist alpha motoneurones. RC's and IaIN's could therefore contribute to switching between antagonist motoneurone groups during walking, and Miller and Scott (Exp. Brain Res., 30, 387, 1977) have proposed that this neuronal system constitutes the spinal locomotion generator. If this model is correct, motoneurone firing during locomotion must be produced through disinhibition, hence the depolarization giving rise to motoneurone firing should be accompanied by reduced membrane conductance, as compared to the conductance when the membrane is hyperpolarized during the opposite phase of locomotion. In addition, interneurones which are rhythmically active during locomotion should be influenced by activation of montoneurone recurrent collaterals.

In order to test this model, experiments were performed on acutely decerebrated cats induced to walk by stimulation of the mesencephalic locomotor region (MLR) (Shik et al, Biofizika, 11, 659, 1966). Persistence of MLR evoked rhythmic motor axon firing after neuromuscular blockade by Flaxedil was evidence of "fictive" locomotion. Conductance changes in motoneurones were monitored by intracellular recording of the amplitude of short hyperpolarizing current pulses applied through the recording electrode. It was found that there was an increased rather than a decreased conductance associated with the depolarizing phase of the locomotor cycle. Such data indicate that motoneurone discharge during fictive locomotion requires input from excitatory interneurones and is not entirely due to disinhibition.

When interneurones which were rhythmically active during fictive locomotion were examined, only IaIN's were inhibited as a result of ventral root stimulation, and only 1 of a total of 26 rhythmically active interneurones was excited by antidromic stimulation of the ventral roots. It must be concluded that the RC-IaIN system cannot alone constitute the spinal locomotion generator. (Supported by M.R.C. of Canada.)

1220 IDENTIFICATION OF A NON-DECAYING OUTWARD CURRENT IN AN ELECTRICALLY COUPLED BURSTING NETWORK IN THE SNAIL. Michael Merickel and Richard Gray*. Dept. Physiol. and Biophysics, Univ. of Illinois, Urbana, IL 61801.

A single electrode voltage clamp has been used to examine the membrane currents in the bursting cyberchron network in the snail Helisoma. The cyberchron network is a population of approximately 20 small, electrically coupled neurons whose rhythmic output controls the feeding behavior of the animal. Voltage clamp experiments have been performed on these neurons in an attempt to understand the slow membrane currents and neuronal interactions underlying burst generation by this network.

A particularly interesting finding is a slow outward current activated by large depolarizing commands (0 mV and above) which does not undergo inactivation decay during a command potential step maintained for 10 sec or more. In addition, these neurons have an anomalous rectification region in their steady-state I-V plot due to a persistent inward Ca-current. These membrane properties appear to be also reflected in the action potential characteristics of cyberchron neurons. The action potentials of these cells typically have a long duration (at least 20 ms half-amplitude duration) and undergo a two to five fold duration increase with repetitive, low frequency stimulation. The action potentials of these cells, as well as the broadening process, are strongly Ca-dependent.

The lack of inactivation of the outward current in cyberchron neurons is remarkably similar to the behavior of the slow outward current in vertebrate heart cells. The shape of cyberchron action potentials, as well as their Ca-dependence are also very similar to heart action potentials. It is believed that the lack of inactivation of the outward current in cyberchron neurons plays a similar role to the analogous current in heart cells - to insure repolarization after a maintained depolarization. Repolarization after a burst of action potentials in the cyberchron system is particularly important to insure burst termination.

Support is acknowledged from the Research Board and Bioengineering Program at the University of Illinois, Urbana-Champaign.

1221 Non-spiking neurons in the cockroach. D.J. Meyer*and B. Walcott
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We have studied the properties of non-spiking neurons in the meta thoracic ganglion of the cockroach, Periplaneta americana. The properties of these neruons were virtually identical to those of the non-spiking interneurons described by Pearson and Fourtner (1975). Depolarization of type I non-spiking neurons resulted in the excitation of coxal flexor motoneurons and the inhibition of coxal extensor motoneurons. The latency of these effects was short (2-6 ms) The membrane potential of type I non-spiking neurons oscillated in phase wtih motoneuron bursts during rhythmic leg movements. The neurons depolarized during flexor bursts and repolarized during extensor bursts. As revealed by intracellular staining with cobalt, the structure of this neuron was very similar to that of interneuron I (Pearson and Fourtner 1975) Depolarization of type 2 non-spiking neurons inhibited flexor motoneuron discharge while hyperpolarization had the opposite effect effect. During rhythmic leg movements the membrane potential of type 2 non-spiking neurons oscillated in phase with motoneuron bursts. The cells depolarized during extensor bursts and hyperpolarized during flexor burst. A depolarizing current pulse applied to a type 2 non-spiking neuron during rhythmic leg movements reset the timing of motoneuron bursts. When depolarized by injected current, type 3 non-spiking neurons inhibited the discharge of the slow extensor motoneuron, and had the opposite efffect when hyperpolarized. Depolarization of type 3 non-spiking neuron reversibly abolished rhythmic leg movements. These observation are consistent with the hypothesis that the non-spiking neurons are part of the central pattern gemerator for stepping, however there are alternative interpretations of the data.

Reference:
Pearson, K.G. and Fourtner, C.R. Non-spiking interneurons in the walking system of the cockroach. J. Neurophys. 38: 33-52 (1975)

1222 TESTING DIRECT CONNECTIONS IN THE STOMATOGASTRIC GANGLION WITH TEA. Brian Mulloney and Karen A. Sigvardt. Dept. of Zoology, University of California at Davis, Davis, CA 95616.

The synaptic organization of the stomatogastric ganglion of the spiny lobster, Panulirus interruptus, has been described from recordings made from the somata of the ganglion's neurons. The diagram of direct connections which summarizes this description predicts the nature and distribution of synapses which should be discoverable using other anatomical and physiological methods. To test these predictions, we have iontophoretically injected TEA into presynaptic neurons to alter the duration of their impulses, and looked for corresponding changes in PSPs of putative postsynaptic neurons (Kehoe, J. Physiol. 1972). Our results confirm that several connections between motorneurons in the stomatogastric system are monosynaptic. For example, injecting TEA into LGN broadens its spikes, and the resulting PSPs in both the GMs and LPGNs are greatly augmented. One other suspected direct synapse, LPGN's inhibition of MGN, was not confirmed by this test; we think this synapse does not exist and should be removed from the summary diagram.

These experiments are most easily done and most readily interpretable when the putative presynaptic neuron is not a spontaneous pacemaker or endogenous burster. Spontaneously active neurons rapidly begin to fire high-frequency trains of impulses which are difficult to control. As a result, the PSPs in putative postsynaptic neurons sum, the membrane potentials are clamped at the reversal potential of these synapses, and the changes in PSPs amplitude have no meaning. Pacemakers and bursters are best injected iontophoretically by passing current between two intracellular electrodes, and then hyperpolarizing the cell while TEA diffuses.

Controls: King (J. Neurocytol. 1976) demonstrated with EM that several known inhibitory interactions in the ganglion are monosynaptic. We injected some of these same neurons (PD and LP) and confirmed that neurons known from anatomical evidence to be monosynaptic give positive results with the TEA test.

TEA will cross electrotonic synapses (Deschenes and Bennett, Brain Res. 1974). Many neurons in the stomatogastric ganglion are weakly coupled by electrotonic synapses, and if TEA spread rapidly to other neurons, our results would be difficult to interpret. We injected TEA into one of two closely coupled neurons (the PDs) and monitored the rates at which spike width and burst pattern changed in both neurons. The spikes and burst pattern of the injected neuron had changed within one-half hour, while the non-injected control was unchanged after two hours. So, leakage of TEA across electrotonic synapses does not affect the interpretation of results obtained in this system shortly after injection of TEA.

Supported by USPHS Grant NS12295 and the A.P. Sloan Foundation.

1223 COMMAND FIBER TO MOTOR NEURON CONNECTIONS IN THE LOBSTER SWIMMERET SYSTEM. John A. Paton. Dept. E.E.C.S. and Biol., Princeton U., Princeton, NJ 08540.

Swimmerets are active in a number of different oscillatory behaviors such as righting and larval swimming. The circuitry underlying this behavior consists of command neurons (CN's) which turn on segmental oscillators, which in turn drive swimmeret motor neurons (MN's). Davis and Kenneday (1972) suggested that connections might also exist between CN's and MN's and that these connections would allow different CN's to produce rhythmic motor output and yet use the same oscillator.

The CN to MN pathway was confirmed and characterized by analysis of the time-locking of MN spikes to CN stimulation. In a whole abdomen preparation of Homarus americanus, single CN fibers were dissected from the connective between ganglia one and two and stimulated electrically with pulse trains. The activity of single MN's was identified from multiunit recordings from nerve branches in the base of the swimmerets.

MN spikes were time-locked by about half (14 of 31) of the CN's examined (18 animals). Both powerstroke and/or returnstroke MN's could be time-locked. For stimulation of a given CN the time-locking varied from 1:1 following to undetectable within the group of MN's to a single muscle. In the strongest cases of time-locking the MN spikes were limited to time windows of as little as 5 to 10 ms. In summary then, some CN's which cause oscillatory motor output cause time-locked spikes in some MN's.

The pathway between CN and MN is currently being studied to determine whether the functional connection found is mono- or polysynaptic.

1224 P CELLS: DISTRIBUTION AND COUPLING OF PATTERN GENERATORS IN THE LOBSTER STOMATOGASTRIC NERVOUS SYSTEM. David F. Russell. Biology Dept. B-022, UCSD, La Jolla, CA 92093.

"P cells" are neurons in the commissural ganglia (CG's) of the lobster CNS which fire in bursts coordinated with the pyloric motor rhythm produced in the stomatogastric ganglion (STG). In isolated preparations in which the bilaterally paired CG's are left connected to the STG, a striking feature of the subthreshold activity in many STG neurons is that a train of EPSP's accompanies each cycle of the pyloric rhythm in a rigidly coordinated 1 train: 1 cycle manner. Such EPSP's are observed in the LP, PL, PE, VD, and IC pyloric-follower motorneurons, and in the AM, CP(DG), and LG(LPG) motorneurons and Interneuron 1 of the gastric system. The EPSP's do not originate in the STG since they are abolished by removing the CG's. The EPSP's are due to, and reflect the firing pattern of, P cells which send axons into the STG. P cell inputs to LP had a variable axon pathway, via either the superior- or inferior oesophageal nerves (SON's or ION's).

Several lines of data support a model for P cell organization in which the P cells would intrinsically fire tonically; their "pyloric" firing pattern would be controlled by an ascending axon from the AB "efferent copy" interneuron (in the STG), postulated to make direct inhibitory synapses to the P cells within the CG's; the P cells would then feed back to synapse onto STG neurons. (i) Nerve records showed that the AB sends an axon into the CG's via the SON's. (ii) The P cell EPSP's in CP or LG's became tonic while firing of the AB was shut off for a few seconds by hyperpolarizing it (or an electrically coupled PD neuron). (iii) Blockade of the SON's caused the EPSP's in LP (from P cell inputs travelling in the ION's) to become tonic for long periods of time. (iv) Records from two points along the stomatogastric n. showed that each cycle of the pyloric rhythm was accompanied by a burst of P cell units, travelling towards the STG, which ended at the next burst of AB spikes, travelling towards the CG's. (v) Intracellular records from a P cell in a L-CG showed trains of IPSP's coordinated with the pyloric rhythm (presumably from AB); the IPSP's disappeared and the P cell fired tonically while a PD cell (in the STG) was hyperpolarized for a few seconds. (vi) Bathing the CG's in low Ca++ saline to block synapses caused the P cell EPSP's in LP to become tonic. (vii) Bathing only the oesophageal ganglia in low Ca++ saline to block synapses there did not affect the "pyloric" timing of P cell EPSP's in LP.

The P cell inputs to pyloric follower neurons may be regarded as part of the pyloric pattern generator. The P cell inputs to gastric-system neurons contribute to a marked 1-2 hz modulation, in time with the pyloric rhythm, of their membrane potential and firing pattern. The P cell circuits are remarkably analogous to phasic supraspinal control systems for locomotion of mammals.

NEURONAL SHAPE AND FUNCTION

1225 QUANTITATIVE DESCRIPTION OF THE BIAS OF EXTRACELLULAR UNITARY RECORDING TECHNIQUES TOWARDS LARGER NEURONS. Charles Abzug. Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201.

In the mammalian CNS the majority of neurons is of relatively small size. However, it has long been known that extracellular recording techniques are selective for the larger neurons. Previous attempts to describe this bias quantitatively(A.L. Towe & G. Harding. Exptl. Neurol., 29: 366-381, 1970; D.R. Humphrey. Fed. Proc., 34: 445, 1975; D.R. Humphrey & W.S. Corrie. J. Neurophysiol., 41: 216-243, 1978.) have been oriented towards comparison between the distribution of experimentally determined speeds of axonal conduction and the histologically established distribution of axonal diameter. In the present model perikaryal size(cross-sectional diameter, D) is the major independent variable. The probability of detection, $P(\Delta)$, is calculated as the product of two terms. The first term, $P(\gamma)$, is the probability that the track of the recording electrode will pierce a virtual surface of detectability(SD) surrounding the neuron. At any point within the SD the voltage caused by the neuron's action current exceeds the minimum detectable level, V_{min}, of the recording system. $P(\gamma)$ is proportional to the cross-sectional area of the SD, and therefore depends upon biophysical characteristics: the total quantity of neuronal action current and the source/sink distribution along the somato-dendritic tree. The second term, $P(\Delta|\gamma)$, is the probability that an action potential will be generated while the electrode tip lies within the SD. The value of this term depends upon the neuron's firing rate and upon the duration of the electrode's passage through the SD. This duration in turn depends upon the speed of electrode advancement, s, and on the size and shape of the SD. Specific solutions for these terms, and therefore for $P(\Delta)$, have been determined for two different models of somato-dendritic current distribution and for two experimental cases. In one case, where the firing rate, f, is constant(e.g., repetitive antidromic driving), there is an exact solution over the entire range of f and s. For the case of spontaneously firing neurons an exact solution is not possible over the entire range without knowledge of the distribution of impulse intervals. Specific solutions for this case, however, are available for two limiting situations: where s/f is much smaller than the maximum linear dimension of the SD, and where it is much greater. All solutions are of the form: $P(\Delta) = k\, D^{j}\, V_{min}^{-m}$. The values of the positive exponents j and m depend upon the biophysical characteristics of the neurons, which may differ among different neuronal populations. However, $j = 2m$, and therefore the value of j can be determined indirectly for each neuronal population .

Supported by USPHS grant number NS12736(NINCDS).

1226 IMMUNOLOGICAL RESPONSES TO REPEATED INTRAVENTRICULAR AUTOLOGOUS BLOOD INJECTIONS MAY INDICATE A NEW MECHANISM IN THE PATHOGENESIS OF STROKE. Maurice S. Albin, Bruce S. Rabin*, Leonid Bunegin*, and Julio A. Martinez*. Depts. Neurosurg. Anesth., and Path. (NeuroImmuno), Sch. Med. Univ. Pgh., Pittsburgh, PA 15261.

These experiments were designed to evaluate the immunological, neurological and histological reactions to autologous intraventricular blood. We were testing the hypothesis that the development of neurological symptoms after intraventricular (and subarachnoid) hemorrhage might be associated with a localized immunological response in the brain. Following injections, macaque brains were evaluated by direct immunofluorescence for immunoglobulins, complement and fibrinogen and by routine histological sections - with the tissue adjacent to the (non-injected) contralateral ventricle serving as control. The experimental groups were: 1 - Blood-Blood; 2 Blood; 3 - Blood-Saline; 4 - Saline-Saline. All monkeys were sacrificed one week after their final injection. Multiple injections were given seven days apart.

Immunofluorescence for IgG and C_3 as well as neurological deficits (plegia and paresis) were found only in Group 1 (Blood-Blood) monkeys. Infiltration with lymphocytes was found in Group 1, two and three animals but not in Group 4 (Saline-Saline). No immunoglobulins, complement deposits, lymphocytic infiltrations or referable neurologic symptoms were found in all the noninjected contralateral ventricles. The immunofluorescence appeared to be localized in the microglial cells and the lymphocyte infiltration was predominantly perivascular in localization. These observations indicate that a single exposure to autologous blood was not associated with either neurological changes or deposition of immunoglobulins or complement. It was only after the second injection of blood that IgG and complement were encountered and a neurological deficit occurred. It is possible that the development of marked neurological sequelae after repeated intracranial hemorrhage (i.e., aneurysm rebleed) may involve immunological factors. Critical to this process may be a breakdown in blood brain barrier permeability due to hemorrhage and accompanying lymphocyte infiltration.

1227 CENTRAL ANATOMY OF THE CRAYFISH STRETCH RECEPTOR NEURONS. Michael Bastiani and Brian Mulloney. Dept. of Physiology and Zoology, University of California at Davis, Davis, CA 95616.

The crayfish muscle receptor organs (MRO), tonic MRO1 and phasic MRO2, monitor position and movement of the abdomen. The sensory receptor neurons (SN) associated with MRO1 and MRO2, SN1 and SN2 respectively, send their axons into the ventral nerve cord via the second roots of the abdominal ganglia. The central anatomy of SN1 and SN2 was determined using Co^{++} backfills of second roots followed by Timm's Intensification of the wholemounts. Both SN1 and SN2 send axons anteriorly to the supraesophageal ganglion and posteriorly to the 6th abdominal ganglion. Each have secondary processes lateral to their axons in the neuropile of ganglia through which they pass.

SN1 and SN2 have elaborate bilateral structures in the 6th abdominal ganglion. Bilateral SN1 and SN2 from all of the abdominal ganglia, except the 6th, project to the same area of dorsal neuropile in the 6th ganglion. The structures of SN1 and SN2 are very similar. The primary process of both is U-shaped (open-anterior), centered around the midline with secondary processes projecting bilaterally. All SN1 on the same side and on opposite sides from all abdominal ganglia show considerable overlap of their primary and secondary processes. The same is true for SN2. SN1 and SN2 also have overlapping primary and secondary processes, but to a lesser extent than among homologues.

Intracellular recordings were made from cells post-synaptic to the SNs in the 6th ganglion. Three classes of cells were found: 1. cells receiving input from SN1; 2. cells receiving input from SN2; 3. cells receiving input from both SN1 and SN2. Cells of each class receive input from the appropriate SNs of all abdominal segments. The post-synaptic cells have not yet been identified.

Ipsilateral SN1 and SN2 in the 6th ganglion.

Supported by U.S. P.H.S. Grant NS 12295 and the Alfred P. Sloan Foundation. M.B. is an NIH trainee.

1228 **A COMPARISON OF SYNAPTIC JUNCTION LOCALIZATION ON LAMPREY SPINAL CORD NEURONS BY PHYSIOLOGICAL AND MORPHOLOGICAL METHODS.** **Burgess N. Christensen and Wm. P. Teubl*. Dept. of Physiology and Biophysics, Univ. of Texas Med. Br., Galveston, TX 77550.**

Monosynaptic e.p.s.p.'s were recorded in lamprey spinal cord interneurons following stimulation of a single presynaptic giant axon through an intracellular microelectrode. These e.p.s.p.'s consist of an electrotonic and chemical synaptic component. Estimates of the cable parameters were determined from an analysis of voltage transients produced and recorded at the soma. Using these estimates and the half-width of the electrotonic synaptic potential measured from the e.p.s.p., the location of the electrotonic synaptic junction on the equivalent cylinder was estimated by the procedure of Jack and Redman (J. Physiol. 215: 321, 1971). These same neurons were injected with horseradish peroxidase and the spinal cord processed for light and electron microscopy. The electrotonic distance from the soma to likely sites of synaptic contact on dendrites of the interneuron was determined from a direct morphological analysis. Electrotonic lengths of dendrites likely to receive synaptic contacts were estimated from physical measurements. The analysis indicated that the electrotonic lengths of individual dendrites varied and were considerably shorter than the equivalent cylinder. This suggests that the termination of dendrites at the same electrotonic distance is an invalid assumption of the Rall model for these neurons. Synaptic junctions were distributed over several dendrites but they tended to be at approximately the same electrotonic distance from the soma. This was confirmed by the physiological estimate of synaptic location. In spite of violating two assumptions, simulation of the electrotonic e.p.s.p. indicated that the Rall model can accurately predict the time course of the synaptic potential. It is concluded from this analysis that functionally identical presynaptic neurons tend to make synaptic contact at approximately the same electrotonic distance on functionally similar postsynaptic neurons.

1229 THE MAUTHNER CELL OF THE PREMETAMORPHIC ANURAN. S.L. Cochran, J.T. Hackett, S.M. Hou, and D.L. Brown. Dept. Physiology, Univ. of Virginia Medical School, Charlottesville, Va. 22901.

In fish, a single neuron--the Mauthner cell (M-cell)--acts as a direct interneuron between sensory input and tail movement. Present also in the tadpole (Rana pipiens) is this cell, which degenerates with the tail and lateral line systems after metamorphosis. The M-cell perikaryon is the largest in the tadpole's brain and is situated ventro-lateral to the sulcus limitans. Examination of 5 μm sections reveals that the M-cell possesses two principal dendrites, a lateral and a medial dendrite. A densely-textured material surrounds all portions of the M-cell. Electronmicroscopy of aldehyde-osmium fixed tissue shows this material to be composed of afferent terminals upon the roughly corrugated M-cell surface. Junctions are of three general types: Gray type I; Gray type II; and type I synapses with gap junctions. These mixed synapses are greatly outnumbered by the plethora of type I junctions. Club endings and axon cap, common in fish, are absent. The M-cell axon increases in diameter as it projects spinally in the contralateral ventral funiculus. Functional characterization of this cell was accomplished after removing the nervous system from the tadpole and maintaining it in vitro. Intracellular recording from M-cell somata show that VIIIth nerve activation occurs at a very short and at longer latency ranges, followed by an increase in transmembrane conductance with little change in potential, suggesting inhibition. Spinal cord activation orthodromically occurs only in the longer latency range. Antidromic activation occurs in less than 1 msec. with contralateral stimulation. Absent is the extrinsic hyperpolarizing potential evident in fish, reflecting the absence of the axon cap. The M-cell conduction velocity increases with increasing distance from the soma, verifying the observations that the axonal diameter increases with distance from the soma. Identification of the impaled cell was obtained after inward injection of horseradish peroxidase and subsequent localization of the reaction product within the M-cell. The M-cell thus has three types of afferent activation: short latency VIIIth nerve activation possibly mediated through active, mixed type I-gap synapses; longer latency VIIIth nerve and spinal activation due to active type I synapses; and the subsequent inhibitory phenomena appear to correlate with the type II junctions upon the M-cell. We would conclude that the anuran tadpole M-cell is rudimentary in relation to the M-cell of the teleost. Functionally, however, the tadpole retains many features of the fish M-cell.

Supported by RSDA 5K02 DA 00009 from NIDA and NSF grant BNS 77-155271.

1230 AN ON-LINE COMPUTER-AIDED TECHNIQUE FOR THE RECONSTRUCTION OF NEURONAL STRUCTURES FROM SERIAL SECTIONS. M.L. Dierker* and T.A. Woolsey. Dept. of Anat. & Neurobiol., Washington U. Med. Sch., St. Louis, MO 63110.

In the past decade a number of computer-aided techniques for collection of the quantitative aspects of neuronal morphology from light and electron microscopic images have been introduced. A serious problem with light microscope based systems has been the difficulty in or inability to quantitate data from serial sections. Reconstruction systems utilizing electron micrographs usually require a series of intermediate photographic steps before digitization of the image. The present system allows on-line quantification of neuronal structure, with the alignment and quantification steps sequentially ordered. A continuous gray level T.V. image of the structure(s) being reconstructed (e.g. from the phototube of a microscope) is digitized (8 gray levels) and stored in a computer. By a subsequent digital to analogue conversion, the stored image may be retrieved and displayed at any time. The recalled image can be displayed simultaneously with the T.V. image of the next section and the two images aligned by rotation and translation of either the stored image or the specimen. In this way it is possible to sequentially align a number of sections in a series and to match and measure features, examined at higher magnification, such as neuronal processes which are continuous from one section to the next. Since the digitized T.V. pictures can be stored on magnetic tape, they may be used for subsequent analysis of other objects in the sections without recapitulating the serial section alignment procedure. While at the moment we do not produce a "movie" of a series of sections, to do so would be straight forward. The utility of this procedure has been shown in a computer-aided system for the reconstruction of Golgi impregnated neurons from serial sections. This system allows the on-line quantification of neuronal structure, as well as the number of dendritic spines, process diameters and somal areas. The procedure also has been used in the reconstruction of other objects such as cortical cytoarchitectonic areas. In principle the method is limited only by the range of magnifications available from input devices and the system can be used equally well to reconstruct images of organs from gross anatomical material, outlines of brain nuclei, individually stained neurons or parts of cells seen in serial electron micrographs. The time required to perform a reconstruction of a Golgi stained neuron on the current system varies with structure complexity, but averages 10-15 minutes per section processed.

Supported by N.I.H. Grants T01GM01827-08, NS 10244, and EY01255.

1231 A MECHANISM FOR THE PRODUCTION OF VERY LOW FREQUENCY REPETITIVE FIRING FROM NERVE ENCODERS UNDER CONSTANT CURRENT STIMULATION. Jürgen Fohlmeister (SPON: C. A. Terzuolo). Laboratory of Neurophysiology, University of Minnesota, Minneapolis, Minnesota 55455.

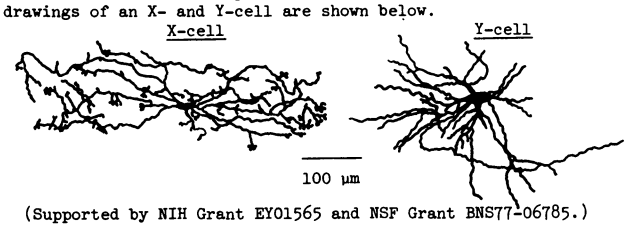
Many neurones (eg. the slowly adapting stretch receptor neuron of crayfish) are capable of producing very low impulse frequencies of less than 1 imp/sec when stimulated with very small, constant currents. Further, the crayfish neuron shows a sizeable nonuniformity of excitability and reduced current threshold at the impulse trigger zone near the axon hillock. Longitudinal potential differences in the neighborhood of the trigger zone will be small during most of the interspike interval because the trigger zone dimensions are a small fraction of an axonal space constant in that state. Passive properties of neighboring membrane will then dominate the control of membrane potential which, for the electrically isolated trigger zone, would otherwise be controlled solely by the excitation kinetics of local membrane. This dominance can allow threshold potential to be reached at the trigger zone for very small stimulus currents leading to very low impulse frequencies. This behavior is demonstrated with a model for which the trigger zone kinetics are given by "voltage clamp" equations. Ionic channel rate "constants" are derived from neural- and model dynamics. The crayfish neuron threshold condition determines $\bar{g}_K(Tz)$ and $\bar{g}_{Na}(Tz)$. Membrane potential then follows an equation of the form $(C_{Ax}+C_{Tz})\dot{E}=-g_{Tz}(E-E_K)-g_{Ax}(E-E_K)+I$ where $g\equiv g_K-g_{Na}(|E-E_{Na}|/|E-E_K|)$.

The magnitude of C_{Ax}, acting as a distributed capacitance, is time varying and reflects the "amount" of axonal membrane that is influential in moderating the effect of trigger zone channel kinetics on membrane potential control. C_{Ax} is zero for a transverse current density such as during an action potential but can become very large in interspike intervals. The term $g_{Ax}(E-E_K)$ must remain <I in order that stimulus current can reach the trigger zone. This condition is sufficient to remove the excitation kinetics of axonal membrane from the encoding process and to consider it as a subtraction from the stimulus current. The mechanism accurately reproduces the stimulus/impulse frequency behavior of the crayfish neuron to very small frequencies. (Supported by NSF #BNS77-22532 and NIH #EY00029312.)

1232 STRUCTURAL AND FUNCTIONAL CORRELATION FOR SINGLE NEURONS IN CAT DORSAL LATERAL GENICULATE NUCLEUS. Michael J. Friedlander*, C.-S. Lin, and S. Murray Sherman (SPON: Lennart Heimer). Dept. Physiol., Sch. Med., UVA, Charlottesville, VA 22901.

Functional properties of neurons of the dorsal lateral geniculate nucleus of the cat were correlated with cell structure using the intracellular HRP injection technique. Recordings were made using glass micropipettes filled with 3% HRP in 0.2 M KCl at pH 8.6. Electrode tip diameters were <1 μm. Electrodes were beveled to a final impedance of 100-175 MΩ at 200 Hz. A section of tissue approximately 4 mm in diameter and 4-5 mm deep was aspirated from cortex overlying the lateral geniculate nucleus (LGN). This facilitated penetration of the LGN with the electrodes which tended to clog or break if the entire overlying cortex had to be penetrated. Several parameters were tested during extracellular recording which allowed us to classify the geniculate neurons as X- or Y-cells. These parameters included receptive field size, tonic or phasic responses, type of center and surround, latency to optic chiasm shock, linearity of spatial summation, and responsiveness to a large, rapidly moving disc. The electrode was then advanced to impale the cell. A second determination of the receptive field properties was quickly made during intracellular recording to verify that the impaled cell was identical to the unit from which data were obtained with extracellular recording. HRP was passed into the cell by applying 5-10 nA depolarizing pulses for 5-10 min. The brains were subsequently prepared for histology, cut frozen into 120 μm sections, and the sections were reacted with diaminobezidine. To date 8 filled cells have been located and traced under oil with a drawing tube attached to a microscope. Extensive dendritic branching, long sections of axons, and axonal and dendritic processes and varicosities were clearly visible.

Compared to 5 Y-cells, 3 X-cells had smaller somas and finer dendritic and axonal processes. Also, X-cells had complex varicosities and swellings along their dendritic arborizations, whereas Y-cells had few processes on their dendrites. Line drawings of an X- and Y-cell are shown below.



(Supported by NIH Grant EY01565 and NSF Grant BNS77-06785.)

1233 THE MIGRATIONS OF PIGMENT GRANULES WITHIN CRAYFISH RETINAL PHOTORECEPTORS UNDER DIFFERENT IONIC COMPOSITIONS. Eugenio Frixione†, Victor Tsutsumi* and Hugo Aréchiga. Depts. Neurosc. and Physiol. and Biophys., CIEA, IPN. México 14, D.F.

A previous account has been presented on the mechanisms of migration of accesory pigment granules in the retinula cell of the crayfish (Frixione et al. 1977, Soc. for Neurosc. Abs.3, 176), where two phases were described for the nucleofugal movement towards the axon in the dark, and an apparently single expansion from the axon towards the nucleus upon illumination. Since light is known to induce conductance changes in photoreceptor membranes, experiments were performed to explore the dependence of these light-controlled movements on different ions. Isolated eyestalks in the light-adapted (LA) or in the dark-adapted (DA) condition were pre-incubated for two hours in the test solution, and either kept in darkness or exposed to light for adequate periods of time before assessing the position of the pigment in the instantaneously fixed organ. An inhibitory effect on dark-adaptation was found with an increase in concentration of Na^+ (300 mM, i.e. 1.5 fold normal) and K^+ (80 mM), or by substitution of Na^+ with Li^+. The inhibition was restricted to the second phase of movement and was linearly related to the concentration of K^+ below 80 mM. DA eyestalks were also unable to maintain the pigment accumulated along the axons when incubated in the above solutions in the dark, but migration towards the nucleus in the light was normally achieved except for the Li^+ treated eyestalks, where its completion was arrested. In contrast to the action of monovalent cations, the nucleofugal migration of pigment seemed favoured by isotonic $CaCl_2$ or 15 mM $CoCl_2$ added to Van Harreveld's solution, without any appreciable effect on the movement in the opposite direction during light-adaptation. High Ca^{++} opposed an inhibitory effect of elevated temperature (25°) upon nucleofugal migration. Pigment movements in isotonic $MgCl_2$ were indistinguishable from controls. Ouabain prevents DA and promotes a partial LA position in DA eyestalks kept in the dark.
These findings permit to consider the second phase of the nucleofugal migration as being closely associated to a hyperpolarization of the photoreceptor cell in the dark. Thereby conditions opposing such a process, as high K^+ and Na^+ or Li^+ and Ouabain, result in inhibition, whereas those increasing membrane resistance, as Co^{++} or high external Ca^{++}, facilitate the migration towards the DA position. Upon the incidence of light, an influx of Na^+ would depolarize the cell, triggering an expansion of the pigment in a process for which Li^+ could be less suited.

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1234 A SYSTEM FOR DETERMINING THE ALIGNMENT OF SERIAL SECTIONS. A. N. Gentile* and E. Harth. Dept. of Physics, Syracuse University, Syracuse, NY 13210.

A method is introduced which achieves the alignment of serial sections, using features appearing at adjacent slide surfaces. An instrument for the measurement and recording of coordinates taken from microscope sections is described. X, Y, and Z coordinates are obtained by a combination of digital encoding of microscope stage, video cursor and microscope focusing. A computer link allows recording and processing of the data via a dedicated PDP-11 minicomputer. It also gives access to the graphics and analysis capabilities of larger computers. The computer assisted digitizer records the positions of easily recognizable gross structures along the surfaces of the sections. These data are used in the determination of areas for alignment. Points in these regions on adjacent slide surfaces are digitized for use in computer programs that perform a spatial filtering process using the two dimensional fast Fourier transform (FFT). Shrinkage and rotation are determined by first taking the complex logarithm of the data from both regions and applying the FFT. These data are then filtered and correlated revealing the precise magnification and rotation between slides. The digitized data are corrected for shrinkage and rotation and then directly reprocessed by the FFT, filtered, and correlated. This determines the precise translation between slides. Hardware implementations of the FFT will permit very rapid alignment of the sections. An example is presented and the significance of each stage in the process is discussed. The method is used in our laboratory for the reconstruction of fiber geometry of Golgi-stained neural tissue, using light microscopes. It may be applied, however, to the spatial analysis of any features appearing in serial sections using light or electron microscopy.

Research supported in part by grants NS10917 and EY01215 of the National Institutes of Health.

1235 INTRACELLULAR STAINING OF PHYSIOLOGICALLY IDENTIFIED CELLS AND AFFERENTS IN CAT VISUAL CORTEX. Charles D. Gilbert* and Torsten N. Wiesel. Dept. of Neurobiology, Harvard Medical School, Boston, Mass. 02115.

To study intracortical connections and the means by which the receptive fields of cortical cells are synthesized, we used the method of staining physiologically identified cells with horseradish peroxidase. Intracellular recordings were made in the cat primary visual cortex, and after we characterized their receptive field properties, the cells were injected with horseradish peroxidase by pressure or by iontophoresis. Previous studies using extracellular recording techniques have demonstrated a relationship between receptive field type and cortical layer[1,2]. The present technique enabled us to expand upon these studies by making a more precise localization of the cell soma, and by distinguishing cells within a given layer on morphological and functional grounds. The morphological differences between cells were helpful in evaluating which of a given cell's receptive field properties were especially important in defining a particular cell class.

In many instances the filling of the cells appeared to be complete, so that we were able to characterize their axonal as well as dendritic arborizations. This made it possible to extend the general correlation between receptive field type and cell morphology made by Kelly and Van Essen[3] who examined cells after procion yellow injection. We have, for example, been able to penetrate and inject afferents from the lateral geniculate nucleus. By filling their terminal processes and boutons we could determine the columnar and sublaminar distribution of the terminals of particular classes of geniculate neurons. The construction of cortical receptive fields from those of a particular geniculate channel could be deduced after characterizing and injecting cells that lie within the terminal field of a given afferent class, and this analysis could be made for subsequent stages by following the axonal projections of these cells within the cortex. (supported by NIH grants EY00082, EY07042, and EY00606)

1 Hubel and Wiesel (1962) J. Physiol. 160: 106-154.
2 Gilbert (1977) J. Physiol. 268: 391-421.
3 Kelly and Van Essen (1974) J. Physiol. 238: 515-547.

1236 THE EFFECTS OF NEURONAL GEOMETRY ON TRANSIENT POTENTIALS IN DENDRITIC SYSTEMS. Barry Horwitz, Physics Department, Texas Woman's University, Denton, Texas 76204.

A theoretical study has been undertaken which seeks to examine the way in which the geometrical structure of a neuron's dendritic tree affects the time course and amplitude of transient potentials generated at different locations on dendritic branches. The model which is used is based upon the work of Butz and Cowan (Biophys. J. 14, 661-689, 1974). They extended the Rall model (Rall, Exp. Neurol. 1, 491-527, 1959), which treats a dendrite as a passive cable whose membrane properties can be represented electrically by a distributed network of resistors and capacitors, to dendritic systems of arbitrary geometry. Butz and Cowan produced a graphical calculus which generates analytic expressions for the Laplace transform of the transmembrane potential change at any point on the dendritic tree in response to a synaptic "current" input at any other specified point along the tree. This work discusses a method which has been developed for determining the inverse transform (which is the transmembrane potential change) in analytic form, thus generating expressions for the time course and amplitude of the transient potential changes. The method involves essentially the following: (1) For a given dendritic geometry one associates a set of symmetric geometries for which the inverse Laplace transforms are either known, or can be fairly readily determined; (2) the transient potential changes for the asymmetric geometry are obtained by adding correction terms to the inverse transforms corresponding to the set of symmetric geometries. This method, which will be illustrated with several examples, provides a way to assess the influence of the dendritic geometry upon the neuronal input-output relationship.
(Supported in part by TWU Institutional Grant 0997)

1237 A COMPARATIVE STUDY OF VENTROLATERAL AND RECURRENT EXCITATORY POSTSYNAPTIC POTENTIALS IN LARGE PYRAMIDAL TRACT CELLS IN THE CAT. A. Labelle* and M. Deschênes* (SPON: A. Roberge). School of Medicine, Laval Univ., Quebec City, Quebec, G1K 7P4, CANADA.

In acute cats deeply anesthetized with Nembutal, monosynaptic excitatory postsynaptic potentials (EPSPs) triggered by stimulation of the ventrolateral (VL) thalamic nucleus and the pes pedunculus were recorded in large pyramidal tract cells (PT cells). Deep anesthesia, low intensities of stimulation and an averaging technique were used in order to get VL and recurrent EPSPs free of polysynaptic potentials. Comparison of the time course of both EPSPs revealed a much faster rise time and shorter half-width for VL EPSPs than for recurrent EPSPs. This would suggest a more proximal location for VL synaptic contacts than for recurrent ones with respect to the soma of PT cells. The separation of the sites of origin of both EPSPs is further suggested by their almost perfect linear summation. It is suggested that VL EPSPs are produced on the apical dendritic tree while recurrent EPSPs could originate on the basilar dendritic branches. (Supported by MRC grant MA-5788)

1238 STRUCTURAL AND FUNCTIONAL CORRELATION FOR SINGLE NEURONS IN CAT STRIATE CORTEX. C.-S. Lin, Michael J. Friedlander*, and S. Murray Sherman. Dept. Physiol., Sch. Med., UVA, Charlottesville, VA 22901.

We attempted to correlate structure with function by intracellular filling with horseradish peroxidase (HRP) of single neurons in cortical area 17 of adult cats. Beveled micropipettes (3% HRP in 0.2 M KCl buffered at pH 8.6; <1 μm diameter tip; 100-175 MΩ impedance at 200 Hz) were used for recording. Cells with receptive fields within 10^{o} of the *area centralis* were classified as simple or complex by previously described criteria. Several other functional parameters were also measured. The electrode was then advanced to impale the cell. Criteria for intracellular recording included resting potential, spike amplitude, polarity and waveform, and synaptic potentials. The receptive field was quickly remapped to ensure that the impaled cell was identical to the unit from which data were obtained with extracellular recording. HRP filling was then accomplished by applying 5-10 nA depolarizing pulses for 5-10 min. The brains were then prepared for histology, cut frozen in the coronal plane into 120 μm sections, and the sections were reacted with diaminobenzidine. Filled cells were located and traced under oil with a drawing tube attached to a microscope. Extensive dendritic branching, dendritic spines and long sections of axons and axon collaterals were clearly visible.

Simple cells were found to be of several morphological types. Somas were both of pyramidal and non-pyramidal forms. The non-pyramidal group included circular, fusiform, and crescent shaped somata. The pyramidal group contained from 1 to 3 main dendritic ramifications. These included a basal group, a group from 50-100 μm above the soma along the apical dendritic shaft, and a terminal apical group. The apical dendrites of some simple cells were followed to the pial surface, showing only a terminal branching at the surface. In some cases, axons of simple cells were traced into white matter. Axon collaterals were also occasionally seen to ramify locally.

To date, too few complex cells have been successfully filled for analysis. Those that have been are of pyramidal morphology.

These data suggest that the simple/complex functional classification does not correlate in a straightforward manner with the stellate/pyramidal morphological classification.

(Supported by USPHS Grant EY01565 and NSF Grant BNS77-06785.)

1239 STIMULATION OF THY-1 SURFACE GLYCOPROTEIN LEVELS ON PC-12 NEUROBLASTS IN RESPONSE TO NGF AND cAMP. Roger Morris*, Wendy Colby*, Bruno Betschart*, and S. E. Pfeiffer. Dept. Microbiol., Sch. Med., U Conn Health Center, Farmington, CT 06032

Pheochromocytoma cell line PC-12 (Greene et al.) has been studied with respect to the levels of the surface glycoprotein Thy-1 as a function of physiological conditions leading to neurite outgrowth. A two-step serological binding assay using either mouse or rabbit antisera against purified rat brain Thy-1 was used, with glutaraldehyde-fixed mouse thymocytes (Thy-1.1) as target cells for PC-12 absorbed antisera. When round proliferating neuroblasts are presented with partially purified mouse salivary gland NGF (streptomycin sulfate precipitation, G-100 column chromatography) at 2 ug/ml, Thy-1 levels increased from 1.5×10^{6} molecules/cell at t_0, to 2.3×10^{6}, 4.8×10^{6}, and 7.4×10^{6} molecules/cell at 24, 48, and 72 h., respectively, at which time the concentration increase had not reached a plateau level. More highly purified samples of NGF are currently being tested. Detergent treatment studies indicated that these increases represented new synthesis rather than exposure of previously synthesized antigen. The phenomena was independent of initial cell density. Treatment of cells with db-cAMP (0.25 mM) or inhibitors of phosphodiesterase (e.g. IBMX, 10^{-5}M) resulted in similar stimulations of Thy-1 with respect to time course and extent. The early elevation of Thy-1 in response to NGF suggests its utility as a biochemical marker for the study of the mode of action of this hormone. (Supported by PHS grant 10861 and NSF grant BNS77-15818; Dr. Morris was a Fellow of the Jane Coffin Childs Foundation).

1240 SUDDEN INFANT DEATH SYNDROME (SIDS): RETICULAR DENDRITIC SPINES IN INFANTS WITH SIDS. James J. Quattrochi*, Nobuhisa Baba*, and Leopold Liss. Dept. Pathology, Sch. Med., Ohio State University, Columbus, Ohio 43210.

Sudden unexpected death in infancy continues to present a problem of major medical significance with the peak incidence occurring between two and four months of age. The role of the central nervous system in the pathogenesis of SIDS has not been fully investigated. The rather narrow age range during the critical period of CNS maturation might suggest a transient developmental abnormality in these infants. Theories have alluded to a dysfunction of central respiratory control, however investigation of specific brainstem pathology has been lacking in SIDS. This study was designed to determine whether the presence of reticular dendritic spines can be implicated as a consistent finding in SIDS victims. It is postulated that during the first month of life in the kitten, spines on reticular dendrites are concerned with primitive control of respiratory rhythms and the rest-activity cycle. The loss of these spines, described by Scheibel, during normal postnatal maturation encodes a more sophisticated program on the dendritic membrane. In the adult cat, smooth, spineless dendrites are seen which then assume a more complex control of sleep-wakeful states of respiration and modulation of chemoreceptor activity. (Exp. Neurology 38:301, 1973.) In our study, Golgi-stained serial sections from the apex of the pons to the caudal end of the medulla exhibited a persistence of reticular dendritic spines in sixteen infants who died of SIDS, while the dendritic spines in the control infants follow a normal maturational scheme,and also confirms findings in cat brainstem. Conceivably, the persistence of reticular dendritic spines in the SIDS infants may indicate a specific lag in the normal maturation of the respiratory control centers. This may bear a causal relationship to prolonged apneic episodes and neuronal dysfunctions often seen in these infants including the final event.

1241 NEURONAL INTEGRATION CONSIDERED AS A DIRECT FUNCTION. Bernard Racine* (SPON: Dalbir Bindra). Dept. Psychol., Univ. de Montréal, Montréal, Québec, Canada, H3C 3J7.

It is shown that the integration of information on the somatodentritic membrane could be considered as a direct function. Formally, a function f: $w_i \longrightarrow w_j$ is a direct function, noted f_d, iff the result-word w_j is a part of the data-word w_i:

$$f_d: w_i \rightarrow w_j \text{ iff } w_j \in P(w_i)$$

(e.g. if w_i = bababc and if w_j = aac, then bababc→aac is a direct function because the result-word aac is a part of the data-word bababc). Neuronal integration could be considered as a direct function f_d if it is accepted that on the somatodentritic membrane (1) an excitation noted e and an inhibition noted i constitute the two letters of a neuronal alphabet noted A, (2) a sequence of excitations and inhibitions constitute a word of the set A* of all the neuronal words where

$$A^* \{\emptyset, e, i, ee, ii \ldots, eiei, \ldots\}$$

and where the empty word $\emptyset$ corresponds to the lack of stimulation. Following these considerations, it happens that the grand slow potential issued from spatial and temporal sommation is a neuronal word $w_i \in A^*$ and that this neuronal word w_i is a part of the neuronal word $w_j \in A^*$ issued from the juxtaposition of the excitatory and inhibitory postsynaptic potentials (e.g. if w_j=eee and w_i = eieieee, then eee ∈ P(eieieee)). Thus, it is possible to conceive neuronal integration as a direct function (e.g. f_d:eieieee→eee because eee ∈ P(eieieee)). From the formal properties of a direct function, it can be shown that the neuron saves at least 300,000 unities of time if its integration of 100,000 postsynaptic potentials is a direct function. More generally, it is suggested from formal properties of a direct function that the neuronal integration would be a direct function for reasons of (I) time economy (II) errors economy and (III) materials economy.

1242 PHYSIOLOGICAL AND MORPHOLOGICAL IDENTIFICATION OF A PROBABLE HIPPOCAMPAL INTERNEURON. Philip A. Schwartzkroin and Lawrence H. Mathers. Depts. Neurol. and Struct. Biol., Stanford Univ. Med. Sch., Stanford, CA 94305

In intracellular recordings from the CA1 pyramidale layer of hippocampal slices, a nonpyramidal cell type has been found which may be a type of hippocampal interneuron. Such cells are seen infrequently compared to the predominant pyramidal cells (about 1:50 ratio). Stimulation of the alveus evokes a short latency, fast rising EPSP; pure antidromic activation such as seen in pyramidal cells, is not observed. These cells also differ from pyramidal cells in the following respects: 1) shorter duration action potentials (0.4-1.2 msec as opposed to 0.8-1.7 msec in pyramidal cells); 2) large hyperpolarizing undershoot following spikes rather than the depolarizing after-potential; 3) high spontaneous activity; 4) considerable baseline "noise" which appears to reflect unitary EPSP activity.

Intracellular labelling of these cells with HRP has shown them to be, morphologically, a nonpyramidal cell type, with extensive dendritic and axonal branching. The orientation of the cell is generally parallel to the pyramidal cell dendritic tree. HRP staining reveals no dendritic spines on these cells (in contrast to stained pyramidal cells), but does show frequent dendritic swellings.

This non-pyramidal cell type has been impaled during electrode penetrations in stratum radiatum as well as in pyramidale. The characteristics of its recorded activity, however, differentiate it plainly from pyramidal cell dendritic impalements. Becuase of their vertical orientation, because of their location, and because no basket plexes have been seen following HRP labelling, these cells appear not to be basket cell interneurons. Their firing characteristics, however, are similar to those often associated with hippocampal inhibitory interneurons (Andersen, Eccles, and Løyning, J. Neurophysiol. 27:592-619, 1964). In addition, the non-pyramidal cell has many of the features of the "theta cell" (which contrasts to the "complex spike cell") recorded in chronically-implanted, behaving animals (Fox and Ranck, Exp. Neurol. 49:299-313, 1975). There are no data showing a definite inhibitory role for these neurons; their function is still to be determined.

Supported by grants NS12151 and NS11669 from the NINCDS, NIH.

1243 AXON CONDUCTION BLOCK IN NERVE TERMINAL REGIONS CAUSED BY AXON DEPOLARIZATION. Dean O. Smith. Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706

In the terminal arborization of the excitor axon innervating the opener muscle of the crayfish walking leg, action potential propagation fails intermittently during prolonged repetitive stimulation (Hatt and Smith, 1976, J. Physiol. 259: 367-393). Using microelectrode techniques for recording and stimulation, it has been found that the blocks occur at axonal branch points and their onset is coincident with (1) decreasing conduction velocity, (2) decreasing sodium inward current, and (3) increasing rate of spontaneous transmitter release. Conduction failure can be reversed at least temporarily by applying hyperpolarizing current (about 12 μA) and jets of low-K^+ saline. It can also be reversed by reducing K^+ concentration in the bath and by decreasing the rate of nerve stimulation. Conversely, propagation fails after fewer impulses in bath solutions containing higher than normal (5.4 mM) concentrations of K^+ and also as the bath temperature is lowered. Action potentials in the nerve cannot be evoked when extracellular K^+ is 3-4 times normal, when muscle membrane potentials are about -50 mV. Thus conduction block results from axon depolarization.

Possible metabolic deficiencies which might cause propagation failure were estimated by assaying ATP and arginine phosphate concentrations in the nerve bundles at various times during block development in the excitor axon. After 10 min of stimulation during which conduction failed intermittently, the ATP concentration did not differ significantly from control values obtained from unstimulated nerves. Therefore, prolonged shortages of ATP do not appear to occur during intermittent conduction block.

Axon geometry at branch points was examined to determine if the safety factor for conduction was unusually low. Using Hoffman modulation-contrast optics, the diameters of the axon branches at a site of failure were measured. The geometrical ratio, $GR = (d_1^{3/2} + d_2^{3/2})/d_p^{3/2}$, where d_1, d_2, and d_p are the diameters of the two daughter axons and the parent axon, respectively, was calculated at regions of block. The average value was 0.89 (±0.20 s.d.), which is slightly less than the value 1.00 at which failure would be expected to occur because of the large load resistance presented by the daughter branches.

It is concluded that conduction block is caused by depolarization. Neither ATP shortages nor resistance mismatches at branch sites appear to underlie propagation failure. The cause of the depolarization may involve accumulation of extracellular K^+. Using K^+-sensitive microelectrodes, this is being studied currently.

Supported by USPHS grant no. NS 13600.

1244 **ESTIMATES OF CABLE PARAMETERS FROM AN ANALYSIS OF VOLTAGE TRANSIENTS IN LAMPREY SPINAL CORD NEURONS.** **Wm. P. Teubl* and Burgess N. Christensen** (Spon: K. I. Naka). **Dept. of Physiology and Biophysics, Univ. of Texas Med. Br., Galveston, TX 77550.**

Voltage transients were produced in lamprey spinal cord giant interneurons by injection of a brief current pulse through an intracellular microelectrode. The transients were recorded by a second intracellular electrode and analyzed according to the procedure suggested by Jack and Redman (J. Physiol. 215: 321, 1971) to estimate the cable parameters governing the passive propagation of transmembrane potentials in neurons. For this approach it was assumed that the Rall neuron model was applicable. This allows reduction of the soma and geometrically complicated dendritic tree to a model soma consisting of a lumped parallel combination of resistance and capacitance attached in parallel to an equivalent cylinder representing the dendritic tree. Membrane time constant (Tm), dendritic to soma conductance ratio (ρ_∞), and electrotonic length of the equivalent cylinder (L) were estimated from the decays of the voltage transients. In twenty two of the thirty two neurons studied it was possible to estimate all three cable parameters. For these twenty two neurons it was found that the electrotonic length of the equivalent cylinder was similar to cat spinal motoneurons (1-2 space constants). A test of the Rall model as an adequate description of these lamprey neurons was provided by computer simulations. Using the estimated cable parameters, a voltage transient was produced from an analytical expression for the Rall model which describes the voltage recorded at the model soma following a brief current pulse. These simulated transients fit closely the experimental transient even during the early part of the voltage decay. This result suggests that the time constant for the soma and dendritic membrane is similar for these lamprey neurons. These results have been used to investigate the location of synaptic junctions made by a specific giant axon on the equivalent cylinder.

NEUROPATHOLOGY AND NEUROIMMUNOLOGY

1245 EFFECTS OF PRENATAL EXPOSURE TO THE CHOLINESTERASE INHIBITOR CARBOFURAN ON MATURATION, BEHAVIOR AND BRAIN MORPHOLOGY OF THE MOUSE. DAVID L. AVERY AND JOAN M. SPYKER, Department of Pharmacology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72201.

Pregnant mice were given a daily dose of 0, 0.01 or 0.50 mg Carbofuran per kg body weight throughout gestation. Mothers of all experimental groups gave birth to approximately equal numbers of viable, overtly normal offspring. Pups born to mothers receiving the higher dose of the carbamate weighed significantly less at birth but their rate of growth equaled that of control pups. Physical maturation and reflex development were evaluated daily in all offspring. Pups born to Carbofuran mothers developed simple righting, acceleration righting and withdrawal responses at the same time and, therefore, in the same sequence as control offspring. Testes descent was delayed in male offspring from both the 0.01 and 0.50 mg/kg Carbofuran groups, while vaginal opening was delayed in female offspring from the lower treatment group only.

Neuromuscular coordination, learning ability, endurance and activity of offspring were evaluated after weaning using several behavioral techniques. Prenatal exposure to Carbofuran significantly affected the swimming behavior of apparently normal male mice. However, swimming posture and style of female offspring did not appear to be affected by the pesticide. Running speed and frequency of errors were measured in a modified Lashley III maze. The performance of Carbofuran offspring was initially indistinguishable from that of control offspring. As learning progressed, the running speed of offspring of the lower dose group fell behind control values, while the speed of offspring of the higher dose group rose above control values. There were no differences in error frequency in the maze between treated and control animals. Prenatal exposure to Carbofuran did not affect treadmill endurance, rod cling endurance, inclined plane performance or open-field behavior.

Subjects were sacrificed at 101 days of age and brains removed for morphological evaluation. Focal defects were found in the forebrains of offspring prenatally exposed to 0.50 mg/kg/day Carbofuran. Dense aggregations of chromatin-containing cells were observed in an area extending from the anterior commissure to the anterior olfactory nucleus. No neuropathological changes were observed in brains from offspring born of mothers receiving either 0 or 0.01 mg/kg/day Carbofuran. (This research was supported by EPA contract 68-01-1925).

1246 DECREASE IN LIPID SYNTHESIS IN FIBROBLASTS FROM PATIENTS WITH DYSTONIA MUSCULORUM DEFORMANS. John Blass, Gary E. Gibson & Adriana Vasil*. Neuropsychiatric Inst., UCLA, Los Angeles, CA 90024.

Dystonia musuclorum deformans is a neurological disorder which appears to be genetic. However, the biochemical basis of the disorder is unknown. The metabolism of [U-^{14}C]glucose, [1-^{14}C]-pyruvate, and [^{3}H]acetate by human skin fibroblasts from dystonia patients was studied, since their utilization provides a general indication of cellular metabolism. If a defect is found using this general approach, then it can be pursued further to determine the precise molecular mechanism.

In the studies with [U-^{14}C]glucose, cells were grown in a balanced salt solution containing heat inactiviated fetal calf serum. Lack of mycoplasma was confirmed routinely using the Hoechst stain and the uridine/uracil ratio method. Cells were harvested with 0.05% trypsin approximately one day before confluence. They were washed with phosphate buffered saline and finally suspended in Krebs-Ringer phosphate buffer. Incubations were for one hour in one ml of Krebs-Ringer phosphate buffer containing 5 mM-[U-^{14}C]-glucose (2 Ci/ mole) under 95% O_2:5% CO_2 and cell protein concentration of 0.25 - 0.5 mg. The incorporation of [U-^{14}C]glucose into $^{14}CO_2$, protein, lipid and nucleic acids was determined. Three dystonia cell lines were studied on six different days and compared with eight different control lines. The incorporation of [U-^{14}C]glucose into lipids was reduced by 33% ($p<0.01$) [from 2108 $\pm$ 230 (n =27) to 1417 $\pm$ 156 (n=18) picomoles of glucose incorporated per mg protein per hour].There was overlap between dystonia culture and control culture in individual expts. Incorporation of ^{14}C into proteins was similar in dystonia (1317$\pm$ 122, n = 17) and controls (1244 $\pm$166, n = 18). Oxidation of glucose to $^{14}CO_2$ was lower in dystonia cultures (4721$\pm$ 416 n = 17, picomoles/ min per mg protein) than in controls (5827 $\pm$ 377, n = 25) but the difference was not statistically significant ($p>0.1$). Furthermore, oxidation of [1-^{14}C]pyruvate did not differ significantly between dystonia and control lines under a number of incubation conditions.

The decreased incorporation of glucose into lipids may indicate a metabolic defect and serve as a molecular "handle" for helping to determine the molecular basis of hereditary Dystonia Musculorum Deformans. (Sponsored by the Dsytonia Medical Research Foundation.)

1247 IMPAIRED ABSORPTION OF CSF DURING EXPERIMENTAL SUBARACHNOID HEMORRHAGE: EFFECT OF BLOOD COMPONENTS ON VESICULAR TRANSPORT IN ARACHNOID VILLI. Albert B. Butler, M.D., Norman H. Bass, M.D. Richard N. Johnson, ScD. University of Virginia School of Medicine, Depts. of Neurosurgery, Neurology, and Biomedical Engineering, Charlottesville, VA 22901.

Elevated intracranial pressure associated with acute subarachnoid hemorrhage has been attributed to a disturbance of cerebrospinal fluid (CSF) efflux through arachnoid villi. In an attempt to assess the role of various blood components to acutely induce this disorder in CSF dynamics we have combined our previously described technique for manometric assessment of CSF outflow resistance (Ann. Neurol. 3: 156-165, 1978) with ultrastructural examination of the arachnoid villi. CSF outflow resistance in the rat was quantitatively assessed before and 15 and 20 minutes after an intrathecal ten minute constant rate infusion (22µl/min) of the following substances: (A) heparinized whole blood, (B) plasma (fibrinogen content: 440mg), (C) dialysate of plasma (containing substances <6000 mol.wt.), and (D) serum as compared with saline controls, with or without heparin.

For electron microscopy, artificial CSF-horseradish peroxidase was infused into the subarachnoid space of control and experimental animals with flow rates adjusted to achieve a steady state CSF pressure of 800mm in all animals. When steady state pressure was achieved, aldehyde fixation was performed to preserve the arachnoid villus in its pressure induced state. Sections containing arachnoid villi were processed for HRP using routine EM techniques.

The data show that both blood and plasma significantly increase CSF outflow resistance, while plasma dialysate and serum show resistance values which cannot be distinguished from saline controls. Ultrastructural changes within the endothelium covering the villi of animals infused with either saline, serum, or plasma dialysate were similar, demonstrating numerous transendothelial channels seen as single enlarged cytoplasmic vesicles, or confluent vesicles in transcytoplasmic chains, all of which contain HRP. In contrast, animals infused with blood or plasma showed a marked reduction in the number of transendothelial channels associated with a fibrin-like material in the subendothelial space at equivalent pressures. Based on these observations we suggest that during experimental subarachnoid hemorrhage CSF outflow through the endothelial covering of arachnoid villi is not impeded by blood born cellular elements, but by macromolecular substances associated with the clotting cascade.

1248 GANGLIOSIDE METABOLISM IN HUMAN BRAIN AND BRAIN TUMORS CULTURED IN VITRO. Mary Thoesen Coleman and Allan J. Yates. Dept. Path., College of Med., OSU, Columbus, OH 43210.

Gangliosides are cell surface components which may play a role in the regulation of cell growth. In this study we examined cultures of normal human white matter and brain tumors for differences in ganglioside patterns after administration of a radioactive precursor. Cells cultured from a malignant astrocytoma, an oligodendroglioma, a glioblastoma multiforme and adult white matter were seeded at subconfluent densities in 7 petri dishes (Corning P-100's). When cells were almost confluent, ^{14}C-1-D-glucosamine was added to the cultures (0.5µCi/ml media). Twenty-four hours later, the medium was removed and cells were washed with phosphate-buffered saline. Cells were harvested by scraping and aliquots of a cell suspension taken for determination of total cellular protein and total incorporation of radioactive label into the cells. After centrifugation, cell pellets were frozen until used for extraction of gangliosides. Cells from one plate were trypsinized and counted. Gangliosides were purified with modifications of the method of Suzuki (1965, J. Neurochem. 12: 629). Gangliosides were separated by thin layer chromatography and radioactive patterns detected using a beta camera. Individual bands were scraped and counted in 0.1 ml water and Aquasol 2.

The population doubling times were: normal glial cells, 7 days; glioblastoma, 4 days; astrocytoma, 33 days; and oligodendroglioma, 30 days. In normal glial cells and glioblastoma cells 71.97 and 67.39% respectively, of the label recovered, was incorporated into bands which cochromatographed with GM_1, GM_2, and GM_3 standards. In the oligodendroglioma and astrocytoma, the percentage of counts in GM_1, GM_2, and GM_3 was only 36.7 and 41.8% of the counts recovered with a substantial percentage of counts in bands cochromatographic with disialogangliosides. When counts in gangliosides were expressed as a function of cell number, the counts in disialogangliosides were higher in the slower growing cells (oligodendroglioma and astrocytoma) than in the normal glial cells and the rapidly growing glioblastoma. All cell cultures showed significant incorporation of label into monosialogangliosides. Label in GM_1, expressed in terms of cell number, showed little variation. Label in GM_2 was higher in the glioblastoma relative to both normal glial cells and other tumor cells. The correlation of ganglioside metabolism with growth rates in these cultures suggests a possible role of ganglioside metabolism in the mechanism of growth control. (Supported by NIH Research Traineeship #784760, American Cancer Society ACS #In-16P and by The Association for Brain Tumor Research.)

1249 MAST CELLS WITHIN THE VENTRICLES OF THE VERTEBRATE BRAIN. John J. Dropp. Dept. Sci. and Math., Mt. St. Mary's College, Emmitsburg, MD 21727.

The brain ventricles of 11 species (frog, toad, newt, congo eel, lizard, horn toad, sparrow, canary, mouse, dog, man) of vertebrates were examined for the presence of mast cells These cells, as adjudged by their morphological and staining characteristics, were found within the ventricles of only adult frogs, young mice, young dogs and humans. They were either entirely free within the ventricle (frog, mouse, dog, man) or wedged between choroidal epithelial cells with approximately one-half of the cell projecting into the ventricle (frog). That these cells were indeed mast cells was strongly suggested by their morphological and histochemical similarities to mast cells in either non-nervous (e.g. tongue) or nervous (e.g. leptomeninges, choroid plexuses) tissues of the same individual.

1250 THE EFFECT OF PRENATAL METHYL MERCURY TREATMENT ON BEHAVIOR IN RATS. Christine U. Eccles* and Zoltan Annau (SPON: A. Goldberg). Dept. Environ. Hlth. Sciences, Johns Hopkins University, Baltimore, Maryland 21205.

It has been well documented that the developing organism is susceptible to the toxic effects of methyl mercury (MeHg) and that the fetus exposed to utero may actually accumulate higher concentrations of it than the treated mother. Behavioral changes in the neonatal rat that occur after in utero exposure to methyl mercury, however, have not been well described.

In the present study, pregnant female Long-Evans hooded rats received 0, 5 or 8 mg/kg of methyl mercury as methyl mercury chloride dissolved corn oil or 50 mM sodium carbonate on day 7 of gestation.

When the pups were born they were weighed and counted; all litters were culled to a standard size of 8. The pups were also weighed on day 7, 14 and 21.

On days 4, 7, 14 and 21, two to three pups from each litter were individually placed in a Stoelting electronic activity meter. Ten minute subtotals of activity were obtained during the course of the hour.

Neonatal weight data for rat pups exposed to mercury in utero were not different from controls. Activity measures revealed that the animals whose mothers received 8 mg/kg MeHg were significantly more active at 7 days of age than control animals of the same age. The mean activity level at 14 days was also higher for controls but the difference was not statistically significant. Offspring of mothers exposed to 5 mg/kg MeHg in utero did not show any significant changes in activity levels on any of the days tested.

Adult males whose mothers were treated with 8 mg/kg MeHg were tested in a two-way shuttlebox avoidance task. Animals were trained to meet a criterion of ten consecutive avoidances during acquisition. Avoidance behavior was extinguished when the criterion was achieved. This was followed by a period of reacquisition in which the animals were again required to make ten consecutive avoidances. There was no significant difference in the trials to reach initial criterion between mercury exposed and control animals, although the performance of the exposed animals was considerably more variable. During reacquisition the exposed animals required a significantly greater number of trials to reach criterion than controls. The results indicate that prenatal methyl mercury ingestion can lead to learning deficits in the adult animal.

1251 ELECTROPHYSIOLOGIC OBSERVATIONS OF CULTURED DORSAL ROOT GANGLIA CELLS INFECTED WITH HERPES SIMPLEX VIRUS. Howard Gitelson*, Robert Pozos, Richard Ziegler*, Paul Lima*, Julie Moore* and Steven Oakes*. Depts. of Physiology and Microbiology, Univ. of Minnesota-Duluth, School of Medicine, Duluth, Minnesota 55812.

Although Herpes Simplex Virus has been reported to reside in the Dorsal Root Ganglia, no studies have been reported as to the effect of the virus on the electrophysiology of the Dorsal Root Ganglia. Rat Dorsal Root Ganglia were grown in culture for 14-40 days. At that time, Herpes Simplex Virus I of a concentration of 10^6 p.f.u. were placed on the culture for 1 hour. After the incubation period of 1 hour, the media was changed. Electrophysiologic studies were made before and after the infection. Resting membrane potential, evoked action potentials and width of the action potential (full width half maximum) were initial parameters studied.

Results of these experiments indicate that in control cultures, two kinds of evoked action potentials are seen. Those with a pronounced falling phase and those with a prolonged falling phase (plateau). The latter were the predominant response recorded. To detect the plateau the evoked action potential was differentiated.

Approximately three hours after virus infection, there is a decrease in the height of the overshoot of the evoked action potential. The resting membrane potential during the initial three hours of observation remains at -40 to -60 mv. From three to six hours there is no overshoot even with maximal stimuli, however there are graded responses. These responses which vary with the stimulus do not show a further widening than seen at three hours. After six hours, it is extremely difficult to elicit action potentials. Control electrophysiologic observations lasting up to 24 hours did not show the changes observed with virus-infected cultures. Light microscopic observations show that there are no observable cytological changes in the nerve cell bodies at six hours when pronounced electrophysiological changes are observed. EM evidence to confirm virus infection of the DRG cells is currently being conducted.

Supported in part in USPHS Grant 1 RO1-NS-13326-01A1

1252 GRAFT-VERSUS-HOST DISEASE IMPAIRS BRAIN DEVELOPMENT. W. Sue T. Griffin, Mauro F. Pacheco, and Judith R. Head*. Department of Cell Biology, Univ. of Texas Health Science Center at Dallas, Dallas, TX 75235.

We previously described production of brain damage in infant rats subjected to graft-versus-host disease (GVHD) caused by foreign lymphocytes reacting against host alloantigens. Presented here are the effects of GVHD on the cytoarchitecture of the cerebellum. Using hematoxylin and eosin stained 7 μm midsagittal sections, various parameters were assessed including: 1) Counting the number of cells in the cerebellar layers; 2) Calculating the total midsagittal area; and 3) Measuring the surface area of the cerebellum. GVHD was procured by injecting 40 x 10^6 parental strain lymph node cells into (Fischer X DA)F_1 hybrid rats on the day of birth. The grafted lymphoid cells attack the lymphomyeloid tissue of the neonatal host, resulting in a fatal wasting syndrome which was well-developed by postnatal day 14, the day of sacrifice. Uninjected littermates served as controls. Total cerebellar area was decreased in GVHD-affected animals by 9% but the perimeter was not significantly altered, indicating a greater change in the internal organization than external surface area. Compared to other layers the external granular layer was most affected by GVHD, having only 67% of the area found in control tissue ($p < 0.05$). The molecular layer was not significantly affected, but the internal granular layer was obviously smaller (87% of control values, $p < 0.05$). The number of cells in the external granular layer was 2.8 ± 0.1 cells per 100 μm^2 in cerebella from GVHD animals compared to 3.7 ± 0.1 in controls (75%, $p < 0.05$). The number of Purkinje cells in lobule VI was also reduced on a per mm^2 basis (84% of control, $p < 0.05$). These data suggest that in addition to a decrease in the number of cells available for mitosis which would result in a smaller internal granular layer, there may be some cell death as indicated by the decrease in Purkinje cells. The cerebellum appeared normal with regard to gross morphological parameters such as layering, and there was no lymphocytic invasion, ruling out the possibility of a cell-mediated attack on cerebellar cells. We postulate that a blood-borne factor is causing a decrease in cell proliferation and function in this disease which is a potential factor in perinatal induction of mental retardation. (Supported by NIH NS14663-01).

1253 ACTIVITY LEVEL MEASUREMENTS AS AN INDICATION OF MUSCULAR WEAKNESS IN EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS. I. J. Griffith, J. A. Lettieri*, N. L. Norcross*, M. E. Eldefrawi*. Dept. of Microbiology, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, and Dept. of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD 21201.

Recently an animal model of myasthenia gravis (MG) has been developed. This model, designated experimental autoimmune myasthenia gravis (EAMG), has proven to be extremely valuable in elucidating the pathogenic mechanisms involved in MG. It is likely that this animal model can also be a valuable aid in evaluating various agents for their effectiveness in the treatment of MG. This type of study has been complicated by the lack of a simple and objective means of precisely determining the extent of muscular weakness in the experimental animal. We have found that measurements of an animal's normal activity can provide an extremely useful means of assessing the degree of muscular weakness associated with this disease.

Studies were performed in which rats of the Lewis/MA f strain were individually housed in Wahman LC-34 activity cages. EAMG was induced in half of the animals through the inoculation of purified acetylcholine receptor protein (AChR), obtained from Torpedo ocellata, together with adjuvants. The remaining animals served as controls and received inoculation of adjuvant only. The experimental animals' activity was assessed by recording the number of wheel revolutions completed by each group during each 24 hour period and comparing the activity of the experimental group to that of the control group. It was found that animals with EAMG experienced two periods of decreased activity which corresponded to the previously reported acute and chronic phases of EAMG. During the acute phase their activity rapidly decreased from 7 days post inoculation (DPI) to 11 DPI. The experimental animals' activity then increased from 12 DPI to 19 DPI until their activity was equal to that of the control group. During the chronic phase the activity of the experimental animals gradually decreased during the period from 28 DPI to 58 DPI. The animals' activity then remained at a stable level until 80 DPI at which time the level of activity began to increase. Correlations between activity levels and immunological parameters of EAMG will also be discussed.

The results of this study indicate that activity measurements can provide a very simple and objective method of determining the extent of muscle weakness in EAMG and may prove to be a valuable method for evaluating new forms of therapy for MG.

(Supported by the Muscular Dystrophy Association of America)

1254 BIPHASIC EFFECTS OF MAM ACETATE ON THE DEVELOPING BRAIN. R.Haddad Ausma Rabe, Judy Shek, Ruth Dumas* and Barbara Canlon.* Neuroteratology Laboratory, New York State Institute for Basic Research in Mental Retardation, 1050 Forest Hill Road, Staten Island, N.Y. 10314.

Methylazoxymethanol (MAM) is a naturally occurring alkylating agent that is carcionogenic, mutagenic, and a CNS teratogen. It has been synthesized and is commercially available as the acetate. A single treatment during pregnancy results in a considerable variety of CNS malformations, the specific forms depending only on the time of gestation at which it is administered. Pathologic ultrastructural changes have been demonstrated within 15 minutes of treatment. Shortly after treatment massive necrotic foci are readily found in the developing brain, their loci depending on the time of treatment. In the rat treated on gestation day (GD) 15, when the cerebral hemispheres are beginning to develop, lesions are found in the cerebral hemispheres and are readily visualized within 8 hours. The result is a massive loss of cerebral neurons, resulting in micrencephaly in the young. Treatment of the rat perinatally, when the highest rate of cellular proliferation is in the developing cerebellum, causes an extensive destruction of Obersteiner's layer resulting in hypoplasia of the cerebellar cortex. However, although the acute lesions determine the subsequent mass of the affected region, there is also a chronic, biochemical, lesion which has effects that contribute to the ensuing pathologic cytoarchitecture of the affected region. Fetal brain DNA, RNA, and protein have been shown to be methylated by MAM. This may be the basis for the neurotoxic effect of MAM during the acute phase. However, the DNA of many of the surviving neurons is also alkylated. A variety of methylated bases have been detected in the brains of adult micrencephalic rats produced by prenatal exposure to MAM. It would appear that these structural alterations of the nucleic acids of the fetal brain result in metabolic changes that affect the course of neuronal migrations in the developing brain. Although all the usual structures are present (including barrels in the somatosensory cortex) in the micrencephalic brain, though on a reduced scale, the cytoarchitecture is altered. Similarly, the perinatally treated rat has not only a hypoplastic cerebellum, but one that is possessed of a grossly abnormal cytoarchitecture. The biphasic consequences of prenatal exposure to MAM Ac have recently been more clearly seen in the lissencephalic ferret (produced by treatment with MAM Ac on GD 32). In this preparation we have been able to experimentally separate the two phases. (Supported, in part, by NIH Grants MH-16610, NS-08856, NS-10409-01A1, HD-08346, 5-S01-FR-05558-08, -09, -10, and allocations from Hoffmann-LaRoche).

1255 INHERITED CANINE NEUROMUSCULAR DISORDER ACCOMPANIED BY HYPERALDOSTERONISM. G. A. Hegreberg and M. J. Hamilton* Dept. Veterinary Micro. and Path., Wash. State Univ., Pullman, WA 99164.

An inherited neuromuscular disorder of the dog characterized by general muscular atrophy and intolerance of cold and exercise stress has been reported. The disorder is not clinically apparent until the affected dogs are 3-4 months old. The disorder has a progressive course in the growing dog. However, the clinical manifestations stabilize after the dogs mature. Preliminary pedigree analysis indicates that the disorder is transmitted as an autosomal recessive trait. Both affected male and female offspring have been produced from apparently nonaffected parent stock. Serum and RBC electrolytes, including Na, K and Mg, appear similar in affected and nonaffected dogs. The disorder is accompanied by an elevation in urinary creatine excretion and depressed urinary creatinine/creatine ratio. Serum creatine phosphokinase (CPK) activity is not elevated in the affected dogs.

The most consistent and severe pathologic change involves the distribution and size of the skeletal muscle fiber subtypes, I and II. The affected dogs have a marked deficiency of type II fibers and increased variation in the diameter size of both type I and type II skeletal muscle fibers. This alteration of fiber type distribution appears to be generalized throughout the skeletal muscles of the body. Other pathologic changes include many fibers with centrally located nuclei, nuclear rowing, and mild increases in perimysial and endomysial connective tissue. Degenerative changes of the skeletal muscle fibers are minimal.

The renin-aldosterone system in adult fasted nonaffected and affected dogs was examined. Plasma aldosterone was measured using a radioimmunoassay (RIA) method adapted from Drewes, et al., on 11 affected and 4 nonaffected dogs. Aldosterone concentrations (mean $\pm$ S.D.) were 276.4 $\pm$ 127.7 pg/ml in affected animals, and 126.8 $\pm$ 54.9 pg/ml in the nonaffected dogs. Plasma renin activity (PRA) was estimated with RIA for angiotensin I, modified from Haber, et al., in 26 affected and 6 nonaffected dogs. PRA (mean $\pm$ S.D.) was 0.930 $\pm$ 0.817 ng/ml/hr in affected dogs and 0.970 $\pm$ 0.912 ng/ml/hr in nonaffected dogs. The plasma aldosterone levels were significantly increased in the affected ($p < 0.05$). The PRA was not significantly different in the affected and nonaffected dogs ($p > 0.1$). Plasma cortisol levels evaluated by RIA in 12 affected and 8 nonaffected adult dogs were not significantly different.

This disorder will provide an important experimental model for the study of the control of aldosterone production and for comparisons to similar human disorders. This disorder will also be useful for comparison with other muscle disorders with similar histochemical changes, including aging changes of muscle.

(Supported by NIH grants RR00515, FR5465, and the MDAA, Inc.).

1256 EFFECT OF INSULIN-INDUCED HYPOGLYCEMIA ON THE BLOOD BRAIN BARRIER. Dora W. Hsu* and E. Tessa Hedley-Whyte. Dept. Path. New England Deaconess Hospital, Harvard University, Boston, MA 02215

Hypoglycemia was induced in mice (30-35 g) by intraperitoneal injection of 8 units of crystalline zinc insulin. Horseradish peroxidase (HRP, 10 mg in 0.1 ml saline) was injected i.v. 15-20 min prior to sacrifice. After per cardiac perfusion with Karnovsky's fixative, brain slices were examined for HRP extravasation along blood vessel walls and into cerebral parenchyma. Plasma glucose levels were determined at time of sacrifice. 16 of 19 mice with severe hypoglycemia (lethargy, stupor) developed generalized seizures. The 3 mice without seizures and 14 of the 16 showed diffuse focal parenchymal extravasations of HRP. A ruptured blood vessel with one or two red blood cells was found in the center of 10 to 18% of the focal exudates. Five of 6 mice with moderate hypoglycemia (40-80 mg%) compared to 2 of 8 normal mice showed occasional HRP parenchymal accumulations. The number of HRP stained segments of vessel walls was less in the insulin treated mice compared to the untreated mice.

These observations contrast with the patterns observed in hypertension and hypervolemia where both the number of stained vessel wall segments and parenchymal accumulation of HRP increase concomitantly. Although experimentally induced seizures are accompanied by increased leakage of HRP into brain, ruptured vessels in the areas of leakage are rarely seen in contradistinction to the severely hypoglycemic mice. We conclude that insulin-induced hypoglycemia is associated with: 1) an increase in cerebrovascular permeability to HRP with focal extravasation into brain parenchyma; and 2) a decrease in the normal transit of HRP across segments of cerebral vessels.

1257 NEUROPATHOLOGY PROBLEM SOLVING EXERCISES. M. Z. Jones, S. Mullis, K. L. Lovell, C. Watson, T. Jenkins, M. Goetting, and B. Vincent. Dept. Pathology, Mich. State Univ., E. Lansing, MI 48824.

Neuropathology Problem-Solving Exercises (NPPSE) were developed to provide the freshman medical student with the opportunity to practice, reinforce and synthesize the basic concepts of cerebrovascular, traumatic and neoplastic diseases of the nervous system. Case materials were adapted by utilizing modified programmed instruction techniques. Objectives, instructions and pretests preceded the clinical problem-solving aspects of the NPPSE. Students were then asked to predict the gross and microscopic pathological findings on the basis of the problem cues. Directions followed which guided the student through the gross and microscopic examination. Completion of a clinicopathological correlation and construction of a final neuropathological diagnosis were expected within one hour. Aids included diagrams, labelled photographs and ancillary diagnostic study reports. The modified programmed instruction format provided immediate feedback. Through this means, real and complex neurological problems were introduced to first year medical students, and objectives relating to common neurological diseases were reinforced. Preliminary analysis of evaluations by students revealed a high level of subjective satisfaction with the effectiveness and efficiency of this learning exercise. Similar exercises can be devised for other clinical or basic neurosciences in order to assure optimal practical experience or to monitor problem-solving ability at different points in a graduate or professional educational program.

1258 IMPROVED METHOD FOR INTRACEREBRAL INPLANTATION OF TUMOR CELLS IN RATS. Naoki Kobayashi*, Nancy R. Clendenon, and Norman Allen. Div. Neurol., Coll. Med., OSU, Columbus, Ohio 43210.

A reproducible tumor model is essential for the study of experimental treatment of tumors. Our present free-hand intracerebral implantation technique, using young rats whose skulls are sufficiently soft so as to permit direct needle puncture has advantages of simplicity and rapidity of procedure. Disadvantages include leakage of cells outside the skull with subsequent growth of large extracranial (EC) masses, intracranial extracerebral (IEC) growth, and intraventricular leakage resulting in spinal cord and brain stem metastases. Studies using a spongioblastoma cell line showed regression of intracerebral (IC) tumor growth with cranial irradiation; however, 38/97 rats inoculated in this manner had spinal or brain stem tumor growth and 8 developed extensive EC masses.

Recently, we compared these results with the following modifications: 1) semi-stereotaxic percutaneous injection with 10 µl tumor cell suspension in phosphate buffered saline; 2) same as 1) but with 1% agar in the cell suspension (Sobue et al,.Igaku no ayumi 101:542, 1977);3) modified stereotaxic injection (Barker et al, Cancer Res.33:976,1973) with the same tumor cell suspension as in 1); 4) same as 3) but with 1% agar; 5) same as 4) but with a 5µl injection volume containing the same cell number. Inbred CDF male rats received 10^4 spongioblastoma cells into the center of the right caudate nucleus. Prior to sacrifice (24 hrs) on day 28, rats were given Trypan blue so that extent of tumor growth and edematous zones would be visually apparent and measurable in formalin fixed samples. Products of the largest diameters (in mm) of three dimensions were taken as indices of tumor size. Dissected tumor samples were blotted dry and weighed. Preliminary studies using Trypan blue established the optimal injection site so as to avoid subarachnoid or intraventricular leakage.

The following results were obtained: in 1) no IC growth was seen in 9 rats studied; 2) IC growth was observed in 6/10 rats but for 5 of 6 the size and weights were very small; in addition, 8/10 had IEC growth of various sizes and 7/10 had EC masses: 3) IC growth was found in 8/9 animals but all 9 had IEC and 2 had EC masses; 4) all 11 rats had adequate IC tumor growth (mean size index = 96.8± 56.3(SEM); 5/11 however, had small IEC and one had a small EC mass; in 5) although 10/11 rats had IC growth, the mean size index was very small (7.8±2.6); 2/11 had small IEC growth, but no EC growth was noted. No spinal cord nor brain stem metastases was detected in these studies. Further studies using method 4 yielded only one IEC, and one EC mass for the 19 animals inoculated.

In conclusion, stereotaxic injection into the center of the caudate nucleus with a 10µl injection volume containing 1% agar (4) appears to be the best method for intracerebral transplantation of tumor cells in rats. (Supported by NIH grant No. CA-20348.)

1259 HISTOENZYMATIC STUDIES ON EXPERIMENTAL BRAIN CONCUSSION. Joseph C. Lee, Hin Ching Liu* and Louis Bakay*. Dept. Anat. Sci., Univ. Okla. Health Sci. Ctr., Box 26901, Okla. City, OK 73190

Brain concussion was produced in rats after absorbing an energy of 1,450gm/cm by a blow of a specially-constructed iron pendulum at the external occipital protuberance. From 5 min to 62 hr, the brain and upper spinal cord were processed for electron microscopic and histoenzymatic studies. Electron microscopy showed severe swelling of neuronal mitochondria in the occipital cortex, cerebral edema in the frontal lobe, and both changes in the craniospinal junction at 30 min, reached a peak at 1 hr and disappeared at 24 hr after concussion. The activities of succinic dehydrogenase (SDH) became stronger in neurons of the cerebellar cortex, medulla and spinal cord 5 min post-concussion, and in the occipital cortex 15 min later, but not in the frontal cortex. The increased activites returned to the normal level 1 hr after concussion. No changes were observed in the activity of cytochrome oxidase in neurons of the above-mentioned regions. In glial cells, these two mitochondrial enzymes remained strong before and after concussion. The alkaline phosphatase (APase) activities reduced in blood vessels at 5 min, disappeared at 15 min, and returned to the normal level 62 hr post-concussion. No significant changes were found in the butyrylcholinesterase activity throughout the experimentation. We conclude that brain concussion activates SDH and reduces APase activities, which result in mitochondrial swelling and cerebral edema, respectively, in those regions of the central nervous system paralleling our electron microscopic observations.

1260 LONGITUDINAL ELECTRORETINOGRAPHIC AND PATHOLOGICAL CORRELATES OF ALUMINUM-INDUCED RETINOPATHY IN RABBITS. A.A. Lidsky, C.M. Miezejeski, G.Y. Wen*, H.M. Wisniewski*. NYS Institute for Basic Research in Mental Retardation, Staten Island, NY 10314.

ERG's were obtained in awake rabbits two and three times weekly, both before and following a single intravitreal injection of 100 µl of 1% aluminum chloride in one eye and an equal volume of physiological saline in the other. Ophthalmic neosynephrine and tetracaine hydrochloride were applied preceeding insertion of Burian-Allen contact electrodes. Each eye was stimulated separately using one per second and 10 µsec duration flashes at each of three stimulus intensities (I=1, 4, and 16 on a Grass PS-22), and 30 Herz flicker of intermediate intensity. Individual animals were sacrificed at intervals from four to sixty days after aluminum treatment and their retinas were assessed morphologically.

Progressive reductions were found in the amplitudes of all components of the ERG, under all stimulus conditions. Single flash stimulation at I=16 produced the earliest nonreversing reductions in b-wave (7-8 days) and c-wave (3-6 days) amplitudes in the aluminum versus saline-treated eye. The a-wave attenuations were delayed to 11-33 days after treatment. Responses to flicker were immediately reduced by approximately 40% in both control and aluminum eyes, but while amplitudes in the former recovered to pre-injection levels within a few days, responses of the aluminum eyes progressively declined to less than 1/3 of either the pretreatment or control eye amplitudes at 40 days. Morphologically, neurofibrillary tangles were identified in both ganglion and bipolar cell layers within 7-14 days after aluminum injection. Other degenerative changes, including fragmentation of rods and cones and focal pyknosis, appeared in individual retinas within 14 days, with progressive loss of pigment epithelium and photoreceptors thereafter. The sequence of tangle formation and cellular degeneration, beginning with ganglion cells, then bipolars and photoreceptors, is consistent with other findings on ocular metallosis, with the ERG identifying both early and progressive events in the bipolar layer, pigment epithelium and photoreceptors.

1261 INFARCTION THRESHOLD IN THE BASAL GANGLIA AND ADJACENT STRUCTURES DETERMINED BY CEREBRAL BLOOD FLOW MEASUREMENT DURING MIDDLE CEREBRAL ARTERY OCCLUSION IN UNANESTHETIZED MONKEYS. Frank W. Marcoux, Richard B. Morawetz*, James H. Halsey, Jr.*, and Umberto DeGirolami*. Neurosciences Program and Departments of Neurosurgery and Neurology, University of Alabama at Birmingham, Birmingham, Alabama, 35294.

Unanesthetized macaque monkeys were subjected to middle cerebral artery (MCA) occlusion. Local cerebral blood flow (CBF) was measured from electrode sites in cortical and subcortical gray and white matter by hydrogen clearance. Two to four weeks following ischemic insult, histologic examination documented cerebral infarction and its precise relation to CBF recording sites.

Residual CBF during MCA occlusion was found to correlate closely with incidence and character of infarction. When residual CBF fell below 12 cc/100/g/min for 2 hrs or more, infarction invariably occurred around the electrode tip. The infarction threshold for ischemic durations of less than 2 hrs is under investigation and appears to fall off rapidly at around 1 hr.

The incidence of infarction correlates with an absolute level of residual CBF rather than a percent fall from pre-ischemic level and this correlation holds for both gray and white matter.

Since blood flow and metabolism are consistently reported lower in white matter than gray matter, it has been presumed that white matter can withstand greater degrees of ischemia than gray. Our data do not support this assumption. When CBF fell and remained below 12 cc/100g/min during 2 or more hrs of MCA occlusion, infarction always occurred in putamen and caudate as well as in capsular and insular white matter.

This finding provides insight into the patho-physiology of ischemic stroke and describes a model with which therapies can be given trial to determine their effectiveness in interrupting the progression to irreversible cell damage.

This work supported in part by NIH Grant NS08802.

1262 FATTY ACID COMPOSITION OF HUMAN ERYTHROCYTES IN DUCHENNE MUSCULAR DYSTROPHY. COMPARISON WITH NORMAL AND NEUROMUSCULAR DISEASE CONTROLS. Jack McLaughlin and W. King Engel. Med. Neurol. Br., N.I.H., Bethesda, MD 20014

Minor compositional changes in phospholipids are among the many reported and disputed "defects" of erythrocyte membranes in Duchenne muscular dystrophy (DMD). Howland and Iyer (Science 198: 309, 1977) have recently reported that a large decrease in palmitoleic acid content of erythrocyte membranes of patients and carriers of DMD was disease-specific and suggested that a defect in membrane triglyceride metabolism is the primary enzymatic lesion in this disease.

We have determined the total fatty acid composition of human erythrocytes from patients with DMD, definite carriers of the disease, and a large number of normal and neuromuscular disease controls. Erythrocytes were thoroughly washed to remove platelets, leukocytes, and plasma, and lipids were extracted, using methods recommended by Nelson (Blood Lipids and Lipoproteins: Quantitation Composition, and Metabolism, Wiley-Interscience, 1972). Sample degradation was avoided by the use of low temperatures, antioxidants, and deoxygenated solvents. Fatty acid methyl esters were prepared with quantitative yield by the method of Morrison and Smith (J. Lipid Res. 5: 600, 1964), purified by thin-layer chromatography, and analyzed using a HP Model 5830A gas chromatograph equipped with a digital integrator. The compositional data obtained for normal controls agreed closely with recent literature values (Nelson, ibid.). We detected no change in any aspect of the fatty acid composition of erythrocytes of patients with DMD or definite carriers of the disease from that of normal controls, from patients with myotonic muscular dystrophy, or from the neuromuscular disease control group (including cases of amyotrophic lateral sclerosis, myositis, polymyositis, myotonia congenita, and other disorders). For example, palmitoleic acid data (area % ± S.D.) were as follows:

Normal Controls (N=11)	0.76 ± 0.14
DMD (N=9)	0.81 ± 0.12
Definite Carriers of DMD (N=3)	0.73 ± 0.08
Myotonic Muscular Dystrophy (N=7)	0.73 ± 0.07
Neuromuscular Disease Controls (N=25)	0.81 ± 0.15

Total plasma levels of palmitoleic acid after an overnight fast were determined in separate experiments and again no significant differences were found: Normal Controls, 2.32 ± 0.38% (N=8); DMD, 2.66 ± 0.51% (N=6); and definite carriers of DMD, 2.43 ± 0.17% (N=3).

We conclude that at this level of analysis erythrocyte total fatty acid composition, including the relatively minor palmitoleic acid component, is not altered in DMD.

1263 PRIMARY TEMPORAL DEGENERATIVE RESPONSE IN THE SEVERED NEWT (Triturus viridescens) OPTIC NERVE. Linda L. Phillips and James E. Turner, Dept. Anat., Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27103.

The primary response to trauma in the CNS involves supportive glia which, invariably participate in the degenerative process. The glial role in removal of cell debris is shown to be underway in the transected newt optic nerve at 6 and 1/2 hours post lesion (p.l.) (Anat. Rec. 187: 291, 1977; Anat. Rec. 190: 510, 1978). The previously reported removal appears to involve an organized interaction of the glia with both non-myelinated (nmf) and myelinated (mf) fibers, however, these studies failed to establish an initiation time and sequential characterization of the response to lesion. The present study examined the transected newt optic nerve at earlier time periods, seeking the initial stages of reactivity. Newts were anesthetized, left orbital nerves cut and animals sacrificed through various fixation procedures 5, 15 and 30 minutes p.l. Nerves were removed, processed for microscopic examination at the light and EM levels. Unoperated and sham operated nerves served as controls. At the light level, nerves examined 5 minutes p.l. appear comparable to controls, with a slight decentralization of the glial nuclei. In addition, a width increase in juxtanuclear cytoplasmic processes of the glia is seen in the 15 minute group. With EM examination, the neuropile at 5 minutes p.l. appears unaltered from that of the controls, intact mf are visible throughout the nerve, and there is absence of any glial organization of the nmf population into 'digestion chambers' seen in the later stages. Movement toward the reactive state of 1/2 hour is seen at 15 minutes p.l., with glia beginning to section groups of nmf into 'chambers' in the central region. Concurrently, 'hillocks' of glial cytoplasm are visible and contain numerous mitochondria at branch points of the 'chamber' segregating processes. These alterations decrease with progression in the central to peripheral direction of the nerve cross-section. At the periphery, glial processes remain attenuated, as in the control nerves. The central mf, adjacent to the reactive glia show a dual population at 15 minutes p.l., some normal in cross section, others collapsed and encircled by the expanding supportive cells. These observations suggest that the degenerative response in the severed newt optic nerve occurs as a temporal sequence of events within the first 1/2 hour p.l. and is carried out in part through a highly reactive glial population.

(Supported by a Basil O'Connor Starter Research Grant from the National Foundation-March of Dimes; the National Society for the Prevention of Blindness made possible through the Alder Foundation and NIH Grant NS 12070 awarded to James E. Turner.)

1264 LOCOMOTOR EFFECTS OF CATECHOLAMINERGIC DRUGS ON HERPES-INFECTED MICE. Richard F. Seegal and John E. Hotchin*. Division of Laboratories and Research, New York State Department of Health, Albany, NY 12201.

Many central nervous system virus infections alter brain catecholamines; yellow fever vaccine virus, West Nile virus, Coxsackie virus (Lycke and Roos, J. Neurol. Sci. 26, 1975). We have employed changes in spontaneous (S) and drug-induced (DI) locomotor activity to assess long-term central nervous system (CNS) infection with herpes type 1 virus (HSV). A dual HSV inoculation procedure was used: the animals received an immunizing foot-pad (FP) dose of .03 ml of a 10^{-2} dilution of HSV followed at two weeks by an identical intra-cerebral (IC) inoculation. Based on IC inoculation in mice, this HSV had a titer of 9.9×10^5 LD_{50}/ml. Animals were tested with IP injections of saline and 0.5 and 2.0 mg/kg d-ℓ-amphetamine (an indirect-acting dopamine agonist) immediately following FP and FP-IC HSV and 4 weeks following FP-IC HSV. FP-HSV had no effect on S or DI activity, whether given as a single or multiple challenge injection. FP-IC HSV when tested days 3-8 post IC depressed both S and DI activity. FP-IC HSV when tested days 28-33 post IC depressed DI but not S activity. IP injection of 5.0 mg/kg d-ℓ-amp overcame the HSV block of DI activity. IP injection of apomorphine (a direct dopamine receptor agonist) in FP-IC HSV mice produced less suppression of DI activity than in controls. These results suggest that chronic CNS HSV produces hypoactivity (compared to hyperactivity in acute IC only herpes, Lycke and Roos, J. Neurol. Sci. 22, 1974) and that based on the differential effects of d-ℓ-amphetamine and apomorphine in HSV infected and control mice, the effect may be due to alterations in either the number of, or the sensitivity of the post-synaptic dopaminergic receptors.

1265 TRANSPLANTED CARCINOMA DEVELOPMENT AND GROWTH: EFFECTS OF ISOLATION, INESCAPABLE SHOCK AND COPING STYLE. L.S. Sklar* and H. Anisman* (SPON: Z. Amit). Department of Psychology, Carleton University, Ottawa, Ontario.

Among the various physiological (ulceration, thymus changes), behavioural (escape deficits) and neurochemical (catecholamine depletions) effects induced by inescapable stressors, several reports are available which indicate that model stressors (e.g. shock, restraint, isolation) will influence the development and growth of carcinomas. Whereas considerable attention has been paid to the role of coping factors in producing the behavioural and neurochemical variations induced by stress, scant attention has been devoted to the role such factors play in affecting carcinogenesis. Moreover, research in this field has not differentiated between other variables (e.g. acute vs. chronic stress, housing conditions) which influence both the neurochemical and behavioural consequences of stress. Work in this laboratory revealed that stress in the form of isolation will exacerbate the appearance and growth of a transplanted tumor line (P815 mastocytoma). Isolation also resulted in reduced survival time among recipient mice. As in the case of isolation, exposure to a single session of 60 inescapable footshocks increased tumor size and rate of death among aggregated mice but not among isolated animals. The effects of a single shock session were ameliorated if mice were chronically exposed to shock. Finally escapable shock was not as effective as inescapable shock in exacerbating the tumor growth. Data are discussed in terms of stress effects on neurochemical activity, hormonal changes and variations in immunosuppression.

1266 A STREPTOZOTOCIN INDUCED MODEL OF DIABETIC NEUROPATHIES IN MICE. Robert W. Stach* and Norman R. West. Depts. Biochem. and Anat., SUNY Upstate Med. Ctr., Syracuse, NY 13210.

Destruction of pancreatic islet β cells with streptozotocin consistently produces hyperglycemia in Jackson Lab C57BL/KSJ mice, a high percentage (>90%) of which express neurological disorders within 3-8 weeks, making this a useful model for studying diabetic -like neuropathies. This is the mouse strain from which the mutant diabetic strain C57BL/Ks(db/db) is derived and in which Sima and Robertson (Acta Neuropath. 41:85,1978) recently reported spontaneously occurring neuropathies after long survival times. Streptozotocin injected male mice, 200 mg/Kg i.p., become progressively hyperglycemic such that 30 days after a single injection their blood glucose levels, obtained by retro-orbital bleeding and the serum assayed with a Beckman Glucose Analyzer, reaches 816 ± 40 mg/100 ml versus control levels of 151 ± 14 mg/100 ml. Despite their greater food and water intake per week, the hyperglycemic mice lose weight, 25.6 ± 1.7 gm and 25.5 ± 0.7 gm in 60 and 90 day old controls respectively versus 19.9 ± 1.3 gm at 30 days post injection (n=12/group). Behavior modification can be described in 5 stages; 1)fore and hindlimb extension, 2)arching of thoracic spine and decreased grooming habits, 3) hindlimb weakness, general slowing of movements, 4) decreased mobility, intension tremor and ptosis, and 5) death. Stages 2 through 5 typically occur over a 3 to 5 day period. The time course for onset and duration of the behavioral affects is quickened in older mice. Muscular alterations, observed by Stearns, et al. (personal communication) are undoubtably responsible for some of the observed behavioral changes. Structural changes observed in hindlimb mixed sensory-motor nerve fibers were similar to those described by Jakobsen (Diabetol. 12:539,1976) in streptozotocin induced hyperglycemia in rats. These include decreased axonal diameter and a slight widening of the nodal gap. In addition, the sympathetic superior cervical ganglia are observed to decrease in size, apparently due to the reduced size and number of unmyelinated fibers which is demonstrable in the postganglionic carotid nerves. The ganglionic neurons do not appear altered although they are more closely packed. The ganglion capsule does not constrict in size. Satellite and Schwann cells do not appear altered when associated with neurons or fibers, but some Schwann cells are associated with very few fibers and therefore more of their cytoplasm is evident. Thus, in later stages of streptozotocin induced hyperglycemia, autonomic neuropathies are present along with the previously described sensory-motor lesions.

(This work was supported by grant NS12325).

1267 CRITERIA FOR HISTOPATHOLOGICAL EVALUATION OF MENINGEAL & CORTICAL CHANGES RESULTING FROM CHRONIC ELECTRICAL STIMULATION. Suzanne S. Stensaas, Dept. of Anatomy, Univ. of Utah, S.L.C., Ut. 84132

A method for quantitatively analyzing histopathological changes resulting from electrical stimulation of meningeal and cortical tissue has been devised, since previous attempts to qualitatively estimate changes had failed to discriminate between different stimulus parameters. The new method combines light and electron microscopy, thus allowing a detailed analysis of all the reactive cellular constituents. Standard values for a square area of normal tissue were established. An average was taken from 3 sample fields of experimental material for comparison. This procedure allows for an analysis of acute changes following short periods of stimulation as well as the detection of subtle changes following long periods of stimulation. Counts were made at fixed levels and consistently revealed a gradient of reactive changes.

Meningeal and cortical samples from beneath electrodes which had been stimulated for various periods of time through a chronic indwelling teflon-platinum array were studied. The reactive changes extended from the electrode surface toward the cortex with clear signs of attenuation by the capsule, neurothelium and leptomeninges. These changes were most pronounced under the reference electrode which served as a common ground. Macrophages within the capsule were engaged in the breakdown and removal of this necrotic material. Analytical electron microscopy revealed the presence of platinum within these capsular macrophages. Leucocytes and plasma cells in the leptomeninges indicated that the localized inflammatory response could extend to the cortical surface.

In general, the cortical changes were less pronounced than those of the capsule. Specific cortical measurements were made in the following general categories: neuronal constituents, glia, blood vessels and exogenous cells. Large dendrites, degenerating myelinated axons, degenerating neurons and normal neurons were counted in the first category. Edematous profiles, hypertrophic astrocytes, phagocytic astrocytes and reactive glial cells were counted in an effort to identify glial changes. In addition, the distribution of macrophages and leucocytes in the parenchyma and around blood vessels as well as the presence of plasma cells was noted.

A standardized quantitative method has now been developed which should permit investigators from different laboratories to evaluate their material in a similar manner. This could facilitate comparisons between different electrode sizes, parameters, materials and species. (Supported by the Surdna Foundation.)

1268 MODULATION OF LYMPHOCYTE RESPONSES BY GANGLIOSIDES. Scott Stewart*, Ronald Whisler* and Allan Yates. Depts. of Path. and Med., Ohio State Univ., Columbus, Oh. 43210.

Gangliosides are sialic acid-containing glycolipids present in high concentration in nervous tissues, especially in cerebral grey matter, but have been found in many extraneural tissues as well. Although their biologic functions are unknown, specific gangliosides present in lymphocytes[1] can modulate B lymphocyte responses to antigen stimulation.[2] In the present study we have investigated the effects of several gangliosides on lymphocyte reactivity in response to concanavalin A (Con A) and to allogeneic cell stimuli.

Normal human peripheral blood mononuclear cells were incubated with an optimal dose of Con A or in a one-way mixed leukocyte reaction (MLR) in the presence of 3.1 nanomoles of a purified ganglioside isolated from normal human cerebral cortex. Cell viability exceeded 85% in all cases as assessed by trypan blue exclusion.

Of the four gangliosides tested, G_{D1B} and G_{T1B} completely inhibited tritiated thymidine uptake both in response to Con A and in the MLR. In contrast, G_{M1} resulted in only 58% and 8% inhibition for Con A and the MLR respectively. A preparation of G_{D1A} containing 10% G_{D1B} inhibited the Con A response 28% and the MLR 37%. These results indicate that G_{D1B} and G_{T1B} can modulate lymphocyte responses to a greater extent than can G_{D1A} and G_{M1}. The mechanism of modulation may involve recognition of the 2→8 sialic acid-sialic acid moiety, the only structure common to G_{D1B} and G_{T1B} but not shared by G_{M1} and G_{D1A}.

This work was supported by the Roessler Foundation and the American Cancer Society.

1 Stein, K., Marcus, D., Biochem. 16:5285-5291 (1977).
2 Esselman, W., Miller, H., J. Immunol. 119:1994-2000 (1977).

1269 REPEATED THIAMINE-DEFICIENT ENCEPHALOPATHY IN THE MOUSE: LIGHT AND ELECTRON MICROSCOPIC STUDY. Itaru Watanabe and Masayo Watanabe.* VAH, Kansas City, MO 64128 and Dept. Path. Oncol., Univ. Kans. Med. Ctr., Kansas City, KS 66103.

Pyrithiamine-induced acute thiamine-deficient encephalopathy in the mouse (PIATDEM) is an animal model of human Wernicke-Korsakoff syndrome, produced in 10 days by combined administration of thiamine-deficient diet and daily injection of pyrithiamine (PT). With a sufficient amount of thiamine (T) (100 X dose of administered PT), the neurologic symptoms can be reversed, if T is given within a few hours after the onset. The early morphologic changes in the brain consist of edema of astroglia and myelin sheath and multiple petechiae, and can also be reversed by T treatment.

In this experiment, 10-day courses of PIATDEM attack were repeated three times within a total 10-weeks period, which also included two alternating courses of T administration consisting of initial therapeutic dose and, after neurological recovery, of maintenance dose.

In the brains of the mice sacrificed at the end of the second T treatment period, gliomesenchymal scar lesions were seen in the thalamus, mammillary body and pontine tegmentum. The mild lesions were recognized only by the presence of some microglial cells and a few hemosiderin-containing macrophages. No cell edema was seen. In the 12 mice sacrificed immediately after the neurological onset of the third PIATDEM attack, there were astroglial and myelinic edema in these sites of the brain. The edematous lesions were fewer, smaller and milder than those after the first attack. In the scarred areas, where no astroglial cells remained, only myelinic edema was seen. In some lesions of the thalamus, only astrocytes in the gray matter selectively showed edema necrosis. In the mammillary body, only hemorrhagic lesions were seen, but edema-necrosis did not occur. In all the lesions examined, the nerve cell alteration was slight. These findings suggest that PIATDEM is primarily a vascular and glial disorder in the brain. There are significant differences in sensitivity to this condition among vasculature, astroglia and myelin (or oligodendroglia) from a lesion to the other, depending upon local anatomical and pathological conditions. This has become more obvious in the repeated encephalopathy.
(Supported by the Medical Research Service of the Veterans Administration.)

1270 NEUROPATHOLOGY OF "VIBRATOR" - A NEUROLOGICAL MUTATION OF THE MOUSE. William R. Weimar and Richard L. Sidman, Dept. of Neuroscience, Children's Hosp Med. Center and Dept. of Neuropathology, Harvard Medical School, Boston, MA. 02115.

Vibrator (vb) is an autosomal recessive mutation on chromosome 11 (P. Lane, The Jackson Laboratories, Bar Harbor). Affected mice display a coarse tremor of trunk, head and limbs during activity, beginning on about postnatal day 9 (P9) (Sidman et al., "Catalogue of Neurol. Mutants of the Mouse"). Initially vb/vb executes tasks such as grooming, exploring and nibbling food with almost the same facility as its +/- littermates. However its condition deteriorates rapidly during the 4th week of postnatal life and affected mice are not known to live beyond 30 days.

Following fixation by perfusion with 1/2 strength Karnovsky's fixative, brains were embedded in celloidin and sectioned serially at 20 um. Alternate sections were stained with cresylecht violett and by the Loyez method for myelin respectively. The brains of 5 vb/vb (P24) and 3 +/- littermate controls were surveyed in coronal (2 + 1), sagittal (2 + 1) and horizontal (1 + 1) planes of section Macroscopically, the vb/vb CNS appeared normal.

In all vb/vb brains examined, abnormal neurons were a consistent and prominent feature of the following CNS regions: (1) lat. vestibular nuc. ++++; (2) red nuc. ++++ (esp. magnocellular div.) (3) large neurons in the brain stem reticular formation +++ (including magnocellular nuc., central-caudal pontine nuc., reticular nuc. of pontine tegmentum, central-oral pontine nuc., and the central nuc. of ventral medulla oblongata); (4) the medial, interpositus and lateral deep cerebellar nuclei +++; (5) mesencephalic reticular formation +/++; (6) thalamic reticular nuc. +/++; (7) nuc. of the incertotectal tract +; (8) zona incerta +; and (9) lat. corticohypothalamic tract +. Signs of cellular abnormality included perinuclear chromatolysis, nuclear eccentricity, and ballooning of the soma with varying degrees of severity. In addition, most abnormal neurons contained large, clear cytoplasmic vacuoles, sometimes to the apparent exclusion of the usual organelles. Larger neurons were particularly prominently affected.

The Loyez method revealed no primary abnormalities in myelin. Also of interest is the lack of pathological changes in either the cerebellar cortex or the olivary nuclei, despite the marked changes in deep cerebellar nuclei and in most cerebellar-related nuclei of the brain stem.

Allelism tests between vb and cerebellar outflow degeneration (cod), a mutation showing a somewhat similar distribution of neuron loss were negative.

Supported by NIH Research Grant NS 11237 and Training Grant T32 NS07017-02.

1271 HEXACHLOROPHENE RETINOPATHY IN THE DEVELOPING RATS. G. Y. Wen* and A. L. Rose*(SPON: A.A.Lidsky). Dept. of Neurology,Downstate Medical Center, Brooklyn, NY 11203 and Dept. of Pathological Neurobiology, NYS Institute for Basic Research in Mental Retardation, Staten Island, NY 10314.

Myelinopathy caused by hexachlorophene(HCP) in the CNS and PNS of rats was found to be reversible except in the optic nerve (Rose et al, 1975). This study of the retina was undertaken to clarify the pathogenesis of optic nerve degeneration in the developing rats.

A series of suckling rats (Sprague-Dawley) were intoxicated with 100-500 ppm of HCP added to the mother's diet from 9-23rd day of life and the weanling rats' diet from 23-37th day of life. There was no mortality in the animals intoxicated with 100 and 250 ppm of HCP. The LD_{50} was 400 ppm of HCP. Twelve rats were sacrificed by perfusion 7,14,21,28 days after the onset of treatment. Another eleven rats were intoxicated for 28 days and were then switched to normal diet. They were sacrificed after 30,60, 90,120,150 days of recovery period.

No retinal pathology was found in the rats acutely intoxicated with 100 ppm of HCP. The retinae of all rats treated with 250, 400, 500 ppm of HCP showed pathological changes. Mild vacuolation first appeared in the outer segment layer of retina 7 days after intoxication. Severe vacuolation in the same layer and degeneration of photoreceptor cell bodies appeared after 14-28 days. Electron micrographs showed that the vacuolation of the outer segment was due to the breakdown of disc membranes and outer membranes of rods.

In the recovery group there was no improvement in the outer segment of the photoreceptor cell. Further loss of photoreceptor cell bodies and degeneration of the inner nuclear layer were found to occur after several weeks.

This study showed that photoreceptor cells in the newborn rats are highly sensitive to HCP. The lesions are irreversible and progression due to trans-synaptic degeneration continues after withdrawal of the toxic agent. The optic nerve pathology appeared to be due to direct effect of HCP on it.

1272 EFFECT OF 5-HYDROXYTRYPTOPHAN ON THE AMPLITUDE OF LUMBAR MONOSYNAPTIC REFLEXES IN RATS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE). Susan R. White. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3V6.

Recent evidence indicates that there is depressed sensitivity of peripheral neuronal receptors to 5-hydroxytryptamine (5-HT) in guinea pigs paralyzed with EAE (Weinstock et al., Brain Res. 125 192, 1977). The purpose of the present study was to examine responsiveness of the central nervous system to the increased levels of 5-HT in EAE paralyzed animals. The effect of 5-hydroxytryptophan (5-HTP), the immediate precursor of 5-HT, on the amplitude of lumbar monosynaptic reflexes (MSR) was tested in normal rats and in rats with hindlimb paralysis following the induction of EAE.

Intravenous injections of 50 or 75mg/kg 5-HTP failed to increase the amplitude of L_4 or L_5 MSR in EAE paralyzed rats while having a marked facilitatory effect on normal rats. Since post-tetanic potentiation tests revealed that the amplitude of the MSR could be increased above control levels in EAE paralyzed rats, these results suggest that sensitivity to 5-HT is reduced in the central nervous system as well as in the peripheral nervous system in EAE rats with hindlimb paralysis.

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

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1273 β-ENDORPHIN-LIKE IMMUNOREACTIVITY BY RIA IN RAT BLOOD: NORMAL LEVELS AND COMPARISON TO HUMAN PLASMA. Huda Akil, Stanley J. Watson, Jack D. Barchas, and C. H. Li*.

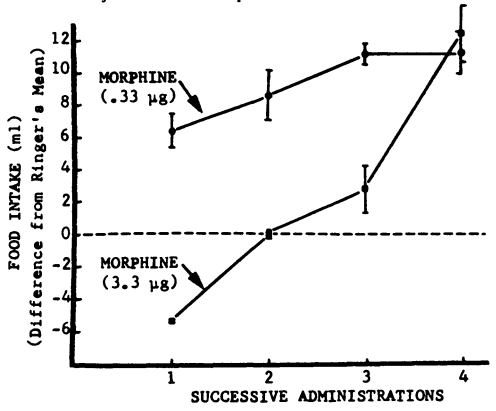
Antiserum against the C-terminus of β-lipotropin (β-LPH) has been obtained from rabbit. β-Endorphin (β-END) conjugated by carbodiimide with immunoglobulin was injected and harvested from New Zealand rabbits. The antiserum, diluted 1/1500, bound I^{125}-β-END, demonstrating an effective range from 4 pM to 10 nM. The sensitivity of the assay is 1-2 fmoles. This antibody exhibits 6-10% cross-reactivity with β-LPH. β-END-like immunoreactivity in rat blood has been detected in unextracted samples when compared to blood from hypophysectomized rats. The whole assay and calibration curves are carried out in plasma from hypophysectomized animals. β-END-like immunoreactivity can be detected in normal rat plasma (110 ± 9 fmole/ml), and exhibits substantial increases with adrenalectomy (1200 ± 24 fmoles/ml). In contrast, samples from 5 healthy normal human males gave significantly lower values when compared to plasma from hypophysectomized humans (12 fmoles ± 3.9 per ml of plasma). The human levels are at the limits of sensitivity of the assay and may be due to β-LPH or other cross reactivities.

1274 CRUSTACEAN NEURO-DEPRESSING HORMONE ACTIVATES A SODIUM PUMP IN CRAYFISH MOTONEURONS. Hugo Aréchiga and Jorge Aceves*. Dept. Physiol. and Biophys. CIEA, IPN, México 14, D.F.

The nervous system of crustaceans synthesizes and releases to the circulation a peptide which diminishes the responsiveness of sensory and motor elements, hence the denomination of Neurodepressing Hormone (NDH) (Aréchiga, H., Huberman, A. and Martínez-Palomo, A. Brain Res. 128: 93-108, 1977). The effect of NDH on crayfish motoneurons is a lowering of the spontaneous firing rate. On the other hand, in some motoneurons, the rate of firing is modulated by the activity of an electrogenic sodium pump, thus suggesting as a likely possibility, that NDH could exert its effect by acting on this mechanism. To explore this possibility, three types of experiments were conducted on isolated abdominal ganglia of the crayfish Procambarus bouvieri. a) The spontaneous electrical activity of the superficial flexor motoneurons was extracellularly recorded under continuous flow of Van Harreveld solution. NDH and other test substances were topically applied to the bathing fluid. Ouabain, a well known inhibitor of active sodium extrusion was tested on its effect on the depression induced by NDH on the firing rate of motoneurons. At concentrations between 1×10^{-5} M and 1×10^{-4} M, it reversibly blocks the depressing effect of NDH, in a concentration-dependent manner. A complete blockage of NDH action can be also obtained by removing the K^+ from the bathing fluid, another manipulation known to interfere with the activity of Na^+- K^+ exchange systems. b) The intracellular concentration of sodium and potassium was analytically determined in isolated abdominal ganglia, incubated in K^+-free solutions and then transferred to normal Van Harreveld, and the rate of the subsequent Na^+ extrusion and K^+ incorporation was found to be enhanced by NDH. c) The rate of binding of ^3H Ouabain as determined in isolated abdominal ganglia, after incubation in K^+-free solutions was decreased as a function of NDH concentration.
These results are consistent with the view that NDH depresses the spontaneous activity of motoneurons by stimulating a sodium pump.

1275 DO ENKEPHALIN SYSTEMS MEDIATE DRIVE REDUCTION? James D. Belluzzi and Larry Stein. Wyeth Laboratories, Philadelphia, PA 19101.

The possibility that enkephalin systems may regulate some aspect of reward function has been suggested by the results of self-administration, self-stimulation, and learning experiments (Belluzzi & Stein, 1977). Since narcotics "seem to produce a state of total drive satiation" (Jaffe, 1965) it has been proposed that enkephalins may mediate drive-reduction reward. If so, administration of enkephalin-like compounds should produce satiation in hungry animals. Rats had access to sweetened milk during daily 75-minute sessions. After intakes had stabilized, D-Ala^2-D-Leu^5 enkephalinamide (L, 1 μg), morphine (1 μg) or the Ringer's solution vehicle were intraventricularly administered before the daily session. Contrary to prediction, mean food intake in ml was increased over baseline both by morphine (13.8 ± 4.1) and L (5.8 ± 2.5), but not by Ringer's (1.8 ± 1.1). In a second experiment, different groups of rats received morphine (0.33 or 3.3 μg) or vehicle after partial satiation induced by 15 minutes of free access to the milk. Dosings were repeated weekly for four successive weeks. The Figure shows that on first administration the 0.33-μg morphine dose facilitated feeding whereas the 3.3-μg dose suppressed feeding. This suppressant or drive-reducing effect, like opiate analgesia, exhibited tolerance over successive administrations. Although these results are not entirely consistent with a simple drive-reduction hypothesis, they are reminiscent of, and may be related to, the dual suppressant and facilitatory effects of opiates on self-stimulation reward.



1276 POSSIBLE PHYSIOLOGICAL IMPORTANCE OF METAL BINDING BY CARNOSINE Charles Eric Brown and William E. Antholine*. Depts. of Biochemistry and Radiology, The Medical College of Wisconsin, Milwaukee, WI 53233.

Carnosine (β-alanyl-L-histidine), which has been postulated to have a role in olfaction, was observed with ^{1}HMR spectrometry to bind to the crude particulate fraction of nasal olfactory epithelium in a sterically specific orientation (Neurochemical Research 2 (1977) 555-579). In an attempt to obtain additional information that could suggest both the significance of and a possible mechanism for the observed binding, we investigated the ability of carnosine and several of its analogues to bind metal cations. The binding of Mn^{++}, Co^{++} and Cu^{++} was measured with ^{1}HMR, ESR and UV-visible absorption spectroscopy at varying concentrations of peptide and metal cation. Both the strength of binding and the structure of the complex are highly dependent upon the identity of the metal cation and of the peptide.

Mn^{++} binds only very weakly to the imidazole ring of carnosine, anserine, homocarnosine and glycyl-L-histidine ($K_{diss} \approx 40$-100mM). In contrast, Cu^{++} exhibits well defined ESR spectra when complexed to these peptides. Cu^{++} binds to the imidazole ring of these peptides when they are present in excess. In addition, carnosine and anserine produce a different complex when the concentrations of both Cu^{++} and peptide are equal. This complex is a dimer of two Cu^{++} cations that is stable in aqueous solution at room temperature and physiological pH. This complex appears to have the same structure as Cu^{++}-carnosine in the crystal. Co^{++} binds only with carnosine. The Co^{++}-carnosine complex yields an ESR signal immediately after mixing that decreases to undetectable intensity over a period of hours. Several hours after mixing the Co^{++}-carnosine complex exhibits a UV-visible absorption spectrum that is characteristic of diamagnetic Co^{++} dimers containing oxygen. Histidine and cysteine do not compete with carnosine for Co^{++}-binding; instead they produce stable mixed complexes.

Thus carnosine and anserine should affect the metabolism and availability of at least certain metal cations in those tissues that contain these peptides in high concentration (i.e. nasal olfactory epithelium and skeletal muscle). Considering reports that metals regulate heme metabolism (Maines, M. D. and Kappas, A., Science 198 (1977) 1215-1221), metal-binding by these peptides may modify the oxidative metabolism of these tissues. In addition, the observed structures of these complexes can be used to explain the substrate specificity of carnosinase.
(Supported by a Biomedical Research Support Grant from The Medical College of Wisconsin and NIH Grant RR-01009)

1277 HIGH AFFINITY BINDING SITES FOR THYROTROPIN RELEASING HORMONES IN BRAIN AND RETINA: RESEMBLANCE TO PITUITARY RECEPTORS. David R. Burt. Dept. Pharmacology & Exp. Ther., Sch. Med., University of Maryland, Baltimore, MD 21201.

Thyrotropin releasing hormone (TRH) binds with high affinity (K_D = 10-30 nM) to sites in the anterior pituitary which appear to represent its physiological receptors. Similar sites were previously detected in rat brain (Burt and Snyder, Brain Res. 93: 309, 1975), but their properties were mostly obscured by the presence of a large excess of low affinity binding sites. Recently these studies have been extended to calf and sheep brains using higher specific activity [^{3}H]TRH (115 Ci/mmol) with the more potent and specific 3-methylhistidyl TRH in blank tubes. Incubations were performed in dilute phosphate buffer at 0° C for 30 or 60 min. Under these conditions no breakdown of [^{3}H]TRH was detected. Bound radioactivity was separated by filtration. High affinity binding was over half of the total binding in the results discussed below.

Discrete regions reported to contain relatively numerous variscosities immunoreactive for TRH (Hokfelt et al., Eur. J. Pharmacol. 34:389, 1975) have been found to possess relatively greater [^{3}H]TRH high affinity binding, with the nucleus accumbens possessing the greatest number of sites (1-3 pmol/g tissue wet weight in preliminary results). The retina has been found to possess a similar high level of high affinity binding, in interesting agreement with the previous report of its light-dependent TRH content (Schaeffer et al., Proc. Nat. Acad. Sci., 74:3579, 1975). Both nucleus accumbens and retina binding sites resemble pituitary receptors in their affinities for TRH and for 15 structural analogs of TRH (generously supplied by Abbott Laboratories). All 3 classes of sites appear to have the same kinetics of association and dissociation. Other aspects of this resemblance are being explored. (Supported by NIMH grant MH 29671-01.)

1278 STUDY OF OPIATE RECEPTOR SITES USING A FLUORESCENTLY LABELLED ENKEPHALIN ANALOGUE. Robert G. Canada* and M. Ashraf El-Bayoumi (SPON: J. I. Johnson). Biophysics Dept., Coll. of Human Med. and Natural Sci., Michigan State Univ., East Lansing, Mi., 48824

The objective of this investigation was to develop a new technique for visually mapping the distribution of the opiate receptor sites on the soma and/or neurites of the single amygdaloid neuron, and to examine the conformation of the met-enkephalin pentapeptide and to examine the nature of the opiate receptor's activation site(s). To achieve these goals we have successfully labelled met-enkephalin with a dansyl group at the unprotonated amino terminal, yielding N-dansyl-met-enkephalin. The attachment of the dansyl group to the pentapeptide was detected by a blue shift in its emission peak and an increase in its quantum yield; because of the nonpolar nature of the dansyl binding site on enkephalin. The unbound dansyl group in ethanol exhibited a fluorescence peak at 528 nm hence the fluorescence maximum of N-dansyl-met-enkephalin was found to be at 472 nm, and its fluorescence intensity was more than 10 times that of the free dansyl group. The fluorescence excitation spectrum of N-dansyl-met-enkephalin in ethanol has a maximum of 340 nm and a secondary peak at 270 nm. The single neurons were taken from the amygdaloid nuclear complex of a 15 da. old albino rat, maintained in dissociated cell culture for 52 days, and stained with 1.57×10^{-6}M N-dansyl-met-enkephalin in ethanol. The enkephalin-dansyl-receptor complexes appeared as a continuous green covering on the surface membrane of the neuron, with discrete blue or turquoise patches; with a fluorescence peak at 442 nm and a shoulder around 478 nm. It is argued that the hydrophobicity of the opiate receptor sites caused the blue shift in the fluorescence peak of the bound N-dansyl-met-enkephalin. The blue clusters were varied in size and shape, and located at soma-soma and neurite-soma contacts between two cells and near the connection of the neurite and soma of the same cell. Evidence suggests that the N-dansyl-met-enkephalin may stereospecifically bind to opiate receptors on isolated membrane from rat brain. The fluorescence emission maximum of 3.6×10^{-10}M N-dansyl-met-enkephalin bound to isolated membrane fragments, from rat brain regions containing a high concentration of opiate receptors, was found to be at 455 nm in 0.05M tris buffer, pH 7.0.

1279 A CLUE TO THE MECHANISM OF ACTION OF ASPIRIN: ROLE OF THYROTROPIN RELEASING HORMONE (TRH). Marthe Cohn, Stephan J. Cohn* and Major L. Cohn. Dept. Anes., Magee-Womens Hosp., Univ. Pgh., Sch. Med., Pittsburgh, PA 15213.

We have previously shown that TRH, luteinizing hormone releasing hormone (LHRH) and substance P-induced tight head to tail rotations are dopamine mediated. We have also shown that somatostatin-induced barrel rotations are acetylcholine mediated. To determine whether dopamine and acetylcholine are the only neurotransmitters mediating or modulating the peptides' functions, we studied here the effects of TRH on body temperature (T^o). We selected this particular system because: 1) TRH-induced hyperthermia in hypophysectomized and thyroidectomized rats strongly suggests that a) TRH acts centrally, b) its regulation of T^o is not contingent upon pituitary-thyroid axis; and 2) investigators reported that T^o is regulated centrally by controlled release of endogenous monoamines (i.e. epinephrine, norepinephrine, serotonin) from nerve terminals in the hypothalamus where the monoamines are found in high concentrations. Groups of male Sprague-Dawley rats were anesthetized and cannulae stereotaxically implanted into either the lateral ventricle (ICV), cerebral cortex, caudate nucleus, thalamic nuclei, hypothalamus or preoptic/anterior hypothalamic (PO/AH) areas, at least one week prior to experiment. After filtration through a millipore filter, freshly prepared solutions were microinjected into the different brain areas in concentrations ranging from 0.03 to 0.14 µM in a constant volume of 0.5 µl. While at room temperature (21-22°C), sterile solutions of saline 0.9%, LHRH, substance P, leu and met enkephalin were without effect, TRH increased T^o from 0.8 to 2.4 C for 2 to 3 hours in all brain sites tested. Exposure to either heat (38°C) or cold (6 to 8°C) did not alter TRH-induced hyperthermia, suggesting that TRH does not elicit paradoxical temperature responses. However, the PO/AH responded most dramatically to microinjection of TRH (0.014 µM) with T^o increases up to 3°C lasting from 6 to 8 hours and occasionally 24 to 48 hours. Pretreatment with cholinergic, serotonergic and catecholamine blockers (atropine, propranolol, phentolamine, pimozide, haloperidol, α-methyl-paratyrosine, parachlorophenylalanine, 5, 6 dihydroxytyramine or 5, 7 dihydroxytyramine) did not alter TRH-induced hyperthermia. However, indomethacin (50 mg/kg) injected intraperitoneally or lysine acetylsalicylate (.22-2.2 nM) injected ICV, dose-relatedly, inhibited hyperthermia induced by TRH in all brain areas. Our findings suggest that 1) T^o is centrally regulated but not in one specific brain center; 2) TRH-induced hyperthermia is not mediated by monoamines; and 3) based on dose-related inhibiting actions of indomethacin and soluble aspirin, prostaglandins may be involved in TRH T^o regulation.

1280 DISTRIBUTION OF BRADYKININ-LIKE IMMUNOHISTOFLUORESCENCE IN THE RAT BRAIN. Fernando M.A. Corrêa*, Robert B. Innis and Solomon H. Snyder. Dept. Pharmacol., Johns Hopkins Sch. Med., Baltimore, MD 21205.

In the last 10 years, several peptides first described in blood and peripheral organs have also been found in the central nervous system.

Bradykinin, a nonapeptide derived from the α_2-globulin fraction is involved in several pathophysiologic phenomena. In addition, autonomic and behavioral effects following intracerebral injection suggest a neurophysiologic role for bradykinin (J. Pharmacol. Exptl. Ther., 193:1, 1975; Neuropharmacol., 15:713, 1976). However, bradykinin and other related kinins have not been consistently shown to exist in the brain.

We have identified bradykinin-like immunoreactive structures in frozen brain slices of colchicine-treated rats by indirect immunohistofluorescence methods. The fluorescence labeling is selective since preincubation of the antibody with bradykinin but not with other peptides tested prevents its development. Cell bodies are observed only in the hypothalamus. Groups of cells are located lateral and dorsal to the third ventricle (in the hypothalamic periventricular nucleus); in a narrow arcuate zone comprising the reuniens nucleus, the zona incerta and the lateral hypothalamus; and in the ventral portion of the hypothalamic dorsomedial nucleus. Fiber-like structures appear as clearly stained, moderately dense varicosities with fine intervaricose segments. They are found in the dorsal and lateral portions of the cerebral cortex; in the pre-hypothalamic area, oriented from the diagonal tract of Broca to the lateral septal area; in the caudate and the border between the lateral hypothalamus and the globus pallidus; in the medial preoptic area, anterior hypothalamus and periaqueductal grey. Fibers are most dense in the reuniens nucleus and the ventromedial portion of thalamus and are radially oriented relative to the dorsal portion of the third ventricle. F.M.A.C. is recipient of a fellowship from FAPESP, Brasil.

1281 NEUROPEPTIDES AND PROTEIN PHOSPHORYLATION IN BRAIN MEMBRANES. Leonard G. Davis and Yigal H. Ehrlich. Univ. Mo.-Columbia, Sch. of Med., Mo. Inst. of Psychiatry, St. Louis, Mo. 63139

Recent investigations indicate that neuroactive peptides may play both mediatory and modulatory roles in synaptic function. However, the nature of the biochemical interactions underlying the involvement of peptides in neuronal activity is still largely unknown. We have selected to study the molecular mechanisms of action of neuropeptides by investigating the opiate-receptor system. In previous studies, we reported that long-term morphine treatment causes a 70% decrease in the endogenous phosphorylation of a group of specific membrane proteins designated H (MW 15-20K) and suggested that such phosphorylative modifications may be affected by changes in the balance of naturally occurring neuropeptides (Ehrlich et al, Life Sci. in-press). In the present study, we have investigated the effects of methionine-enkephalin (Met-enk) on the phosphorylation of endogenous protein substrates in brain membranes. Fractions enriched in synaptic membranes were prepared by differential centrifugation, osmotic shock and dialysis. These membrane preparations were incubated with γ-^{32}P-ATP in the presence and absence of met-enk. Reactions were stopped by the addition of SDS and solubilized membrane proteins were separated by slab polyacrylamide gel electrophoresis. Specific protein components which incorporated radioactive phosphate were identified by autoradiography of the gels. Inclusion of met-enk in the reaction mixture resulted in a decreased incorporation of radiophosphate into one group of specific proteins (MW 15-20K). The selective inhibition of phosphorylation by met-enk was found to be concentration dependent as well as a time dependent phenomenon. Both Leucine- and met-enk altered the phosphate incorporation into apparently the same specific proteins, but in a different fashion. The inhibition of the phosphorylation of these protein(s) by met-enk could be blocked by including naloxone in the reaction. Naloxone itself caused a slight elevation of radiophosphate incorporation into most all ^{32}P accepting proteins. The findings that chronic morphine treatment in vivo and met-enk in vitro selectively affect the phosphorylation of the same synaptic-membrane proteins support our suggestion that endorphine-regulated phosphorylation may play a role in mechanisms underlying narcotic dependence. Moreover, the present results and those of others suggest that neuropeptides may exert their modulatory effects on neuronal activity through mechanisms that also involve the phosphorylation of specific proteins. Supported by intramural funds from the Mo. Inst. of Psychiatry to E. G. Brunngraber.

1282 ENKEPHALINS: NEUROPHARMACOLOGICAL ROLE IN THE EXTRAPYRAMIDAL SYSTEM. B.I. Diamond, H.S. Havdala* and R.L. Borison. Anesthes. Dept., Mount Sinai Hospital, Chicago, Illinois 60608

Studies on the distribution of opiate receptors in brain have shown them to be most heavily concentrated in both the limbic and extrapyramidal systems. Moreover, it has been demonstrated that enkephalin regional distribution is largely localized to the corpus striatum. Given these facts, what role must the enkephalins play in regulating the activity of the extrapyramidal system? We have now studied this role by investigating the action of methionine-enkephalin and agents which modulate its brain levels, upon two different animal models of Parkinson's disease. One model is the induction in white male Sprague-Dawley rats of hunched back ptosis, tremor, rigidity and akinesia after the administration of reserpine (10 mg/kg). We found that the specific narcotic antagonist, naloxone, by itself, produced a reversal of reserpine-induced neurological signs (latency to reversal of 3.5 minutes). Similarly, in animals also pretreated with the catecholamine depleter α-methyl-para-tyrosine, naloxone alone was still capable of reversing the parkinsonian-like reserpine syndrome. We have also used another animal model in which rats received a unilateral injection of 6-hydroxydopamine (8μg in 4μl) into their substantia nigra. These animals rotate ipsilateral to the side of the lesion after the administration of agents which work via presynaptic dopamine release, namely d-amphetamine or phenylethylamine. The animals rotate in a direction contralateral to the lesion after the administration of directly acting postsynaptic agents such as L-dihydroxyphenylalanine (L-DOPA) or apomorphine. We found that pretreatment of animals with naloxone antagonized the rotations produced by the presynaptic agents while potentiating the number of rotations induced by the postsynaptically active agents. In contrast, administration of the amino acid d-phenylalanine, which blocks the enkephalinergic degradative enzyme carboxypeptidase A, or administration of methionine-enkephalin itself, both potentiate the actions of presynaptically active drugs, while antagonizing the effects of those compounds with a postsynaptic site of action. These data suggest to us the presence in the nigro-striatal pathway of a presynaptic enkephalinergic neuron with a facilatatory action, and a postsynaptic enkephalinergic neuron which produces an inhibiting effect. Manipulation of these endogenous systems may lead to new treatment modalities for Parkinson's disease and other extrapyramidal disorders.

1283 DEETECTION OF ENKEPHALINS IN THE SYMPATHETIC GANGLIA. A.M. Di Giulio*, B.E. Lutold*, W. Fratta*, H.-Y.T. Yang* and E. Costa (SPON: N.H. Neff). Lab. Preclin. Pharmacol., NIMH, Saint Elizabeths Hosp., Washington, D. C. 20032

Methionine-enkephalin (ME) as well as leucine-enkephalin (LE) have been detected in some sympathetic ganglia of Sprague Dawley rats, i.e., the paravertebral paired superior cervical ganglion (SCG) and the prevertebral unpaired coeliac ganglion (CG), by the radioimmunoassay with ME and LE specific antisera. The identity of the two peptides was tested in tissue extracts by Biogel P-2 chromatography column, followed by radioimmunoassay. Enkephalins levels in rat sympathetic ganglia, expressed as ng/mg prot±S.E.M, are: 3.3±05 ME and 0.95±0.1 LE for coeliac ganglion and 3.0±0.6 ME and 1.4±0.3 LE for superior cervical ganglion. Both methionine and leucine-enkephalins were found to be present also in rabbit coeliac and superior cervical ganglia. We were not able to detect any beta-endorphin and beta-lipotropin in the ganglia studied. Enkephalins were measured in CG and SCG of spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto controls (WKY), aged 4,8 and 12 weeks. The content of ME and LE in the CG was lower in spontaneously hypertensive rats than in Wistar Kyoto controls. No significant difference was found in enkephalins levels of the two experimental groups in the superior cervical ganglion, caudate nucleus and hypothalamus. Whether these alterations in the enkephalin content of coeliac ganglion in spontaneously hypertensive rats is dependent on the integrity of the afferent innervation or on the efferent vagal brunch will be discussed.

1284 MOUSE BRAIN ENKEPHALINS: STUDIES OF LEVELS AND SYNTHESIS CORRELATED WITH NOCICEPTIVE SENSITIVITY. R.C.A. Frederickson, D. L. Hesche*, J. D. Edwards*, C. E. Harrell* and V. Burgis*. The Lilly Research Laboratories, Eli Lilly and Co., and Department of Pharmacology, Indiana University, Indianapolis, IN 46206 & 46202.

There is substantial evidence that small opioid peptides may function as neurotransmitters in brain and elsewhere. We have observed a diurnal rhythm in responsivity of mice to noxious stimuli and a corresponding time dependence of naloxone-induced hyperalgesia (Frederickson et al., Science 198: 765, 1977). Further studies performed on hypophysectomized mice compared to sham-operated controls have indicated that this rhythm is mediated by brain rather than pituitary. In order to examine the role of brain opioid peptides in this phenomenon we first compared whole brain total opioid activity at the times of minimum and maximum sensitivity to noxious stimuli. Mice were sacrificed by microwave irradiation, the brains were homogenized in acid, the homogenate was centrifuged and enkephalins were extracted from the supernatant over columns of Amberlite XAD-2 resin and bioassayed on the mouse vas deferens. The levels were 55.8 ± 3.3 ng/brain for mice sacrificed at 7:30 a.m. and 93.7 ± 12.1 ng/brain for mice sacrificed at 3:30 p.m. ($p<0.01$, df = 22, Student's t test). Studies with radioimmunoassay methodology are underway but we have not yet conclusively established whether the diurnal change occurs specifically in Met^5- and/or Leu^5- enkephalin. We are also utilizing the incorporation of ^{3}H-tyrosine into opioid peptides after intraventricular injection to compare the rate of synthesis of various opioids as a function of time of day. Thin layer chromatography and high pressure liquid chromatography methodology have provided strong evidence for incorporation of ^{3}H-tyrosine into Met^5-enkephalin and Leu^5-enkephalin. There was substantial incorporation into Met^5-enkephalin but that into Leu^5-enkephalin was barely detectable. The greatest incorporation, however, was into a third component which may be a unique opioid peptide, a precursor to the enkephalins, or merely a breakdown product of one of the brain endorphins. A study of the time course of incorporation into the various components is in progress and the nature and role of the third component is being investigated. The data to date support a role for opioid peptides in modulating reaction to noxious stimuli but we have not yet identified the specific opioid peptide which mediates the diurnal rhythm in behavior and total opioid activity.

1285 MORPHINE OR STRESS INDUCED INCREASES OF PLASMA β-ENDORPHIN AND PROLACTIN ARE PREVENTED BY DEXAMETHASONE PRETREATMENT. Edward D. French*, Floyd E. Bloom, Catherine Rivier, Roger Guillemin, Jean Rossier. A.V. Davis Center for Neurobiology, The Salk Institute, La Jolla, CA 92037.

Under conditions of stress, plasma levels of β-endorphin (B-E) and ACTH are concomitantly increased. Opiates are also known to alter anterior pituitary function and to increase plasma levels of corticosteroids (CS) through hypothalamic mechanisms. Rats receiving morphine sulfate (MS) 20 mg/kg s.c.showed a 9-fold increase in B-E immunoassayable like material 1 hr after injection and a return to control values by 6 hr. Prolactin (PRL) (as determined by radioimmunoassay) levels also were elevated 3-fold at 30 min post injection but returned more rapidly to preinjection levels. Additional studies also showed that MS i.v. and i.p. (20 and 10 mg/kg, respectively) produced comparable increases in plasma B-E and PRL. Prior treatment with two injections of dexamethasone (DX) (400 µg/kg at 24 hrs and 200 µg/kg at 2 hrs before) blocked the MS induced rise of both B-E and PRL. Also, the simultaneous administration of either 0.2 or 10 mg/kg of naloxone with MS completely blocked the MS produced elevations of plasma B-E and PRL. Tolerance to the plasma hormonal changes were found to occur by 7 days of MS (twice daily-stepwise increased injections). In the MS-tolerant rat, anterior lobe B-E content increased 50% over controls while posterior lobe content remained unchanged. However, the long-term administration of MS failed to alter regional brain levels of either B-E or enkephalins. In an additional study, rats were fitted with indwelling venous catheters and footshocked for 30 min. Plasma B-E increased 6-fold with peak levels at 15 min and returning to control values by 150 min. Footshock (15 min) also increased plasma PRL. In both instances DX pretreatment blocked the stress-induced rises of these hormones. In rats subjected to even more prolonged stress, (footshock-1 hr) anterior pituitary B-E was decreased with no change in posterior lobe content. These results indicate that common central mechanisms may mediate the MS- and stress-induced increases of plasma B-E and PRL.

Supported by DA 01785-02; E.F. supported by NIMH Fellowships F32-MH 05626-02; J.R. supported by INSERM, France.

1286 TRANSMITTER-LIKE EFFECTS OF ENKEPHALIN ON CULTURED SPINAL NEURONS. D.L. Gruol, L.M. Huang*, J.L. Barker, and T.G. Smith*, LNP, NINCDS, NIH, Bethesda, Md. 20014

The direct effects of the opiate peptide, leucine-enkephalin (ENK), and an antagonist, naloxone (NAL), on cultured fetal mouse spinal neurons were studied using intracellular recording techniques and extracellular iontophoresis of ENK and NAL. When applied to the neuronal soma, ENK frequently produced dose-dependent membrane polarizations which were accompanied by a conductance increase and which varied in amplitude as a function of membrane potential (Vm) and position of the iontophoretic pipette. The responses could be divided into two categories. The most common response was a slowly developing and desensitizing polarization (several sec. to peak of response), most clearly seen when ENK was applied for a long period of time (> 1 sec.). The second type of response was less frequently observed and required critical placement of the iontophoretic pipette for detection. This response was faster in time course (≤ 1 sec. in duration), desensitized rapidly and had a more positive reversal potential than the slow ENK response. Occassionally both fast and slow responses were simultaneously evoked by ENK application. The fast ENK response evoked spikes and thus appeared to be excitatory in nature. The functional significance of the slow response remains to be determined. NAL reversibly supressed both ENK responses but interpretation of this result has been complicated by the direct effects of NAL on membrane properties. Two types of NAL responses were observed: (1) a depolarization accompanied by a conductance increase and (2) a hyperpolarization accompanied by a conductance decrease. Both NAL effects were reversible, dose-dependent and varied in amplitude as a function of Vm.

Thus, ENK can alter membrane properties of cultured spinal neurons in ways which are similar to responses evoked by putative neurotransmitters. Like transmitter actions, the peptide responses are associated with a conductance increase, have a reversal potential, desensitize and show a topographical variation in amplitude of the response. These results, coupled with previously published observations demonstrating non-transmitter-like (neuromodulatory) effects of ENK (Science 199 (1978) 1951) suggest that ENK can have multiple actions in the nervous system, both as a neuromodulator and as a neurotransmitter.

1287 SPECIFICITY OF ACTION OF SUBSTANCE P (SP) IN THE LOCUS COERULEUS (LC). P. G. Guyenet, E. Mroz*, S.E. Leeman, and G.K. Aghajanian, Depts. Psychiat. and Pharmacol., Yale Univ. Sch. Med., New Haven, CT 06508 and LHRRB, Harvard Med. Sch., Boston, MA 02115.

In the LC of the rat, SP is localized within boutons that make axodendritic synapses with the noradrenaline-containing neurons. The iontophoretic technique was used in the present study to determine the effect of SP on the firing rate of LC neurons in this species. In addition, the specificity of para-chlorophenyl-GABA (baclofen) as an SP antagonist was examined and a pharmacological approach was used to assess whether SP might modulate cholinergic transmission or have any opiate agonist potency as recently suggested.

SP had an excitatory effect on all LC neurons tested. This effect was also produced by the 4-11 octapeptide derivative of SP and by tachykinins with SP-like activity (elidoisin and physalaemin). By contrast, TRH, bradykinin and neurotensin were inactive while metenkephalin (ME) inhibited all neurons tested. SP excited the neurons with a latency that varied greatly with the intensity of the retaining current applied between ejection periods (3 to 6 sec to more than 40 sec at 10 nA). In order to investigate this variability further, the release of SP (3 mM, pH 4.5) from iontophoretic pipettes was monitored "in vitro" using a sensitive radioimmunoassay. Its transport number was found to vary from 1.6 10^{-2} in the presence of 17.5 mM Na^+ to 0.08 10^{-2} in presence of 170 mM Na^+. The effect of a retaining current on subsequent ejections paralled exactly the kinetics of the neuronal response "in vivo": higher retaining currents resulted in prolonged delays in release. These characteristics of SP release can be accounted for by its low diffusibility, the latter being a direct consequence of its high molecular weight. Thus, the reported sluggishness of neuronal responses to the iontophoretic application of SP might be due for the most part to the physicochemical characteristics of its ejection from glass micropipettes.

Baclofen did not exhibit any specificity as an SP antagonist since it also blocked the response to ACh and glutamate. Muscarinic antagonists and naloxone were remarkably specific in antagonizing the excitation caused by ACh and the inhibition due to mE respectively. Neither of these compounds, however, had any influence on the increase in firing rate due to SP, thus providing evidence that SP in the LC neither interferes with cholinergic transmission nor possesses any opiate agonist potency. In conclusion, the present data suggest that neuronal responses to SP are rapid in the CNS and are due to an interaction with highly specific receptors (USPHS Grants, MH-17871, MH-14459, the State of Connecticut, and the CNRS, France).

1288 GENERAL ACTIVITY CHANGES INDUCED BY D-ALA^2-MET^5-ENKEPHALINAMIDE IN RATS. Craig T. Harston*, Morris A. Spirtes, William P. Dunlap* and David H. Coy*. Dept. Pharm., Tulane Med. Sch.; Veterans Admin. Hospital; Dept. Psych., Tulane U., New Orleans, La.

Morphine is known to increase behavioral activity with low doses and following a period of decreased activity with higher doses. To test whether intracranioventricular (ICV) injections of endorphins act similarly, cannulae were implanted stereotaxically into the right lateral ventricles of male albino rats. Seventeen to 18 days after surgery, groups of 6-9 rats were injected ICV with either 10 µl .9% saline, 12.5, 25, 50 or 100 µg D-ala^2-met^5-enkephalinamide (ENK). Uncannulated controls were not injected but handled similarly. Following injection, each animal was observed for 5 hr in a 31 cm diameter activity chamber. Quadrant crossing, rearing, grooming, head movement, and wet-dog shakes were observed via a video system. Activity was also recorded automatically with 2 photocells.

Quadrant crossing, rearing and photocell interruptions of the ENK groups decreased initially but later increased as compared to the saline-injected controls. Grooming and head movement were decreased and wet-dog shakes were increased by ENK.

In the second experiment, the activity chambers were modified to maintain the rats on ad lib. food and water. Seven days post surgery the rats were put in the activity chambers and injected each morning with saline for three days to habituate them to the procedure. Beginning on day 4, 16 subjects were injected ICV with 25 µg ENK and another 16 with 2.5 µl saline. Twenty-five min after the ICV injection, half of each group was injected IP with 5 mg/kg naloxone or 1 cc/kg saline.

On day 4, the overall activity of the ENK-injected group was higher than that of the saline-injected group. Also on day 4, naloxone reduced activity of both the ENK- and saline-injected rats. The overall activity of the ENK-treated rats increased further over successive days of ENK treatment.

These results suggest that the increased activity was due to the direct effects of ENK and not to a rebound or recovery from the depressive effects. Some naturally occurring activity may involve endorphins, since activity was decreased by naloxone in saline controls.

1289 DISSOCIATION OF EFFECTS OF SOMATOSTATIN ON TURNOVER OF CATECHOLAMINES IN THE CNS. Viktor Havlicek, Robert Herchl and Milan Rezek, Dept. of Physiology, U. of Manitoba, Winnipeg, Canada.

Somatostatin (SRIF) administered into rats intracerebroventricularly (ICV) or into various brain regions (neostriatum, amygdala, hippocampus or supracortically) produces a graded excitatory response: animals begin running in circles; grooming activity may be replaced by excessive and stereotyped scratching, animals become hyperexcitable, uncoordinated, tremulous and finally akinetic and some rats develop tonic-clonic motor seizures. Milder forms of central irritation resemble those produced by amphetamines. Since dopamine (DA) and norepinephrine (NE) are implicated in the mechanism of action of several CNS active drugs, we have examined the effect of SRIF on turnover of catecholamines. SRIF (10 μg) was administered ICV twice (a) 5 min prior to intraperitoneal (i.p.) injection of α-methyl-p-tyrosine (α-MT 250 mg/kg) and (b) 25 min after α-MT injection. Controls were injected with saline or α-MT i.p. and with saline ICV. Rats were sacrificed by decapitation 60 min after receiving α-MT. The brain was rapidly dissected into three regions: forebrain, hindbrain and cerebellum. The fluorescence method for the measurement of DA and NE was used. Results are presented in table below:

Brain Region	Treatment	N	NE ng/g	P	DA ng/g	P
Forebrain	sal & sal	24	291 ± 8	> 0.001	835 ± 28	> 0.001
	α-MT & sal	18	230 ± 8	> 0.05	484 ± 20	> 0.05
	α-MT & SRIF	17	202 ± 12		603 ± 68	
Hindbrain	sal & sal	10	495 ± 11	> 0.001	27 ± 10	
	α-MT & sal	6	328 ± 16	> 0.1	a	
	α-MT & SRIF	6	282 ± 22		a	
Cerebellum	sal & sal	19	190 ± 4	> 0.001	a	
	α-MT & sal	17	118 ± 9	N.S.	a	
	α-MT & SRIF	16	121 ± 10		a	

a-quantities were too small for accurate estimations

As shown in the table α-MT decreased NE by 21% and DA by 42% in forebrain. Pre- and post-treatment with SRIF significantly altered these changes potentiating the effect on NE and inhibiting that on DA. Similar changes for NE were seen for hindbrain, while cerebellum did not show any differences in NE turnover. Thus there was a dissociation in the effect of SRIF in the metabolism of these amines. Decrease in dopaminergic neurotransmission in neostriatum might be related to parkinsonian symptoms seen after somatostatin. (Supported by MRC of Canada).

1290 β-ENDORPHIN INDUCED EPILEPTIFORM ACTIVITY: EFFECTS OF LESIONS AND SPECIFIC OPIATE RECEPTOR AGONISTS. S.J. Henriksen, F. McCoy,* E. French* and F.E. Bloom. The Salk Institute, A.V. Davis Center for Behavioral Neurobiology, La Jolla, CA 92037.

Opiate agonists and endogenous opioid peptides can induce limbic epileptiform activity that can be electroencephalographically recorded from a variety of subcortical loci. β-endorphin (B-E) is the most potent in eliciting this activity when compared on a molar basis to either morphine sulfate or the naturally occurring enkephalin pentapeptides. Intraventricularly (i.c.v.) administered B-E (1-3mm) induces, in rats, subcortically generated paroxysmal waves and cortical slow-waves at doses devoid of other opiate agonistic effects, such as analgesia or behavioral immobility and rigidity. Similar electroencephalographic (EEG) abnormalities are seen in mice, guinea pigs and squirrel monkeys following (i.c.v.) B-E. However, both guinea pigs and squirrel monkeys exhibit less intense epileptiform activity particularly at the onset of paroxysmal activity(initial ictal episode). Intravenous injection of B-E (20-26 mg/kg) in albino mice, but not in rats, produces paroxysmal activity, analgesia and rigidity. In an attempt to anatomically specify the subcortical loci participating in either the generation or elaboration of this phenomena, radio frequency lesions have been made in a variety of sub-cortical limbic loci in rats who were later implanted with chronic electrode arrays and tested for the effect of B-E on spontaneous EEG activity. Lesions have been made in the amygdala (bilateral; medial, central and basal nuclei), the pre-optic area of the anterior medial hypothalamus, and the dorsal-medial nuclear complex of the thalamus (including periventricular, anterior medial and lateral portions, as well as the nucleus reuniens). For pharmacological studies rats implanted with similar chronic electrode arrays have been treated with specific opiate agonists of the μ,κ, and σ type (Martin et al, 1976) alone or following B-E administration. The following agents have been utilized: SKF-10047, a σ agonist; WIN-35, 197-2, a κ agonist; cyclazocine, a κ and σ agonist and ketocyclazocine, a κ agonist. B-E (I.C.V.) induced substantially the same electrographic response in lesioned rats as compared to controls, indicating generating loci located in areas other than amygdala or medial thalamus.

Pharmacological studies will be presented indicating B-E interaction with specific opiate receptors.

(Martin et al J.P.E.T. 197:517, 1976)(supported by DA-01785-02 and the Klingenstein Foundation)

1291 IMMUNOHISTOCHEMICAL AND RADIOIMMUNOASSAY STUDIES ON THE DEVELOPMENT OF SOMATOSTATIN CONTAINING PRIMARY AFFERENT NEURONS IN THE RAT. Raymond H. Ho, Robert Elde, and Robert Sorenson*, Departments of Anatomy, The Ohio State University, Columbus, Ohio, 43210; University of Minnesota, Minneapolis, Minnesota, 55455.

Some small diameter, primary afferent neurons have been shown to contain somatostatin immunoreactivity in their peripheral and central processes, as well as in some small diameter dorsal root ganglion perikarya. Neurons with these characteristics may play a role in nociception. Antibodies raised in rabbits against synthetic somatostatin were used for immunofluorescence histochemical localization and radioimmunoassay studies in a series of fetal and neonatal Sprague Dawley rat spinal cords. Immunofluorescence histochemistry was performed on 10μ cryostat sections of spinal cords that were fixed by whole-animal intracardiac perfusion of 4% paraformaldehyde in phosphate buffer. Somatostatin-like-immunoreactivity was demonstrable in nerve fibers and/or terminals in Laminae I and II of the spinal cord dorsal horn in animals as early as the day 4 neonate. The specificity of immunostaining was established in control experiments in which anti-somatostatin serum pretreated with an excess of somatostatin was unable to localize such structures. Radioimmunoassay data from whole spinal cord extracts indicated very small quantities (approaching limit of assay sensitivity) of somatostatin in cords younger than the day 4 neonate. There was an abrupt rise of somatostatin content in the spinal cord between the day 3 and the day 4 neonate; beyond that stage of development there was a general tendency for the spinal cord somatostatin content to increase with age. Although the rat somatostatin-containing primary afferent neuronal system may be capable of synthesizing and transporting the peptide at an earlier time, it does not store sufficient quantities of somatostatin to be detected until day 4. Supported in part by the Graduate School, University of Minnesota.

1292 EVIDENCE FOR ENDORPHIN RELEASE DURING ELECTROCONVULSIVE SHOCK. John W. Holaday, Gregory Lucas Belenky, Horace H. Loh*, and James L. Meyerhoff. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20012.

Available evidence implicating an endorphin involvement in various behavioral disorders has been contradictory and inconclusive. We have demonstrated the possibility that the therapeutic consequences or side effects of electroconvulsive therapy (ECT), which uses electroconvulsive shock (ECS) to produce a generalized seizure, may involve a functional release of endorphins.

Male Wistar rats (Walter Reed Strain) weighing 250-300g were affixed with wound clips on the pinna of each ear to serve as electrodes for transauricular ECS. Naloxone (10 mg/Kg) or saline were injected intraperitoneally in a volume of 0.1 ml/100g body weight. Ten min. later, electroshock was delivered (2 sec, 210 v, 62 mA, 60 Hz). The first group of rats were evaluated for duration of seizure and ECS-induced catalepsy, as well as for changes in tail-flick latencies and respiratory rates. Raters were blinded as to whether naloxone or saline had been injected. In a second group of similar rats, tail arteries were cannulated [Chiueh and Kopin, Am. J. Physiol. (in press)] two days prior to experiments. Cardiovascular parameters were then monitored via the tail-artery cannula both prior to and following ECS in these conscious, freely moving rats following preinjection with naloxone or saline as before.

ECS produced a tonic-clonic seizure, the duration of which was unaffected by naloxone. Following the seizure, a state of catalepsy characterized by a loss of righting reflex was observed. This catalepsy was behaviorally indistinguishable from opiate-induced catalepsy. Naloxone preinjection significantly diminished this ECS-induced cataleptic state. Tail-flick latencies were elevated following ECS treatment, an effect that was significantly decreased in the naloxone injected rats. The decline in respiratory rates following ECS was also significantly less in the naloxone group.

Blood pressure in the ECS-treated rats rose from approximately 108 mm Hg to 228 mm Hg within the first second of electroshock. Naloxone was without effect on this hyptertensive surge. Comparison of saline and naloxone treated rats following this surge demonstrated significant effects of naloxone on blood pressure and heart rate which persisted during the first 35 sec. post-shock.

Our data suggest that endorphins are released during ECS in rats and that they play a role in post-ECS behavior. We suggest that a release of endorphins may play a role in the therapeutic effects or complications of ECT in man.

1293 ELEVATION OF β-ENDORPHIN-LIKE SUBSTANCE LEVELS IN THE VENTRICULAR CSF BY CENTRAL GRAY STIMULATION (CGS) IN HUMANS. Yoshio Hosobuchi*, Jean Rossier*, Floyd Bloom*, and Roger Guillemin* (SPON: Nancy M. Lee). Dept. Neuro. Sur. UCSF Sch. Med., San Francisco, CA 94143 (YH) and The Salk Institute, La Jolla, CA (92037) (JR,FB,RG).

Electrical stimulation of the periaqueductal and periventricular gray matter provides significant relief from severe intractable pain in humans; it is totally reversed by the specific opiate antagonist naloxone. At the initial implantation of chronic brain electrodes, ventricular CSF was collected from six patients undergoing the operation; base levels of β-endorphin-like substances were determined on each sample by a radioimmunoassay. This technique may also detect molecules larger than β-endorphin; it will certainly exclude smaller peptides such as enkephalin. Series of pre- and post-stimulation CSF samples were collected from a ventricular catheter placed at the foramen of Monro during the stereotactic surgery necessary to perform a ventriculogram. In three patients electrodes were implanted in the posterior limb of the internal capsule; stimulation of this area produced no increase in ventricular levels of β-endorphin-like substances. However, in three other patients who had electrodes implanted in the periaqueductal area, stimulation of this area produced a two- to four-fold increase in the levels of β-endorphin-like substances. Intraventricular administration of human β-endorphin has been shown to produce a dose-dependent, prolonged analgesic effect in humans. The current results represent the first evidence of *in vivo* release of β-endorphin-like substances in humans and suggests that stimulation may have produced the release of the substances.

1294 ENKEPHALIN MODULATION OF AMINO ACID RESPONSES ON CULTURED SPINAL NEURONS. L.M. Huang*, D.L. Gruol, J.L. Barker and T.G. Smith*, (SPON: H.G. Wagner) LNP, NINCDS, NIH, Bethesda, Md. 20014.

The effects of the opiate peptide leucine-enkephalin (ENK) and its antagonist naloxone (NAL) on amino acid responses of cultured fetal mouse spinal neurons were studied using intracellular recordings of membrane potential and current made with conventional and voltage clamp techniques. The putative inhibitory amino acids γ-aminobutyric acid (GABA) and glycine and ENK and NAL were applied by iontophoresis. The results reported here were mainly obtained from cells where ENK evoked a slow membrane polarization. NAL also had direct effects on membrane conductance in some neurons. However, similar modulatory effects were seen in the absence of any change in membrane properties due to ENK or NAL. ENK and NAL depressed the GABA responses in a reversible, dose-dependent manner. A depression of the GABA currents by ENK was also observed under voltage clamp. Pharmacological analysis of the ENK-GABA interaction showed a parallel shift to the right of the GABA dose-response curve without a change in the limiting slope of the log-log plot. In some neurons a depression of V_{max} was also observed. ENK and NAL enhanced or depressed the glycine response in a dose-dependent, reversible manner. In those cells where the glycine response was enhanced there was a parallel shift of the glycine dose-response curve to the left in the presence of ENK without a change in the limiting slope of the log-log plots. In neurons studied under voltage clamp, low iontophoretic ENK currents enhanced the glycine response while at higher ENK currents the glycine response was depressed.

While some of the depressant effects of ENK on amino acid responses are likely due to shunting of the membrane, enhancement of the glycine response and depression of amino acid-induced currents cannot be explained by this mechanism. Two alternative explanations are that ENK may directly modulate inhibitory amino acid responses (1) by acting on the receptor-coupled conductance mechanism as has been observed for ENK modulation of glutamate events (Science 199 (1978) 1451) or (2) by altering the Cl^- ion gradient across the membrane and thus altering the driving force on the Cl^- conductance activated by GABA and glycine in these cells.

1295 DISTRIBUTION OF CHOLECYSTOKININ OCTAPEPTIDE-LIKE IMMUNOREACTIVITY IN THE RAT BRAIN. Robert B. Innis, Fernando M.A. Correa* and Solomon H. Snyder. Dept. Pharmacol., Johns Hopkins Sch. Med., Baltimore, MD 21205.

Several peptides, including neurotensin, vasoactive intestinal peptide, enkephalin, cholecystokinin (CCK), and the C-terminal octapeptide of CCK (CCK-8) are present in both the brain and the gut and CCK-like immunoreactivity has been observed histochemically in rabbit brain (Proc. Natl. Acad. Sci., 74:3033, 1977). With an antiserum raised against CCK-8 we have identified and mapped the distribution of CCK-8-like peptides in the rat brain by indirect immunohistofluorescence. At every level, control sections were stained with antiserum preadsorbed with CCK-8 and with pre-immune serum. Cellular and fiber staining was removed by preadsorption of the antiserum with CCK-8 of gastrin but was unaffected by preadsorption with a large series of unrelated peptides.

Immunoreactive cells are observed diffusely throughout the cerebral cortex. These neurons are usually 10-20 μ in length, bipolar, and radially oriented to the surface of the brain. They are located in layers II-VI but predominantly in layers II and III. In rostral portions of cortex, positively stained cells appear in the midline and shoulder regions' caudally, these cells tend to be in lateral cortical areas. Varicose fibers are located diffusely in the cortex with the most dense network near the rhinal sulcus, lateral to the central nucleus of the amygdala. Sparse cell bodies are observed in the hippocampus. Dense cell clusters occur in the periventricular and dorsomedial nuclei of the hypothalamus. Varicose fibers run dorsally and laterally from these two hypothalamic clusters. Caudally, a neuronal cluster is found in the midline, just ventral to the aqueduct at the level of the substantia nigra. Fibers tend to run dorsally and ventrolaterally from this cluster. In the brainstem, a neuronal cluster is located in the midline, just ventral to the fourth ventricle at the level of exit of the fifth cranial nerve. F.M.A.C. is recipient of fellowship from FAPESP, Brasil.

1296 "OPIOID" BEHAVIORAL EFFECTS FOLLOWING ADRENOCORTICOTROPIN OR β-ENDORPHIN INJECTIONS IN PERIAQUEDUCTAL GRAY OF RATS: SIMILARITY TO "PARADOXICAL" MORPHINE EFFECTS SUGGESTS MECHANISM FOR OPIATE DEPENDENCE. Yasuko F. Jacquet. NY State Research Institute for Neurochemistry, Ward's Island, New York City, N.Y. 10035.

Microinjection of adrenocorticotropin (ACTH 1-24) in the periaqueductal gray (PAG) of rats resulted in a dose-dependent hyperreactivity characterized by repeated and rapid high leaps ("flying") similar to the behavior seen in opiate-dependent rats undergoing precipitated abstinence. This was followed by a period during which other opiate abstinence signs were manifested, i.e., wet-dog shakes, teeth chatter, abnormal posture, squeal on touch, etc. Shorter analogs, i.e., ACTH 1-13, ACTH 4-10, resulted in an attenuated form of the abstinence syndrome. Naloxone pretreatment failed to block this behavior.

We previously reported (Jacquet and Lajtha, Science, 1974) that morphine microinjection in the PAG resulted in 2 "paradoxical" effects: (1) analgesia/catatonia, and (2) hyper-reactivity, characterized by rapid and repeated high leaps. Pretreatment with naloxone blocked (1) but not (2). Significantly, injections of β-endorphin in the PAG resulted only in (1) but not (2) (Jacquet and Marks, Science, 1976), while injections of the unnatural (+)-morphine in the PAG resulted only in (2) and not (1) (Jacquet et al, Science, 1977). These observations suggested that morphine effects in the PAG were mediated by 2 classes of receptors, one being stereospecific for opiates and naloxone-sensitive, of which the endogenous ligand appeared to be β-endorphin, and the other which was not stereospecific for opiates and was naloxone-insensitive. Our present evidence suggests that ACTH may be the endogenous ligand of this latter class of receptors.

Systemic injections of morphine never resulted in "flying." Moreover, pretreatment of rats with systemically-administered morphine blocked "flying" following morphine microinjection in the PAG, but did not block analgesia/catatonia. (Similarly, pretreatment with systemically-administered morphine blocked "flying" following ACTH microinjection in PAG while naloxone did not.) These differential effects of morphine following systemic or local administrations suggest that following systemic administration, morphine achieves distribution throughout the CNS, activating endorphin receptors in neuronal circuits which exert an inhibitory influence on the excitatory effects of morphine acting at ACTH receptor sites. The opiate abstinence syndrome may be due to excitation by morphine of the ACTH receptor following removal by naloxone blockade, or weakening by tolerance development, of the inhibitory influence exerted by the endorphin receptor.

1297 ACTION OF INTRAHYPOTHALAMICALLY INJECTED β-ENDORPHIN ON THE BODY TEMPERATURE OF THE RAT. G. E. Martin and C. B. Bacino.* Merck Institute for Therapeutic Research, West Point, PA 19486.

Injected into the lateral cerebral ventricle (LCV), β-endorphin evokes hypothermia in the rat (Bloom et al., Science, 1976, 194: 630). The site of this action could be thermoregulatory neurons in the preoptic/anterior hypothalamus (POAH), but the LCV route of administration does not preclude the involvement of other structures which form the walls of the ventricles. Hence, we measured rectal temperature (T) after the injection of β-endorphin into the POAH.

A 24 ga guide cannula was implanted above a POAH or LCV site in 34 male Sprague-Dawley rats. POAH and LCV injections were made via a 30 ga injector over a 30 sec interval. The volume of injection was 0.5 or 1.0 µl in the POAH and 5 µl in the LCV. Artificial CSF vehicle or β-endorphin, in doses of .74, 1.48 and 3.7 nmoles (nM) was microinjected in the POAH (n=6), whereas the LCV doses (n=5) were .74, 3.7 and 7.4 nM. T was measured by inserting a thermometer into the rectum of the unrestrained rat.

β-Endorphin evoked a hypo- or hyperthermia depending on the dose and route of administration. Compared with the increase in T to 38.4 ± 0.2°C (Mean Peak T, MP) from the 37.2 ± 0.1°C baseline after the microinjection of the vehicle, β-endorphin, at doses of 0.74, 1.48 and 3.7 nM, caused a significant but non-dose-related rise in T. The MP was 39.7 ± 0.1, 39.6 ± 0.2 and 39.3 ± 0.3°C for the respective doses of β-endorphin after the POAH microinjections. In the LCV, on the other hand, 0.74 and 3.7 nM of β-endorphin caused no significant change in T (MP = 37.7 ± 0.3, 38.1 ± 0.5°C). The LCV injection of 7.4 nM of the opiate, however, evoked a marked but naloxone reversible drop in T to 34.0 ± 0.9°C which was accompanied by catatonia and respiratory depression. Pretreatment with either naloxone (2 or 5 mg/kg) or Indocin (15 mg/kg), slightly attenuated the rise in T seen after the POAH injections. Indocin, but not naloxone, blocked the slight rise in T evoked by the CSF injection into the POAH. Pretreatment with both naloxone and indocin reduced significantly the hyperthermia evoked by POAH injections of β-endorphin. These data suggest that the β-endorphin-induced rise in T may be mediated in part by synthesis or release of prostaglandins in the POAH.

Since catatonia and respiratory depression accompanied the hypothermia caused by the LCV injection of β-endorphin, the drop in T may not reflect a specific action on thermoregulatory processes in the POAH region. In fact, at the doses of β-endorphin examined, a marked increase in T followed the direct injection of β-endorphin into the POAH. Further studies will be required to determine whether β-endorphin has an endogenous role in T regulation, and whether its hyperthermic action is, at least partly, mediated by prostaglandins.

1298 CORRELATIVE ANALYSIS OF HYPOTHALAMIC PEPTIDES AND CATECHOLAMINES IN THE AGED NON-HUMAN PRIMATE. J.A. McConnell, T.H. McNeill and J.R. Sladek, Jr., Univ. of Texas Med. Schl., Houston, TX 77030 and Univ. Rochester, Rochester, NY 14642.

Aging is accompanied by a decreased adaptability of certain homeostatic mechanisms under hypothalamic regulation. One such function, water balance, is controlled by vasopressin. This magnocellular peptide is synthesized in the paraventricular (PVN) and supraoptic (SON) nuclei, both of which are heavily innervated by catecholamine (CA) varicosities.

Hypothalami from six female macaques (M. nemestrina), three each of 4 and 20 years of age, were prepared for the simultaneous visualization of peptides and CA by the technique of McNeill and Sladek (Science 200:72-74, 1978). Quantitative analyses were performed on one animal of each age at comparable levels of the PVN. Various analyses were made of cell size and number in Nissl and PAP stained sections. Sections were stained with the PAP technique for bovine neurophysin (BNP), human estrogen-stimulated neurophysin (ESN), and human nicotine-stimulated neurophysin (NSN). Sections adjacent to those stained for neurophysin were examined for CA fluorescence and the degree of CA varicosity/neurophysin perikaryal interaction was determined with the use of a microscope comparator bridge system. A range of cell sizes (15-45 µ) was seen in both ages, but fewer of the largest cells (>35µ) were present in the 20 year old. Total numbers of neurophysin-stained cells remained constant, but fewer heavily stained cells were seen in the older animal. This decrease was especially prominent following BNP (84%) and NSN (54%) staining and the change was accompanied by a concomitant increase in lightly stained cells. A proportionate shift was not seen in ESN perikarya. Apart from numerical changes, a general decrease was noted in neurophysin staining in older specimen with all antisera, especially in the processes of the hypothalamo-neurohypophyseal tract. Herring bodies also accumulated with age. CA varicosity patterns within the PVN were similar in both ages, although a somewhat reduced appearance of fine-sized varicosities was noted. The number of CA varicosities and the number of neurophysin (ESN, NSN,BNP) containing perikarya with apparent CA contacts were comparable in both ages. These data indicate that the amount of immunoreactive neurophysin decreased with age and that the use of the more specific antisera focus on a possible alteration in vasopressin content. Whether this represents an alteration in synthesis, storage or another cellular mechanism is unknown.

We thank Earl A. Zimmerman for the antisera and Douglas M. Bowden for the animals. Supported by NS 11642 (JRS).

1299 COMPARATIVE IMMUNOHISTOCHEMISTRY OF MET-ENKEPHALIN, VASOPRESSIN, AND OXYTOCIN IN THE MEDIAN EMINENCE AND HYPOTHALAMUS OF THE CAT. Paul Micevych* and Robert Elde (SPON: David Coulter). Dept. of Anatomy, Un. of Minnesota Medical School, Minneapolis, MN 55455

Several immunohistochemical studies have reported the distribution of enkephalins in the nervous system of the rat. The present study reveals striking differences in enkephalin immunoreactivity in the hypothalamus of the cat. Normal and colchicine treated cats (30 ug in third ventricle, 48 hour survival) were perfused with 4% paraformaldehyde. Ten-micron cryostat sections were taken of the hypothalamus and stained using the indirect immunofluorescence method. Antiserum directed against met-enkephalin (M-ENK) and used in this study cross-reacted less than 0.1% with leu-ENK and β-endorphin. Antisera directed against vasopressin (VP) and oxytocin (OT) were used for comparative purposes.

Immunoreactive M-ENK axons, terminals, and Herring bodies were observed in the internal layer of the median eminence, pituitary stalk, and posterior pituitary. This immunofluorescence distribution agreed closely with the staining pattern obtained with VP and OT. VP and releasing factors have been localized in the external layer of the median eminence. M-ENK immunoreactive axons and terminals were visualized here also, especially near the capillary loops of the hypothalamo-hypophyseal portal system.

In normal animals M-ENK immunoreactive cell bodies were seen in the hypothalamic paraventricular and supraoptic nuclei, as was staining for VP and OT.

Localization with M-ENK antiserum in colchicine treated cats revealed a greater number of supraoptic and paraventricular cell bodies, but additionally, an extensive system of periventricular neurons was positive. In contrast to the supraoptic and paraventricular nuclei, the periventricular cell bodies remained negative after incubation with VP and OT antisera.

VP and OT containing neurons of the paraventricular and supraoptic nuclei project primarily to the posterior pituitary. In the cat, M-ENK perikarya in these nuclei may be the cells of origin of M-ENK fibers, terminals, and Herring bodies in the internal layer of the median eminence. This is totally distinct from what has been visualized in the rat, where there is no M-ENK immunoreactivity in the median eminence, posterior pituitary, supraoptic or paraventricular nuclei. The M-ENK immunofluorescence in the external layer of the median eminence may arise from the supraoptic/paraventricular neurons or the periventricular area neurons. M-ENK immunoreactive terminals in the external layer of the median eminence may participate in the regulation of release of anterior pituitary hormones.

Supported in part by the Graduate School at the University of Minnesota.

1300 EXCITATORY ACTION OF NEUROTENSIN ON CAT DORSAL HORN NEURONES IN LAMINAE I-III. Vjekoslav Miletic and Mirjana Randic, Iowa State Univ. Sci. Tech., Ames, IA 50011.

Neurotensin has been recently shown by immunohistochemical technique to be present in substantia gelatinosa of rat spinal cord. Since it is known that this area contains neurones principally excited by an input in nociceptor afferent fibres, and that, in addition, it is particularly rich in opiate receptor binding sites and enkephalin, it was of interest to study the central effects of neurotensin by applying it microelectrophoretically to dorsal horn nociceptive and other types of neurones at the level of Rexed's laminae I-III.

We have observed that synthetic neurotensin causes a slight to moderate excitation of about 65% of all tested units located in Laminae I-III. Excitation was observed as initiation of firing in a previously quiescent unit, or as an increase in the rate of spontaneous and/or evoked firing. The excitant response to neurotensin was relatively slow in onset and recovery. Neurotensin proved to possess an excitatory action in all categories of neurones recognized in spinal preparations of cats in this area on the basis of their excitability by different kinds of cutaneous afferent input. Units excited by sodium-L-glutamate were also excited by neurotensin. The latter result and the temporal characteristics of neurotensin-produced excitation are consistent with the possibility that neurotensin acts on postsynaptic sites in Laminae I-III of the spinal cord as a neuromodulator. (Supported by PHS Grant NS 12972-01 NSF Grant BNS 23871 and Salsbury Foundation.)

1301 CHARACTERIZATION OF THE BOMBESIN RECEPTOR IN MAMMALIAN BRAIN. Terry W. Moody* and Candace B. Pert (SPON: W. E. Bunney, Jr.). Biological Psychiatry Branch, NIMH, Bethesda, MD 20014.

Bombesin, a tetradecapeptide isolated from frog skin, produces hyperglycemia and hypothermia with a well-defined structure-activity relationship when injected intracisternally in rats. Also, bombesin-like immunoreactivity has been demonstrated in rat brain. Since bombesin-like peptides may function as neurotransmitters or modulators of neural activity in the central nervous system, we undertook the characterization of the bombesin receptor in mammalian brain. A radiolabeled tyrosine^4 analogue of bombesin bound specifically to rat brain membranes with high affinity (K_d = 1 nM). The high affinity binding was noncooperative and saturable, with an estimated 3 pmoles of sites/g wet tissue. The association and dissociation rate constants were 1.1×10^8 $M^{-1}min^{-1}$ and 1.0×10^{-1} min^{-1} respectively. Subcellular fractionation studies revealed that the density of sites is 3-fold greater in synaptic than nuclear or mitochondrial membranes. Regional distribution studies revealed that the density of sites in the hippocampus, the highest region, is 7-fold greater than the medulla pons, the lowest region. Pharmacological studies indicated that those bombesin analogues which possess high biological activity inhibit the binding of tyrosine^4-bombesin with greater affinity than do those analogues which possess lower biological activity. In particular, numerous amino acid residues near the CO_2 terminal, e.g., Trp^8 and His^{12}, appear to be essential for interaction with the bombesin receptor whereas numerous amino acid residues near the NH_2 terminal, e.g., Leu^4, Gly^5 and Asn^6 are not essential. Other putative neurotransmitters and brain receptor antagonists are not competitive for the high affinity tyrosine^4-bombesin binding site. These results suggest that synaptic membranes from rat brain contain a unique receptor which mediates the effects of bombesin-like peptides in the central nervous system.

1302 EFFECTS OF PHARMACOLOGICAL AND ENDOCRINOLOGICAL MANIPULATION ON NEUROTENSIN-INDUCED HYPOTHERMIA. C. B. Nemeroff, G. Bissette*, P. J. Manberg*, A. J. Osbahr III*, G. R. Breese, P. T. Loosen*, M. A. Lipton and A. J. Prange, Jr.*, Biol. Sci. Res. Ctr., Univ. North Carolina, Sch. Med., Chapel Hill, N. C. 27514.

Neurotensin (NT), an endogenous central nervous system tridecapeptide, has been demonstrated to produce a marked dose-dependent hypothermia after intracisternal (IC) administration in microgram quantities in a variety of laboratory animals. The present study sought to determine the mechanism of the hypothermic action by utilizing pharmacological treatments which alter the function of brain neurotransmitter systems. In each experiment adult male albino rats (300 g) were divided into 4 groups (n=6/group): (1) Saline IP + Saline IC; (2) Saline IP + NT (30 µg IC); (3) Pretreatment IP + Saline IC; (4) Pretreatment IP + NT (30 µg IC). After drug pretreatment rats were lightly anesthetized with ether, injected IC with NT or saline and placed in a cold room (4°C). Rectal temperature was monitored at 0,30,60,90 and 120 min after IC injection. Pretreatment of rats with anticholinergic (atropine), antinoradrenergic (propranolol and phenoxybenzamine) or anti-opiate (naloxone) agents did not significantly alter NT-induced hypothermia. Paracholorophenylalanine (PCPA) and 6-hydroxydopamine (6OHDA) were utilized to deplete brain serotonin and catecholamines respectively and these depletions were confirmed by radioenzymatic assay as previously described (Endocrinol. 101, 613, 1977). Specific depletion of brain serotonin using PCPA did not alter NT-induced hypothermia. Although depletion of both norepinephrine (NE) and dopamine (DA) with 6 OHDA potentiated NT-induced hypothermia, selective depletions of either brain NE or DA did not exert a significant effect. Haloperidol pretreatment (2 mg/kg IP) markedly potentiated NT-induced hypothermia, though lower doses of this neuroleptic did not exert this action. Pretreatment with thyroid hormone (T_3) did not alter NT-induced hypothermia but central (IC) injection of microgram quantities of thyrotropin-releasing hormone (TRH), the tripeptide which releases TSH from the anterior pituitary, significantly antagonizes NT-induced hypothermia. Treatment with Pro-Leu-Glu-NH_2 (MIF-I), another hypothalamic peptide, did not significantly effect NT-induced hypothermia. Furthermore, IC NT injection into thyroidectomized (TX), but not control rats, resulted in a significant reduction in the characteristically high levels of serum immunoreactive TSH observed in TX animals. These results demonstrate that two endogenous neuropeptides, NT and TRH, appear to be antagonists in certain systems. (Supported by NICHHD HD-03110, HD-10570, and NIMH grants MH-15631, MH-00013, and MH-22536.

1303 IMMUNOHISTOCHEMICAL IDENTIFICATION AND MAPPING OF αMELANOCYTE STIMULATING HORMONE-CONTAINING NEURONS IN THE RAT BRAIN. Thomas L. O'Donohue* and David M. Jacobowitz. Lab. Clin. Sci., NIMH, Bethesda, Md. 20014 and Dept. of Pharmacology, Howard University, Washington, D.C. 20059.

αMelanocyte stimulating hormone (αMSH) is a biologically active pituitary peptide capable of exerting potent behavioral effects in rats. Recent studies have demonstrated the presence of immunoreactive αMSH in mammalian brain. In this study we have used a highly specific and well-characterized αMSH antibody to identify and localize αMSH immunoreactivity in nerve fibers and cell bodies of the rat brain.

αMSH-containing neurons were observed within discrete varicose beaded fibers distributed throughout the rat brain. In the forebrain particularly dense populations of αMSH-containing fibers were present in the medial preoptic, periventricular, anterior hypothalamic and dorsomedial nuclei. Moderate numbers of fine varicose fibers were also noted in the internal layer of the median eminence, and the arcuate, paraventricular and posterior hypothalamic nuclei as well as in the mammillary body. A heavy fiber distribution is present in the lateral septum, dorsal and ventral parts of the nucleus interstitialis stria terminalis, and a moderate number of fibers were localized in the tractus diagonalis. A substantial number of fibers were also present in the periventricular and rhomboid nuclei of the thalamus. Many fibers were located in parts of the medial, central and basal amygdaloid nuclei.

In the mesencephalon and pons numerous fibers were observed in the central gray adjacent to the cerebral aqueduct with a medio-lateral projection of fibers through the nucleus cuneiformis and also passing ventral to the nucleus parabrachialis dorsalis. The locus coeruleus contained few, if any, fibers. A moderate number of fibers was observed in the nucleus tractus solitarius.

Intraventricular injections of vinblastine (20 µg) demonstrated that αMSH-containing cell bodies were present in the entire rostral-caudal extent of the arcuate nucleus.

It is apparent that αMSH has joined the growing list of biologically active peptides present in nerves in the central nervous system. The physiological significance of this neuropeptide awaits elucidation.

1304 ACCUMULATION OF ^{3}H-THYROTROPIN RELEASING HORMONE (TRH) BY RAT CEREBELLUM SLICES. M. Pacheco, D. J. Woodward and J. F. McKelvy. Depts. of Cell Biology and Biochemistry, Univ. Texas Hlth. Sci. Ctr., Dallas, TX 75235.

The extrahypothalamic distribution of TRH (Endocrinology 95: 540, 1974)suggests a widespread central action for this neuropeptide in addition to its hypophysiotropic function. The current work was done to investigate the possible existence of a transport mechanism for TRH in extrahypothalamic brain areas - which might be related to its synaptic actions. Two hundred µm sagittal slices of rat cerebellum were incubated with (^{3}H)-TRH (5×10^{-5} M final concentration) in a medium containing Bacitracin to prevent its degradation (Biochem. and Biophys. Res. Com. 73: 507, 1976). Time course studies showed a rapid accumulation of labeled TRH in slices incubated at 27°C or 37°C. Maximum uptake occurred at 60 min of incubation. The process responsible for (^{3}H)-TRH uptake had many of the properties of a high affinity transport system: 1) it was temperature sensitive (Q_{10} = 1.48); 2) it showed saturation kinetics; and, 3) tissue: medium ratios of 5 : 1 were attained after 60 min incubation at 37°C. Bacitracin in the incubation medium increased the uptake of label by 38%; however, chromatographic analysis revealed that in these conditions 30% of the total dpms were TRH metabolites and 70% were (^{3}H)-TRH. This indicates that Bacitracin protection is incomplete but that the majority of the label is in the form of TRH. Iontophoretic studies in the cerebellum have shown TRH to produce inhibition of the basal firing rate of the Purkinje cells (Nature 255: 233,1975 and Pharm. Biochem. & Behav. 5 : 171, 1976). Such results in combination with those of the current work support the hypothesis of a functional role for TRH in the cerebellum, as well as in other extrahypothalamic areas of the Central Nervous System.

Supported by NSF grant BNS 78-84506 and NIH grant RCDA I K04 AM 00331-01 (J. F. M.), and NSF grant BNS 77-01174 (D. J. W.).

1305 UPTAKE AND DISTRIBUTION IN MOUSE BRAIN OF A C^{14} LABELED DIPEPTIDE PROTECTIVE AGAINST PUROMYCIN AMNESIA. T. C. Rainbow, J. B. Flexner*, L. B. Flexner*, R. Walter* and P. L. Hoffman.* Dept. Anat., Sch. Med., University of Pennsylvania, Phila., Pa. 19104 and Dept. Physiol., Sch. Med., University of Illinois, Chicago, Ill. 60612.

We have found previously that subcutaneous injection of Cyclo (Leu-Gly), a stable derivative of the C-terminal fragment of oxytocin protects against puromycin amnesia in mice when given immediately after or 24 hr before Y-maze training. Cyclo (Leu-^{14}C (U) Gly) was used to determine the level of peptide in regional and subcellular fractions of mouse brain up to 96 hr after injection of a fully protective dose (1 μ mole = 170 μg). Brains were dissected into the following regions: brainstem + midbrain, hippocampus + entorhinal cortex, diencephalon, basal ganglia and cerebral cortex. At 0.5 hr and all succeeding times there was no significant difference in the level of peptide among the several brain areas. Cyclo (Leu-Gly) was identified intact 4 days after injection in brain and plasma. The ratio of the concentration of the peptide in the intracellular space to that calculated for the extracellular space was greater than 1 by 7 hr after injection and this ratio increased continually up to 96 hr indicating that the peptide was restricted in its passage out of brain tissue.

Cyclo (Leu-Gly) was found in all subcellular fractions. The amount present of Cyclo (Leu-Gly) in the synaptosomal fraction correlated highly with the degree of protection provided against puromycin-induced amnesia ($R=0.92$, $P < .005$). No other subcellular fraction showed a significant correlation. This suggests to us that the anti-amnestic effects of Cyclo (Leu-Gly) may be related to its presence in brain synaptic fractions.
(Supported by USPHS Grant AM 18399 and NSF Grants GB 42753 and BNS 76-11779).

1306 ENKEPHALIN ACTS TO INHIBIT LOCUS COERULEUS MEDIATED BEHAVIORS. D.E. Redmond, Jr., M.S. Gold and Y.H. Huang, Dept. of Psychiatry, Yale University, New Haven, Connecticut 06510.

Both intravenously[1] and iontophoretically[2] administered morphine diminishes the neuronal activity of the noradrenergic nucleus locus coeruleus, an effect directly mediated by opiate receptors,[3] as shown by naloxone blockade.[1,2] Similar effects of iontophoretically or intravenously administered α-adrenergic agonists are specifically blocked by low doses of the α-adrenergic antagonist piperoxane.[4] This paper will present further evidence that interactions of these receptors may be important to rated behaviors in non-human primates which may be related to human anxiety.[5] Behaviors were scored by two raters from videotape recordings. Mean threat-associated behaviors per 5 minute period from four adult *Macaca arctoides* were compared after morphine, naloxone, piperoxane, or a parenterally active synthetic pentapeptide (Sandoz FK 33824) were infused intravenously from outside a sound dampened isolation chamber. Piperoxane (1.0 mg/kg) increases behaviors indistinguishably from the effects of human threats, or of low intensity electrical stimulation of the locus coeruleus.[6] Morphine sulfate (0.2 mg/kg) or FK 33824 (0.4 mg/kg) will reduce these behaviors to below the baseline. The specificity of these effects on opiate receptors is demonstrated by their reversibility by naloxone. Administered alone FK33824 (0.4 mg/kg) blocked the effects of locus coeruleus electrical stimulation at stimulation parameters which had behavioral effects prior to drug administration and after 0.4 mg naloxone. These data suggest that exogenous opiates may have therapeutically useful effects in clinical doses because of their inhibition of noradrenergic nuclei and may be abused partly for the same "anxiety-reducing" effects.[5] Endogenous endorphin or enkephalins may function to diminish anxiety-fear symptoms and behaviors under some circumstances such as combat, shock, or extreme pain, where they would be maladaptive. Unfortunately, these conditions will be difficult to reproduce experimentally to test this hypothesis in humans. Relative opiate deficiency may lead to opposite effects, as suggested by our recent report.[7]

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1307 NEUROHYPOPHYSEAL PEPTIDES, NOREPINEPHRINE AND ETHANOL TOLERANCE. R. F. Ritzmann , Paula L. Hoffman*, and Boris Tabakoff. Dept. of Physiology & Biophysics, University of Illinois Medical Center, Chiago, Illinois 60680.

The functional tolerance which develops during chronic ethanol consumption has been postulated to resemble other adaptive phenomena such as the acquisition of a learned response or memory (Tabakoff and Ritzmann, J. Pharmacol. Exp. Ther. 203:319, 1977). Brain noradrenergic (NE) systems have been shown to be involved in the processes of learning, memory and the development of tolerance to sedative hypnotic drugs. Neurohypophyseal peptides have also been shown to influence learning and memory, and these peptides have in turn been reported to alter NE turnover in various brain regions. We therefore investigated the effect of vasopressin (AVP) and oxytocin (OXT) on ethanol tolerance. Mice were fed a liquid diet containing 7% ethanol or pair-fed an iso-caloric control diet for 7 days. On the morning of the 8th day, ethanol diets were replaced with control diet; 30 hours later mice from each group received a subcutaneous injection of either 10 μg AVP, 10 μg OXT, or an equal volume of saline. These injections were repeated at 24-hour intervals for 9 days. At 3-day intervals after withdrawal, tolerance was assessed by monitoring the hypothermia and duration of the loss of righting reflex produced by the intraperitoneal administration of ethanol (3 g/kg). By the 6th day, tolerance was found to have dissipated in ethanol-fed mice treated with OXT or saline. On the other hand, no diminution in acquired tolerance was observed in the ethanol-fed mice treated with AVP. When AVP treatment was terminated 9 days after withdrawal, tolerance subsequently disappeared with its normal time course of approximately 6 days. In a second series of experiments, mice were removed from the ethanol diets and 8 hours later, injected icv with 6-OHDA (50 μg). Half of these mice were then treated daily with AVP while the remaining half received saline injections. AVP did not prolong the duration of tolerance in the 6-OHDA-injected ethanol-fed mice. Tolerance disappeared with a similar time course in AVP- and saline-injected mice, i.e. within 6 days following the termination of ethanol treatment. These results indicate that AVP may be acting through the NE system to maintain ethanol tolerance.

This work was supported by grants from the National Institute on Alcohol Abuse and Alcoholism, AA 2696-03; the State of Illinois Department of Mental Health and Developmental Disabilities, 720-03 and the National Science Foundation, NSF BNS 76-11779.

1308 Similarity in Structure Between MIF-I and Dopamine: Reason For Anti-Parkinsonian Activity? T. A. Robert*, R. L. Snell*, and R. M. Kostrzewa, East Tennessee State University, College of Medicine and College of Arts and Sciences, Johnson City, Tennessee, 37601.

Although the tripeptide L-prolyl-L-leucyl-glycine amide (MIF-I) has been found to be effective in preclinical trials for the treatment of Parkinson's Disease (Lancet 2:683, 1973), the mechanism of action of this agent has not been determined. Previous studies by this group and others have suggested that MIF-I may be a dopamine (DA) receptor agonist. In the present study, CPK (Ealing Corporation) models of MIF-I and DA were constructed in order to examine the possibility that MIF-I structurally mimics the DA molecule.

Three primary conformations with similarities to DA have been revealed upon examination of the space-filling molecular models. Of these three, one particular conformer appears to possess minimal bond strain and notable similarities to DA. The proline amine and terminal carbonyl oxygen of MIF-I can be superimposed upon the terminal amine and 4-hydroxy groups of DA respectively. Approach to the tripeptide surface which presents these exposed functional groups and resembles the topology of the DA molecule is not sterically hindered. The spacial position and Π-cloud orientation of the oxygen atoms from the two molecules are similar, and the possibility of tautomerism within the peptide bonds of MIF-I may produce a hydroxyl group chemically similar to that of DA. The ability of the MIF-I carbonyl and DA hydroxy oxygens to form hydrogen bonds may prove to be a significant factor in ligand-receptor interaction.

1309 ENKEPHALIN FIBERS ARE PRESENT IN THE PARS NERVOSA OF THE RAT PITUITARY. DEHYDRATION DECREASES MARKEDLY THEIR ENKEPHALIN CONTENT. Jean Rossier, Elena L.F. Battenberg, Alejandro Bayon*, Tamotsu Shibasaki*, Roger Guillemin, Richard J. Miller+, and Floyd E. Bloom. The Salk Institute, La Jolla, CA 92037. +Univ. of Chicago, Illinois 60637.

Radioimmunoassays of β-endorphin (B-E), α-MSH and Leu^5-enkephalin (L-e) were performed on extracts of the pars distalis, pars intermedia and pars nervosa dissected under microscope from the rat pituitary. B-E was concentrated in pars distalis and pars intermedia and was absent from pars nervosa, α-MSH was mostly concentrated in pars intermedia, L-e was found mostly in pars nervosa.

	Total content whole pituitary	Per cent per pars distalis	intermedia	nervosa
B-E i.l.*	1.3 µg	60%	35%	5%
α-MSH i.l.	450 ng	20%	68%	12%
L-e i.l.	750 pg	9%	13%	78%

i.l.= immunoassayable like material.

By immunocytology, fibers visualized with L-e antiserum were seen only in pars nervosa. The L-e i.l. of the pars intermedia extracts was coeluted with synthetic L-e on gel filtration columns. Neither vasopressins (AVP and LVP) nor oxytocin cross-react with our L-e assay at doses up to 100 µg/assay tube. Our assay shows a 3% cross reactivity with Met^5-enkephalin which may thus be a part of our L-e i.l.

After dehydration (NaCl 2% drinking fluid for 5 days) L-e levels in the posterior lobe of the rat pituitary were 37% of the value of the control animals while no changes were noticed for B-E. Enkephalinergic cell bodies were visualized by immunocytology in the paraventricular nuclei of the hypothalamus after cochicine pretreatment. Although we have measured L-e in the hypothalamus there were no changes after dehydration. These results indicate that enkephalinergic fibers may play an important role in the regulation of the response to dehydration. It is known that morphine injections release vasopressin from the pars nervosa and that most of the opiate receptors from the pituitary are located in the pars nervosa. All these data may indicate the existence of an hypothalamic pars nervosa enkephalinergic pathway which may project onto vasopressin fibers and which may play a role in the regulation of vasopressin secretion.

Supported by DA 01785-02; J.R. is supported by INSERM (France).

1310 MIMICRY OF DOPAMINE STIMULATION OF HYPOTHALAMIC PATHWAYS BY C3a ANAPHYLATOXIN. Brian Seeman*, Nicole Schupf and Curtis A. Williams. Div. Nat. Sci. SUNY College at Purchase and Dept. Psychology, Manhattanville Coll., Purchase, New York 10577.

A soluble exogenous antigen reaction (EAR) system has been shown to alter eating and drinking patterns when administered directly to the perifornical hypothalamus (Williams and Schupf, Neurosci. Abstr. 1273, 1977). An immunological mechanism is suggested by the aggregation of PMN cells at the site the day following EAR treatment. The leucotaxic anaphylatoxin (AT) C5a might have been produced in a complement cascade initiated by the immune complex of EAR. The nonleucotaxic AT C3a would also have been produced, however, and both peptides are cytoactive via specific receptors, causing smooth muscle contraction, vasoconstriction and degranulation of mast cells. The purpose of this experiment was to determine the possible receptor specificity and/or releasing activity of AT on the one hand and, on the other, to eliminate the role of PMN cells as a participant factor in the phenomenon. Accordingly we tested the nonleucotaxic human AT C3a (supplied by Dr. A. Hugli) for its ability to modify pharmacologically induced appetitive behaviors in sated rats.

Rats were prepared with cannulae in the perifornical hypothalamus, a region where direct chemical stimulation in sated rats with Norepinephrine (NE) will elicit excessive eating and stimulation with carbamyl choline (CC) will elicit excessive drinking. Treatment with C3a 20 minutes before drug stimulation caused increased eating in NE-stimulated rats and increased drinking in CC-stimulated rats. C3a appears selectively to potentiate ongoing behaviors; no change in consummatory behavior is observed in saline control animals. The enhancement of drug-induced behaviors is confined to the relevant reinforcer: NE-stimulated rats increase food but not water intake, CC-stimulated rats increase water but not food intake. Similar effects on food and water intake in hungry and thirsty rats following injection of L-DOPA and dopamine (DA) into this region of the hypothalamus have been reported by Friedman et.al. (Life Sci, 1973), Coons and Starr (EPA, 1975) and Friedman (EPA, 1978). Since our data suggest that C3a may release endogenous DA or stimulate DA receptor sites, we confirmed the effect of DA in these animals.

The results with C3a, however, do not mimic the effect of the EAR. which potentiates NE-stimulated eating but not CC-stimulated drinking, and which induces eating in saline control rats. Thus while C3a activity is worthy of further study, it does not seem to be the mechanism of action of EAR. C5a will be investigated by similar procedures.

1311 OPIATES AND OPIOID PEPTIDES MAY EXCITE HIPPOCAMPAL (HPC) PYRAMIDAL NEURONS (HPN) BY INHIBITING ADJACENT INHIBITORY INTERNEURONS. G.R. Siggins, W. Zieglgänsberger*, E. French, N. Ling, and F. Bloom. Behav. Neurobiology Lab and Neuroendocrinology Lab, The Salk Institute, La Jolla, CA 92037.

Microiontophoretically applied opiates and endorphin peptides depress single unit activity in most areas of the CNS via specific opiate receptors (Zieglgänsberger and Fry, In: Developments in Opiate Research, M. Dekker, 1978). An apparent exception is the predominantly excitatory response of rat HPN's, which is often naloxone-sensitive (Hill et al, J. Physiol. 272:50P, 1977; Nicoll et al, PNAS 74:2584, 1977) and not due to an interaction with the muscarinic cholinergic input to HPN's (French et al, Proc. Soc. Neurosci. 3:291, 1977). To investigate the mechanism of this excitatory response, HPN's were tested with conventional five-barrel iontophoretic pipettes, or with multi-barrels glued to one or more recording barrels with tip separations of 30-60 µm. When opioid peptides (Met^5-enkephalin, D-ala^2-met^5-enkephalin, β-endorphin) were applied by iontophoresis or by a micro-pressure ejection method most neurons with burst-like firing patterns (probable HPN's) were excited while cells with non-bursting spontaneous activity (probable interneurons) were only inhibited. Employing tip separations up to 150 µm, a probable interneuron at one recording tip on occasion could be inhibited while a bursting neuron at the other tip could be reciprocally excited. Low iontophoretic currents of Mg++ (10-30nA), applied to depress local transmitter release, blocked opioid excitation and/or reversed them to inhibitions, without affecting ACH-evoked excitations. Bicuculline, known to block postsynaptic GABA inhibitory actions, often directly excited HPN's and antagonized opioid-induced excitations. Stimulation of contralateral HPC was used to evoke inhibition of ipsilateral HPN's via recurrent activation of basket cells, thought to release GABA onto HPN's. This basket cell-evoked inhibition was often specifically antagonized by iontophoretically applied enkephalin. These results suggest that the naloxone-sensitive excitatory response of HPC pyramidal neurons is due to inhibition of adjacent inhibitory basket cells, resulting in loss of tonic recurrent inhibition (excitation by disinhibition). Thus, while specific opiate receptors may generally mediate inhibitory responses in all brain regions, including hippocampus (Fry et al, J. Physiol. Mar 78, 21P), the ultimate action of opiates on a brain region depends upon the local circuitry. This excitatory mechanism in HPC could provide a basis for the limbic epileptiform activity produced by intracerebroventricular opiates (Henriksen et al, Proc. Soc. Neurosci. 3:292, 1977). *Permanent address: Max Planck Inst. f. Psychiatry, Munich. Supported by USPHS Grant DA 01785 and a Bosch Fellowship.

1312 NEUROPEPTIDES AND THE BLOOD-BRAIN BARRIER IN GOLDFISH. Dolores M. Smith*, Richard D. Olson, Abba J. Kastin, Gayle A. Olson*, David H. Coy*, and Gary F. Michell*. Dept. of Psychology, Univ. of New Orleans, VA Hospital, Tulane Med. School, New Orleans, LA 70122

The general activity level of a goldfish is easily monitored by placing it in an inch of water in a standard, 10 gallon aquarium placed on top of a Stoelting activity meter. After an initial burst of activity, goldfish become less active over time and usually generate approximately 300 activity counts during a standard 30 minute session.

Using this paradigm, goldfish were administered a 5 µl (80 µg/kg) ICV or IP injection of one of 21 test substances and tested for general activity. Results indicated that activity decreased reliably over time and that significant differences existed as a function of site of injection, with ICV injections producing greater decreases. Of the substances evaluated, no reliable differences existed between the diluent control and melanin, MIF-I, substance P, Met-enkephalin, D-Ala^2-enkephalin, DSIP, and D-Ala^3-DSIP. Significant decreases in activity were obtained using D-Ala^4-DSIP, α-MSH, neurotensin, and somatostatin. Further, all endorphins tested produced reliable decreases: α-endorphin, β-endorphin, γ-endorphin, Leu^5-β-endorphin, D-Thr^6-D-Leu^{17}-D-Lys^{19}-β-endorphin, D-Leu^{17}-D-Lys^{19}-β-endorphin, and D-Ala^2-β-endorphin. Finally, the greatest decreases in activity were produced by two new enkephalin analogs.

General results indicated that activity decreased approximately 3 minutes after a central injection and 6 minutes after a peripheral injection. The longer latency after IP injections may indicate the amount of time required for the substance, either in original or fragmented form, to reach and cross the blood-brain barrier. Accordingly, since both ICV and IP injections produced reliable decreases in activity, it would seem reasonable that the action of the substance is not a function of the site of injection. Some peptides or their metabolites appeared to readily cross the blood-brain barrier (e.g., γ-endorphin) as no difference existed between ICV and IP injections, with both producing reliable decreases from the control. In summary, peptides can exert behavioral effects after both central and peripheral administration in goldfish.

1313 COMPARISON OF PEPTIDE AND AMINO ACID DEPRESSION OF EXCITABILITY ON CULTURED SPINAL NEURONS. T.G. Smith*, J.L. Barker, D.L. Gruol, L.M. Huang* and J.H. Neale, LNP, NINCDS, NIH, Bethesda, Md. 20014 and Dept. Biology, Georgetown University, Washington, D.C.

Spinal neurons derived from mouse embryos were grown in tissue culture for 4 weeks or more. Intracellular recordings coupled with extracellular iontophoresis were used to study the effects of leucine-enkephalin (ENK) and the putative inhibitory amino acid transmitters γ-aminobutyric acid (GABA) and glycine on neuronal membrane properties. ENK depressed spike generation induced by constant-current, suprathreshold pulses by two mechanisms. ENK elevated threshold for generation of action potentials (1 to 20 mV) in a dose-dependent, reversible manner without change in membrane potential or input resistance. The increase in threshold was accompanied by a dose-dependent increase in the current required to attain threshold (rheobase). This effect was observed in the absence of any change in the current-voltage relations of the cell and clearly outlasted the iontophoretic application of the peptide. It did not desensitize and was reversed by naloxone at doses which did not alter membrane properties. A second form of depression of excitability by ENK occurred through a slight increase (∿ 10%) in membrane conductance without observable change in membrane potential. Rheobase was again increased in a dose-dependent, reversible manner but threshold for spike generation was unaltered. This effect did not desensitize and outlasted the peptide application.

GABA and glycine also depressed spike generation induced by constant-current, suprathreshold pulses by two mechanisms. Both amino acids greatly increased (50-1000%) membrane conductance, markedly altering the current-voltage relations of the cell. These effects decreased excitability by hyperpolarizing the membrane potential away from threshold and by shunting the cell. These actions only briefly outlasted the amino acid application. Threshold for spike generation was not altered, but the rheobase increased in a dose-dependent reversible manner. Both amino acid actions desensitized, GABA typically more than glycine.

Thus, there are similarities and differences between the depressant effects of the opiate peptide and the amino acid transmitters. The peptide can directly depress excitability either by elevating threshold or by slightly increasing membrane conductance. The amino acids depress excitability by intensively activating Cl^- conductance (J. Physiol., in press) which then dominates resting membrane properties. The results thus suggest that some of the pharmacologic effects of ENK fall outside operational definitions for neurotransmitter action.

1314 THYROTROPIN RELEASING HORMONE: AROUSING ACTION IN THE HIBERNATING GROUND SQUIRREL. T.L. Stanton, A. Winokur and A.L. Beckman. Depts. Physiol., Psychiat. and Pharm., Sch. Med., Univ. of Pa., Philadelphia, PA 19104.

Several laboratories have demonstrated that thyrotropin releasing hormone (TRH) antagonizes the CNS depression and hypothermia induced by a variety of neurotropic agents. These experiments were conducted to investigate the effects of intracerebral application of TRH in the adult golden-mantled ground squirrel (Citellus lateralis) during deep hibernation, a naturally induced depressed state that is entered through slow wave sleep and considered to be an extension of natural sleep.

Bilateral, 1 µl-injections of TRH (1.0 ng/µl to 1.0 µg/µl) were delivered through injection cannulae inserted via chronically implanted guide tubes into either the hippocampus (HPC) or preoptic/anterior area of the hypothalamus (PO/AH), two areas implicated in the neural control of hibernation. Metabolic rate and brain temperature (T_b) were recorded continuously during the experiments.

Intrahippocampal administration of TRH, in a dose as low as 1 ng/µl, was observed to be a potent agent for arousing animals from a state of deep hibernation. Full arousal, in which a dramatic rise in oxygen consumption accompanied an increase in T_b from approximately 5 to 37 C, was triggered with a dose-dependent decrease in latency associated with increasing dose of TRH. This effect was evident in the early period of the hibernation bout when the animals are most resistant to arousing stimuli. TRH, in the same dose range, was ineffective in triggering arousal from hibernation when microinjected into the PO/AH.

These data compliment previous pharmacological demonstrations of an arousing action of TRH during drug-induced depressed states and suggests that TRH may have physiological significance as a modulator of CNS excitability. Such a role may be particularly evident during the state of hibernation in which the level of inhibition in the CNS appears to be high. Increasing evidence indicates that the HPC may be the likely source of this inhibitory influence. In view of this, and considering the predominantly inhibitory action of TRH at the single unit level, it is possible that intrahippocampal microinjection of TRH may be triggering arousal from hibernation by disinhibiting the ongoing inhibitory control. (Supported by NSF grant # BNS 77-11352.)

1315 ENKEPHALIN CATABOLISM IN THE PRESENCE OF AN OPIATE RECEPTOR PREPARATION FROM RAT BRAIN. Susan O. Sullivan*, Huda Akil and Jack D. Barchas.

A washed membrane preparation from rat brain, containing opiate receptors, was incubated under conditions used for the opiate receptor binding assay (90 min., 0°C). At the completion of incubation, centrifugation was used to precipitate membranes, and the supernatant was analyzed for breakdown products of ^{3}H-Tyr-Met^5-enkephalin. Two thin layer chromatography systems and a Biobead-SM2 column were used. A peak of radioactivity that did not correspond to tyrosine, tyr-gly, tyr-gly-gly or met-enkephalin was observed. This peak may be tyr-gly-gly-phe suggesting that breakdown of met-enkephalin can occur at the phe-met bond. Previous reports in plasma and unwashed whole brain homogenates indicate that the initial cleavage in met-enkephalin breakdown is at the tyr-gly bond. It is suggested that a soluble enzyme is responsible for the tyr-gly cleavage. Upon further washing of the membrane fraction, a membrane bound enzyme capable of phe-met cleavage is exposed. Further identification of the breakdown product and the subcellular localization of this enzyme is under investigation.

1316 EXTRA-HYPOPHYSIAL OXYTOCIN-CONTAINING PATHWAYS IN THE BRAIN AND SPINAL CORD OF THE RAT AND MONKEY. L.W. Swanson. Dept. Anat. and Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

The distribution of fibers cross-reacting with antisera to oxytocin and antidiuretic hormone (ADH), and to bovine neurophysin I, was studied in the rat and monkey CNS with the indirect immunofluorescence method. All three antisera labeled the hypothalamo-neurohypophysial tract. A number of other fiber systems, which appeared to arise within the magnocellular neurosecretory system, were also stained with anti-oxytocin and anti-neurophysin I. In the rat a small group of stained fibers could be traced through ventral parts of the reticular formation to sacral levels of the spinal cord. Branches of this pathway appeared to innervate the locus coeruleus and parabrachial nucleus, the vagal complex, the spinal nucleus of the trigeminal nerve, and the marginal zone and central gray of the spinal cord. The intermediolateral column was particularly densely labeled at levels T1-T2, T11-T12, and L2-L3. Of particular interest, other labeled fibers were found to pass through medial parts of the septal region and subfornical organ to the interior of the choroid plexus of the third ventricle, and still other varicose fibers were found running parallel, and immediately adjacent to some of the major penetrating arteries supplying the paraventricular nucleus. A less complete analysis in the monkey has thus far shown the presence of neurophysin I and oxytocin-stained fibers in the vagal complex and spinal cord (including the intermediolateral column), as well as stained fibers closely associated with hypothalamic blood vessels and ependymal cells of the third ventricle. In the monkey, fewer oxytocin-stained fibers were found in the dorsal motor nucleus of the vagus and the nucleus of the solitary tract, and more were found in the intermediolateral column, than in comparable regions of the rat. The evidence suggests that the paraventricular nucleus projects to the pituitary and to a number of regions within the CNS itself, including cell groups associated with both divisions of the autonomic nervous system. Furthermore, at least some of the oxytocin-stained fibers end in relation to capillaries (median eminence and pituitary), arterioles (hypothalamus), ependymal cells (third ventricle) and the choroid plexus (third ventricle). (Supported in part by PHS Grant NS13267).

1317 EFFECT OF NEUROPEPTIDES ON ACUTE STRESS-INDUCED HORMONAL CHANGES. Y. TACHE* and R. COLLU. Dept. Péd., Hosp. Ste-Justine, U. de Montréal, Montréal, P.Q.

We have recently reported that various neuropeptides distributed throughout the brain and the gastrointestinal track could affect hypothermia and the susceptibility to gastric ulcerations induced by cold+restraint stress (Taché, Y. and Collu, R., 60th Ann. Meet. End. Soc., 1978). In the present study, we further investigated the interaction between neuropeptides and stress-induced hormonal changes.

Adult male rats were injected intraventricularly-through a chronic cannula implanted two days previously into a lateral brain ventricle- with saline or oligopeptides. Immediately after the injection rats were exposed for 1 hr to various stressors (cold, restraint, fasting + restraint at 4°C) and killed at the end of the stress procedure. Bombesin (5-0.1μg) and neurotensin (5μg) further enhanced the hyperglycemic response to stress whereas somatostatin and thyrotropin releasing hormone (TRH) decreased plasma glucose levels in stressed rats; β-endorphin (5-1μg) and substance P had no effect. Stress-induced inhibition of growth hormone (GH) secretion was not affected by any of the neuropeptides tested, whereas prolactin (PRL)-releasing effect of the various stressors was antagonized by bombesin (5-0.1μg), neurotensin (5μg), TRH (5μg) and to a lesser degree by substance P (5μg). β-Endorphin (5-1μg) and somatostatin (5μg) did not modify plasma PRL levels in stressed rats. Adrenalectomy prevented the enhancing effect of bombesin on stress-induced hyperglycemia but did not modify the inhibitory effect on PRL release. Bombesin was 10^3 more potent when given intraventricularly than when injected intravenously in decreasing plasma PRL levels in stressed rats.

These results show that various neuropeptides affect the hormonal response to stress. In particular, bombesin appears a very active neuropeptide capable of interfering centrally with stress manifestations.

1318 SUBSTANCE P-CONTAINING PROCESSES VISUALIZED BY A MODIFIED PAP PROCEDURE IN THE RAT SPINAL CORD. Linda L. Vacca, Susan J. Abrahams*, and N. Eric Naftchi*. Dept. of Path. & Anat., Med. Coll GA, Augusta, GA 30902 and IRM, NYU Med. Ctr., New York, NY 10016

Using a modified peroxidase anti-peroxidase (PAP procedure (J. Histochem. Cytochem. 26: 226, 1978), the peptide substance P (SP) has been localized within beaded processes and punctate bodies in the ventral, lateral, and dorsal horns of the rat spinal cord. The modified procedure, which employs a double bridge and diaminobenzidene in solution at low pH, increases the sensitivity of the PAP method and enables us to see staining patterns not previously reported. These patterns include staining around the motoneurons, in the region of the central canal including the dorsal and ventral gray commissures, in association with myelinated fibers, and in occasional cell bodies in the dorsal horn.

At most cord levels, a sparce number of beaded processes traverse the ventrolateral extent of the ventral horn. At high cord levels, SP-containing processes occur within the lateral horn. Since the processes in the lateral horn cannot be traced for long distances, their relationship with the ventrolateral and commissural processes remains obscure.

In some regions of the spinal cord, SP is closely associated with myelinated fibers. In the dorsal part of the lateral funiculus, punctate bodies containing SP lie within the white matter. Many are associated with fasciculus dorsolateralis, but some occur more laterally. Punctate bodies also appear within the anterior white commissure and ventral to it along the anterior fissure. The punctate bodies are embedded within the matrix of myelinated fibers in these regions where certain spinal cord pathways occur.

In the dorsal horn, as previously reported, numerous SP-containing punctate bodies can be seen in the substantia gelatinosa (SG) region. Rows of punctate bodies stud the large bundles of myelinated dorsal root collaterals which sweep around the dorsal horn medially and penetrate the SG. Fewer punctate bodies follow linear patterns along the myelinated collateral fiber bundles which course through the SG radially. As these fiber bundles penetrate deeper into the dorsal horn and are distributed to various cell groups within the grey matter, the associated SP-containing bodies become less abundant. The close association between SP and these myelinated collaterals from the dorsal root may indicate a modulatory role for the peptide on other primary afferent fibers.

Occasional cell bodies appear stained for SP. They are bipolar and extend their processes along the dorsolateral part of the dorsal horn. Their relationship to immunoreactive fibers is at present unknown. (Supported by Medical College of Georgia Biomedical Research Grant 10-16-04-3611-23).

1319 DETECTION OF TWO NOVEL ENDORPHIN LIKE PEPTIDES IN RAT AND BOVINE STRIATUM. H.-Y. T. Yang*, W. Fratta*, J. S. Hong*, A. M. Di Giulio* and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

Constant rate infusion with ^{3}H tyrosine or glycine (150 μCi) into the cerebral ventricles for thirty minutes causes little or no incorporation of radioactivity into striatal [met5]-enkephalin (MET). The formation of radioactive MET increases during the following two hrs. These observations suggested that the in vivo synthesis of MET in striatum may involve the formation of one or more precursors. Since we failed to detect β-endorphin in rat and bovine striatum we searched for possible precursors of striatal MET. For this purpose we have developed a MET antibody which possesses some affinity for endorphins with molecular weight larger than MET but smaller than β-endorphin. By this antibody, two endorphin-like peptides with molecular weight larger than MET were detected in the eluate of a Biogel P-2 column loaded with the extract of a bovine caudate. The retention volume of these two peptides is smaller than that of MET. The study on the immunoreactivities of these two endorphin like peptides with antibody directed toward β-endorphin and β-lipotropin revealed that the novel endorphin are neither β-endorphin nor β-lipotropin. Moreover we have found that after trypsin hydrolysis they can generate a peptide similar to that generated by trypsin hydrolysis of α-endorphin. With Sephadex G-75 column and thin layer chromatography techniques, we have excluded that the novel peptides are α-endorphins. In order to decide whether these two endorphin-like peptides are formed post mortem during the handling of the bovine brain, we have attempted to identify these two peptides in the brain of rats killed by a focused microwave irradiation. In rat brain we detected at least 2 endorphin-like peptides, which in the Biogel P-2 column chromatography behave similarly to the two endorphin-like peptides of bovine brain. These results suggest that in the striatum of rat or bovine brain, there are two endorphin-like peptides with molecular weight larger than MET which are structurally different from α- and β-endorphins and β-lipotropin. Whether these two peptides function as precursors of MET in striatum will be discussed.

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1320 PROGRESSIVE INCREASE OF SENSITIVITY TO SEROTONIN IN CHRONIC SPINAL RATS. Hugues Barbeau*, Paul Bédard* and Michel Filion. Laboratoires de Neurobiologie, Université Laval, Québec, Canada, G1K 7P4.

A few hours after spinal transsection in rats, 5-hydroxytryptophane (5-HTP), the precursor of serotonin, increases spinal reflexes elicited below the transsection (Anderson et al. (1966) J. Pharmacol. Exp. Therap. 153: 352). The present study provides quantitative data showing that the effect of 5-HTP on the activity of neurons below a complete spinal transsection varies over a period of days. Spontaneous EMG activity of tigh flexor and extensor muscles was recorded chronically and quantified in spinal rats free to move their hindlimbs. A few hours up to about 4 days after the transsection the spontaneous EMG activity is low and is not altered or barely increased by a single standard dose of 5-HTP (100 mg/kg, i.p.). After the 4th day the same dose of 5-HTP clearly increases the EMG activity and induces obvious movements of the paralyzed limbs for about 6 hours. There is a parallel increase in the activity of the autonomic nervous system (micturition, defecation, erection, ejeculation). This effect of 5-HTP is manifest within the first minute after the injection and reaches a maximum within 10 minutes. The amplitude of the effect increases from 3 to 5 times the preinjection level of EMG activity, around day 5, to over 10 times the preinjection level, around day 20. It remains at this maximum amplitude up to day 30. The effect is blocked rapidly by cyproheptadine (10 mg/kg) and various antagonists of serotonin. It is mimicked by LSD (0.5 mg/kg) and several other compounds thought to act as agonists of serotonin. Methysergide (5 mg/kg) shows mixed properties. The changes in the intensity of the response to 5-HTP can be interpreted in terms of denervation supersensitivity of serotonin receptors. The sensitivity to serotonin is thus maximum 3 weeks after spinal transsection and the preparation provides a good model to test agonists and antagonists of serotonin.
(Supported by MRC of Canada)

1321 HIGH AFFINITY BINDING SITES FOR N,N-DIMETHYLTRYPTAMINE ON PURIFIED RAT BRAIN SYNAPTOSOMAL MEMBRANES. L.J. Bearden, L. Burrow* and S.T. Christian. Neurosciences Program and Department of Psychiatry, Medical Center, University of Alabama in Birmingham, Birmingham, Alabama, 35294.

The possibility that the endogenous hallucinogen N,N-dimethyltryptamine (DMT) serves as a neuroregulatory agent is suggested by several observations. Both DMT and an immediate precursor, tryptamine, have been found in brain tissue from humans and animals. Appropriate biosynthetic enzymes and metabolites of DMT have also been found in neural tissue. In addition, DMT has been detected in synaptic vesicle fractions isolated from rat brain. Thus, the in vivo biosynthetic reactions and a possible endogenous storage site for DMT have been demonstrated. However, the precise role which DMT plays in the central nervous system has not been elucidated.

Experiments conducted in our laboratory have shown that $5 \cdot 10^{-10}$M DMT stimulates the production of cAMP in synaptosomal membrane preparations to levels greater than twice those observed in control samples. In the same experiments, lysergic acid diethylamide (LSD) was found to stimulate cAMP production to similar levels. But when 5-hydroxytryptamine was present with either DMT or LSD, similarly additive effects on membrane cAMP production were observed. These findings are supported by other experiments which indicate that DMT does not inhibit the binding of 5-hydroxytryptamine ($5 \cdot 10^{-10}$M) to synaptosomal membranes, whereas DMT ($1 \cdot 10^{-5}$M) displaces LSD which is bound to synaptosomal membranes.

Studies of the binding of DMT to synaptosomal membranes at concentrations down to 10^{-10}M have now been conducted by means of equilibrium dialysis. These experiments have utilized ^{3}H-DMT and synaptosomal membranes prepared in buffered sucrose media by conventional differential centrifugation techniques. Results from these studies have indicated the presence of high affinity, synaptosomal membrane binding sites for ^{3}H-DMT which are sensitive to low concentrations of LSD. (This work was supported in part by a grant from NIDA #2R01 DA01235-03.)

1322 STRESS INDUCED CHANGES IN WHOLE BRAIN INDOLEALKYLAMINE LEVELS IN THE RAT: USING GAS LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY, John M. Beaton and Samuel T. Christian. Neurosciences Program, and Department of Pharmacology and Psychiatry, University Station, Birmingham, Alabama 35294.

Many theories of schizophrenia are predicated on the belief that the illness is caused by a chemical substance which, if isolated and administered to normals, would mimic the clinical aspects of schizophrenia in these normals. The indolealkylamines, (N,N-dimethyltryptamine-DMT; bufotenin-BUF and O-methyl-bufotenin -OMB), have been implicated as possible compounds. Environmental stress is thought to be an important precipitating factor in schizophrenia. Therefore, the aim of the studies to be presented here was to expose rats to various stresses and to examine the effects of these stresses on the levels of DMT and other indolealkylamines in the brains of the rats. Groups of six rats were exposed, individually, to one of the following conditions: no stress, electric shock, electric shock plus restraint, cold, swimming to exhaustion, or swimming to exhaustion in cold water. Purified synaptic vesicles were prepared from the groups of six rats. The vesicles were then extracted with methylene chloride and the extract derivatized with heptafluorobutyryl imidazole. Samples of the derivatized extracts were then analyzed on a GLC-MS. The electron impact mass spectrum of both DMT and OMB (as heptafluorobutyryl derivatives - HFB) exhibits m/e 58.2 as the base peak due to alpha-cleavage of the tertiary amino group. Thus selected ion monitoring of this mass fragment provides simultaneous determination of both compounds in a single run. Aliquots of 1 to 3 ul were chromatographed over a six-foot glass column, 2 mm i.d., of 3% SP-2250 on 160°C for 4 minutes followed by a programmed temperature increase of 10°/min. to 225°C. Helium was used as the carrier gas at a flow rate of 40 ml/min. Under these conditions, DMT-HFB eluted at 4.7 minutes and OMB-HFB at 7.3 minutes. Instrumentation used was a Hewlett-Packard 5985 A GC/MS data system. Identification of DMT and OMB was based upon retention time and simultaneous monitoring of mass fragments other than m/e 58.2 which are diagnostic for these compounds. Absolute sensitivity of this method is 2 pg DMT-HFB and 3 pg OMB-HFB per injection. Quantitation was achieved by an isotope dilution method using alpha, alpha, beta, beta-tetradeuterated DMT and OMB as internal standards. The results of this study indicated that both DMT and O-methyl-bufotenin (OMB) were present in normal rat brain. The control levels of one or both of these compounds was increased to varying degrees by the different stresses. Electric shock plus restraint increased the levels of DMT by the greatest amount.

This work was supported in part by N.I.M.H. Grant #R01 MH29691-01.

1323 EFFECTS OF CHOLINOMIMETICS ON PENTOBARBITAL ANESTHESIA IN MICE. Srinath N. Bellur, M.D., Walter Wojcik, B.S. and Miodrag Radulovački, M.D., Ph.D. Dept. Pharmacology, U of I Med. Center, Chicago, IL 60612.

Previous reports of physostigmine pre-treatment on pentobarbital anesthesia in mice have varied from no effect to prolongation or shortening of duration of anesthesia. Latencies and duration of anesthesia were calculated in mice by measuring the time for loss and resumption of righting reflex after pentobarbital administration (60 mg/kg i.p.). Physostigmine (0.25 mg/kg), neostigmine (0.25 mg/kg), choline chloride (120 mg/kg) and saline control (0.2 ml) were given i.p. 600 sec prior to pentobarbital administration (experiment I) and 1800 sec after the loss of righting reflex (experiment II). Control latency was 147 ± 21 sec (mean ± SD) and duration was 4623 ± 852 sec. There were no significant changes in latency or duration of anesthesia in experiments with choline or neostigmine. Physostigmine significantly shortened latency to 109 ± 16 sec (26%) without changing the duration of anesthesia when given prior to pentobarbital (experiment I). However, it significantly shortened the duration to 3042 ± 745 sec when given during the anesthesia (35%) (experiment II). The above data indicate that a peripheral or central effect of acetylcholine does not seem to be involved in physostigmine action. It is more likely that an enhanced blood-brain barrier permeability to barbiturates may explain the obtained findings (Greig and Mayberry, 1950). These results are of interest in suggesting a role for physostigmine therapy and for its proper timing in barbiturate intoxication.

1324 HIGH AFFINITY BINDING SITE FOR [^{3}H]-L-GLUTAMIC ACID ON BRAIN MEMBRANES. K. Biziere* and J.T. Coyle (SPON: M.E. Molliver). Dept. Pharmacol., Johns Hopkins Univ. Sch. Med. Balto., MD 21205.

L-glutamic acid (L-Glu) has excitatory effects when iontophoresed onto most neurons in the mammalian CNS. There is growing evidence that L-Glu may be the excitatory neurotransmitter for several neuronal pathways including the cortico-striatal projection. With [^{3}H]-L-Glu of high specific radioactivity (50 Ci/mmol), we have examined the binding of the amino acid to brain membranes in an attempt to characterize physiologically relevant receptor binding sites.

Rat brain membranes were prepared by sonication of fresh, frozen brain in Tris-citrate buffer (0.05 M, pH 7.1 at 2° C); the membranes were isolated by centrifugation, washed extensively, and pre-incubated for 20 min at 25° C. Typically, 300 µg membrane protein was incubated for 20 min in 2 ml of Tris-citrate at 2° C with 3 nM [^{3}H]-L-Glu in the absence or presence of 100 µM L-Glu; membranes were then isolated by centrifugation. Specific binding is the difference between total binding and non-specific binding in the presence of 100 µM L-Glu. The binding was rapid and reversible with equilibrium reached by 10 min incubation. The amount of [^{3}H]-Glu bound was linear with membrane protein up to 500 µg. A saturation isotherm for forebrain membranes revealed an apparent K_D of 11 ± 1 nM and a B_{max} of 110 ± 15 fmol/mg protein; however, anomolous kinetics occurred at concentrations of [^{3}H]-L-Glu in excess of 10^{-8} M. The specific binding of [^{3}H]-L-Glu varied in brain regions (fmol/mg protein): parietal cortex, 43 ± 4 ≥ frontal; 38 ± 2 > hippocampus, 29 ± 2 ≥ striatum, 28 ± 2 > cerebellum, 13 ± 2 ≥ pons-medulla, 10 ± 2. The IC_{50} for several excitatory amino acids and analogues were examined: L-Glu, 0.003 µM; D,L-homocysteic acid, 0.7 M; D-Glu, 1 µM, D-aspartic acid, 0.9 µM; L-aspartic acid, 1 µM. Notably, kainate, dihydrokainate, quisqualate, ibotenate did not displace [^{3}H]-L-Glu at 10^{-5} µM; GABA, glycine, taurine, phenytoin, phenobarbital, carbachol, norepinephrine and diazepam were also ineffective at 10 µM.

Decortication produced a 45 ± 5% reduction in [^{3}H]-L-Glu synaptosomal uptake and a 55 ± 4% reduction in endogenous Glu in the striatum but did not significantly alter the specific binding of [^{3}H]-L-Glu; thus, it is unlikely that [^{3}H]-L-Glu is labelling a presynaptic transport site for Glu. In contrast, a striatal lesion with kainic acid, which selectively ablates striatal intrinsic neurons, produced a 51 ± 8% reduction in the specific binding of [^{3}H]-L-Glu. These studies suggest that [^{3}H]-L-Glu is binding to an high affinity site primarily localized on neurons that may be a receptor mediating the neurotransmitter action of Glu. (Supported by USPHS Grants NS 13584, RSDA KO2-MH 00125).

1325 **INCREMENTAL DOSES OF MORPHINE PROVIDE METHOD TO IDENTIFY DIFFERENT PATTERNS OF RESPONSES RECORDED FROM EIGHT BRAIN NUCLEI.** M. Brown*, B.M. Rigor*, and N. Dafny (SPON: T.F. Burks). The University of Texas Medical School at Houston, Houston, Texas 77025.

From preliminary experiments using single doses of morphine (10 mg/kg ip), it has become evident that neurons in the central gray (CG), mesencephalic reticular formation (RF), parafasciculus thalami (PF), caudate nucleus (CN), anterior hypothalamus (AH), ventromedial hypothalamus (VMH), lateral septum (Spt), and dorsal hippocampus (Hipp) exhibit either increased or decreased activity in response to morphine treatment. In the present study, incremental doses of morphine were given in an attempt to identify differences between these nuclei. Experiments were conducted in unanesthetized, unrestrained, morphine-naive rats. Permanent electrodes (60µm in diameter) were implanted stereotaxically one week before the experiment. The amplified signals were then put through a waveform discriminator, and the digital output was interfaced parallel to an integrator connected to a polygraph, to plot online the frequency firing rate in spikes/sec. Recordings were taken for 30 min for control periods and 30 min for each treatment. Each animal was given an ip injection of saline followed by 5 incremental doses of morphine sulfate (0.5, 1.0, 5.0, 10.0 and 30.0 mg/kg) at 30 min intervals and by 1.0 mg/kg naloxone 30 min after the last morphine treatment. A total of 180 units were recorded. In general, as the dose of morphine increased, more units responded. Seven patterns of responses were observed. Each structure exhibited a pattern of response different from that of the other 7 nuclei. In conclusion, the 5 incremental doses of morphine and the dose of naloxone provided data demonstrating that each nucleus in the brain responded to morphine in its own pattern. In addition, it was shown that the dose of 10 mg/kg was the most effective dose for all structures. (Supported by USPHS DA 00803)

1326 BEHAVIORAL ASSESSMENT OF ANTICHOLINERGIC PROPERTIES OF METHADONE, MORPHINE AND ENKEPHALIN. William T. Chance and John A Rosecrans. Dept. Pharmacol., Med. Col. Va., Richmond, Va. 23298.

We have previously reported (Nature 270: 167, 1977) that intrahypothalamic injection of 4.0 nmol (1 µl) of morphine effectively antagonized drinking elicited by either a subsequent (5 min) injection of an equimolar dose of carbachol or 24-hr food and water deprivation. The lack of effect of morphine on food intake indicated that this inhibition was not due to non-specific behavioral depression. Furthermore, we demonstrated that this antagonism of drinking was reversed by pretreatment with an equimolar dose of naloxone. In the present experiment, we have extended these data to other opiate and opioid compounds. Across two experiments, cannulae (24 ga) were implanted into the perifornical hypothalamus of 61 adult, male, Sprague-Dawley rats. These rats were maintained ad lib. on ground rat chow and water. Injection (1 µl) of carbachol (4.0 nmol) was observed to reliably elicit both eating and drinking during the subsequent 1-hr period. Pretreatment (5 min) with l-morphine (4.0 nmol) reduced drinking by 67%, while not affecting eating. Pretreatment with d-morphine (4.0 nmol) had no effect on either behavior. Injection of l-morphine (4.0 nmol) also selectively antagonized drinking elicited by 24-hr (86% reduction) and 48-hr (84% reduction) food and water deprivation, while d-morphine was again without effect. Pretreatment with either 4.0 or 8.0 nmol of d- or l-methadone had no effect on drinking or eating elicited by the subsequent (5 min) injection of carbachol (4.0 nmol), while 8.0 nmol of l-methadone selectively reduced drinking (47% reduction) elicited by 48-hr food and water deprivation. Pretreatment (5 min) with the enzyme-resistant enkephalin analogue, d-ala^2-met^5-enkephalin (4.0 nmol), effectively antagonized drinking elicited by an equimolar dose of carbachol (92% reduction) or 48-hr food and water deprivation (91% reduction), while not affecting food intake. In a subsequent experiment, the reduction of carbachol-elicited drinking by pretreatment with d-ala^2-met^5-enkephalin (66% reduction) was completely reversed by pretreatment (5 min) with 4.0 nmol of the opiate antagonist, naltrexone.

These results extend out initial findings concerning inhibition of the muscarinic-cholinergic drinking response by opiate compounds, suggesting that these effects are specific cns narcotic effects. In addition, differences in anti-cholinergic effects are indicated between the compounds tested in this report, with morphine and enkephalin being much more effective antagonists of exogenously-stimulated drinking (carbachol-elicited) than methadone. These differences may represent a selective action of morphine and enkephalin, acting not only to inhibit the release of acetylcholine (as assessed by deprivation-induced drinking) but also serving to block the post synaptic muscarinic receptor. (Supported by: NIH DA-00296-04 and NIDR DE-00116)

1327 EXCITATORY EFFECTS OF COCAINE ON THE LIMBIC SYSTEM. Jeremiah P. Collins, Henry Lesse and James Gaffney*. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, CA 90024.

The effects of cocaine on epileptiform afterdischarge (AD) activity within the limbic system of cats were studied. Subjects were chronically prepared with bilateral stimulating and recording electrodes in amygdala and hippocampus. Unilateral focal stimulation at low frequency (3 Hz) and at threshold intensity was employed in order to detect initiation of the AD during stimulation. Electrophysiological responses were monitored, and stimulation was discontinued when a focal AD was elicited. The latency for spread of the epileptiform activity to other brain areas was then determined. To separate drug effects from those of repeated brain stimulation, all tests were conducted at 48-hr intervals following alternating administrations of saline or cocaine. Three subconvulsant doses were tested in a counterbalanced order.

Results indicated that cocaine significantly increased the speed at which AD activity spread to amygdala and to hippocampus, both ipsilaterally and contralaterally. This was true whether amygdala or hippocampus was being stimulated, and the effects were dose-related. The latency of propagation of seizure activity to amygdala following hippocampal stimulation was then compared to the latency of propagation to hippocampus following amygdala stimulation. This analysis indicated a greater effect of cocaine in accelerating propagation to the hippocampus. There were changes in the pattern in which limbic seizure activity spread as well. After stimulating amygdala, the AD normally was propagated to contralateral amygdala prior to contralateral hippocampus. Following cocaine administration, this order was altered. Cocaine thus facilitates the spread of epileptiform activity to both hippocampus and amygdala but with a greater effect on hippocampus. This finding of an excitatory effect of cocaine on certain limbic structures is consistent with our previous studies showing that cocaine lowers the current threshold necessary to induce ADs in amygdala and hippocampus. These data suggest that in individuals with temporal lobe dysfunction, subconvulsant doses of cocaine may exacerbate focal epileptiform activity. (Supported by NIDA Grant DA-1351.)

1328 PRODUCTION IN RATS OF RESPONSES CHARACTERISTIC OF NEUROLEPTIC DRUGS IN THE ABSENCE OF DOPAMINE (DA) RECEPTOR BLOCKADE BY ETHANOLAMINE-O-SULFATE, A GABA-TRANSAMINASE INHIBITOR. B.R. Cooper*, K. Viik*, H.L. White*, R.M. Ferris*. (SPON: J.L. Howard). Dept. of Pharmacology, Wellcome Research Labs., Research Triangle Park, North Carolina 27709.

Intracisternal injections of ethanolamine-O-sulfate (EOS), an irreversible selective inhibitor of GABA transaminase (GABA-T: Fowler and John, Biochem. J., 130:569-573, 1972) were used to determine the effects of elevated GABA levels in brain on certain behavioral and pharmacologically-induced responses in rats which are frequently used as screens for antipsychotic drugs. EOS (200 µg - 800 µg/brain) resulted in a dose dependent elevation of GABA (2 fold-5 fold) and a corresponding decrease in GABA-T (52% to 85% inhibition) 24 hours after injection. At this time interval EOS also produced a dose-related antagonism of stereotyped behavior induced by apomorphine or d-amphetamine, and an antagonism of apomorphine-induced aggression. Hypoactivity and catalepsy also accompanied the higher doses of EOS. Study of the relationships of EOS to the function of the dopamine systems in brain revealed that EOS (800 µg/brain) did not alter DA levels, and that EOS (10^{-4}molar) did not compete with H^3-spiroperidol for binding to the presumed "dopamine-receptor" in vitro. Unlike chlorpromazine which blocks DA receptors, 24 hrs pretreatment with EOS (800 µg/brain) did not effect the accumulation of H^3DA in brain after i.v. injection of H^3 tyrosine, or the accumulation of DOPA after decarboxylase inhibition. Even though 800 µg EOS blocks completely the behavioral effects of 5 mg/kg apomorphine, there was no change in the biochemical effects of apomorphine in producing a reduction of H^3DA formed after H^3Tyr injection, suggesting presynaptic DA receptors were functional. Results indicate that EOS shares several behavioral but not biochemical effects with neuroleptic drugs. The mechanism for these unique effects is not presently clear, but the correlation of elevated GABA levels with the actions of EOS may implicate GABA.

1329 ETHANOL: ELECTROPHYIOLOGICAL EVIDENCE OF TOLERANCE REMAINS AFTER ISOLATION OF THE HIPPOCAMPUS IN VITRO. William A. Corrigall and P.L. Carlen, Dept. Medicine (Neurology), Addiction Research Foundation, Toronto, Ontario, Canada, M5S 2S1

While changes due to ethanol tolerance have been observed in the EEG, there have been no electrophysiological studies at a more reduced level in the vertebrate central nervous system. We have therefore examined the effect of acute ethanol exposures on the field potential elicited in the CA1 pyramidal neurons of the in vitro hippocampal slice preparation from animals administered ethanol chronically versus controls.

Male Sprague-Dawley rats were allowed ad libitum but measured access to a nutritionally balanced liquid diet containing 36% of its calories as ethanol; paired control animals received isocaloric amounts of the same diet except with the ethanol calories replaced by maltose-dextrins. Both groups of animals increased their caloric intake and weight over the 5 month duration of the experiment. At the time of electrophysiological experiments there was no significant difference in the weights of the groups. The average dose of ethanol consumed at this time was 14 gm/kg/day, corresponding to blood alcohol levels between 130-260 mg%.

Field potentials were recorded in the CA1 region of the slice with stimulation in the stratum radiatum. In brain slices from control animals, acutely applied ethanol at increasing concentrations up to 600 mM resulted in decrement and, at 600 mM, in block of the field potential. The high concentrations of ethanol necessary to produce an acute effect in this brain slice preparation are typical of studies of ethanol in vitro. When ethanol was bath-applied to slices from animals which had been administered ethanol chronically, significantly less decrement was observed for a given concentration of the drug and complete block of the response did not occur until considerably higher concentration. As yet we are uncertain as to the specific cellular locale of this tolerance; however, we have noted that the component of the field potential which signals the presynaptic fiber volley often decreased in parallel with the population spike. The tolerance observed in this tissue may be due therefore to changes in the presynaptic cells/fibers rather than in transmitter release and/or postsynaptic responsiveness. Irrespective of the cellular site, it is apparent that ethanol tolerance is a local phenomenon within the central nervous system, demonstrable in a single, identifiable neuronal pathway. That is not to say that ethanol tolerance in our animals exists within the hippocampus only, but rather that evidence of tolerance is not contingent on interaction with the remainder of the brain.

(supported by Medical Research Council of Canada grant MA-6019)

1330 EFFECTS OF BARBITURATES ON POSTSYNAPTIC INHIBITORY RESPONSES IN APLYSIA. Ila L. Cote* and W. A. Wilson (SPON: J.Parmentier). Dept. Physiol., School. Med., U.N.M., Albuquerque, N. M., Dept. Pharmacol., Duke Univ. Med. Ctr., Durham, N. C. 27710.

The effects of barbiturates on responses produced by iontophoretic application of acetylcholine (ACh) and γ-aminobutyric acid (GABA) were studied in Aplysia californica using the voltage clamp technique. Dose-response data was obtained for barbiturate concentrations ranging from 10 uM to 3 mM. Previous studies of invertebrate systems have shown that barbiturates selectively depress excitation while sparing or enhancing inhibition. In contrast, we have found that both phenobarbital and pentobarbital depress chloride-dependent inhibitory responses to both ACh and GABA. This depression occurs at approximately the same concentrations at which excitatory responses are attenuated. At these concentrations, potassium-dependent responses were minimally affected. In no case was enhancement of inhibitory responses seen, even at concentrations considerably below those which depressed the responses. These results, taken with previous studies, demonstrate that barbiturates can have varying effects on inhibitory processes.

1331 A STUDY OF THE PROCONVULSANT EFFECTS OF MEPERIDINE AND NORMEPERIDINE IN THE RAT FLUROTHYL TEST. A. Cowan, M.W. Adler, R.F. Kaiko*, C.E. Inturrisi* and M.M. Reidenberg*. Temple Univ. Sch. of Med., Philadelphia, PA 19140, Memorial Sloan-Kettering Cancer Center, New York, NY 10021 & Cornell Univ. Med. Coll., New York, NY 10021.

The proconvulsant effects of meperidine (M) and normeperidine (NM), and their interaction with naloxone (N), have been examined in rats exposed to the convulsant inhalant, flurothyl.

In the first experiment, dose-response curves were obtained for both compounds using male, albino S.D. rats (300-350 g). Different groups of 6-12 animals each received a s.c. injection of saline (S), M, or NM at 0.25,0.50,1,2,3, or 18 hr prior to challenge with flurothyl. The proconvulsant effects of each dose of NM tested (6.25,12.5, and 25 mg/kg) were always greater than the corresponding proconvulsant effects of the same 3 doses of M at 0.25, 0.5,1,2, or 3 hr respectively. The maximum decrease in seizure threshold (S.T.) with the 25 mg/kg dose of M (15-17% relative to controls) occurred between 0.5 and 1 hr whereas peak effects with the 25 mg/kg dose of NM (30-32%) occurred between 1 and 2 hr. All S.T.'s had returned to control levels by 18 hr.

In the second experiment, the effect of N (10 mg/kg, s.c.) on the proconvulsant properties of M and NM was examined (1 mg/kg of N had no influence on M or NM). Different groups of 8-12 rats were treated as indicated in the Table. Each rat was decapitated immediately after convulsing and plasma samples were obtained, frozen, and subsequently analyzed by GLC for levels of M and NM.

Treatment (at 0 hr)	S.T. (at 0.5 hr) mean ± s.e.(sec)	Plasma: mean ± s.e.(ng/ml) M	NM
S-S	387 ± 12		
N-S	387 ± 13		
S-M (12.5 mg/kg)	380 ± 12	65 ± 10	< 9
N-M (12.5 mg/kg)	341 ± 13	67 ± 14	<11
S-M (25.0 mg/kg)	369 ± 10	116 ± 24	125 ± 30
N-M (25.0 mg/kg)	284 ± 18	123 ± 23	100 ± 18
S-NM (12.5 mg/kg)	326 ± 10		1054 ± 51
N-NM (12.5 mg/kg)	303 ± 10		1253 ± 79
S-NM (25.0 mg/kg)	309 ± 9		2687 ± 385
N-NM (25.0 mg/kg)	284 ± 10		2702 ± 176

Three conclusions may be drawn from the 2 experiments: a) M, and particularly NM, can lower S.T. in the rat flurothyl test; b) N can potentiate the proconvulsant effects of M and NM, and c) the lowered S.T.'s are not a consequence of N altering plasma levels of N or NM.

(Supported by grants DA 00376, 01707, and 01457 from NIDA).

1332 ELECTROPHYSIOLOGICAL EVIDENCE FOR TOLERANCE TO MORPHINE: UNIT ACTIVITY RECORDINGS FROM HYPOTHALAMUS AND PARAFASCICULUS NUCLEUS IN FREELY MOVING RATS. N. Dafny and B.M. Rigor**. The University of Texas Medical School at Houston, Houston, Texas 77025.

In previous experiments using 5 different doses of morphine, it was found that 10 mg/kg morphine was the most effective dose to induce changes in spontaneous multiunit activity in naive rats. The present experiment was initiated to investigate whether this dose (10 mg/kg) would induce changes in the electrophysiological activity recorded from physically dependent rats. Rats physically dependent on morphine were obtained by injection of multiple (3) incremental doses of morphine, a maximum of up to 130 mg/kg/day daily for 5 days. The response to morphine (10 mg/kg ip) and the morphine antagonist naloxone (1 mg/kg) were examined on day 1 (naive animals) and on day 5 of the experiment (physically dependent animals). Permanent electrodes (60μm in diameter) were implanted previously in the parafasciculus (PF) and within the ventromedial hypothalamus (VMH) under a stereotaxic guideline. A total of 52 units were recorded. Morphine in naive rats modified the spontaneous activity of all the units (26) recorded from PF and 84% (22) of the VMH units. An increase and a decrease in activity following morphine treatments were observed in both structures. Those units recorded from the VMH which responded on day 1 with increased activity, did not respond at day 5 to morphine. Those units recorded from the PF which responded on day 1 with increased activity, exhibited at day 5 a variety of patterns. All the units recorded from the VMH which exhibited decreased activity (12) on day 1 following morphine; at day 5 the same dose induced increased activity. However, the units recorded from the PF which exhibited decreased activity on day 1 (14) following morphine did not respond to this challenge dose at day 5. In conclusion, both the VMH and the PF exhibited neurophysiological evidence of tolerance to morphine, but the pattern of tolerance in each structure was different. (Supported by USPHS DA 00803)

1333 ANATOMICAL AND ELECTROPHYSIOLOGICAL EXAMINATION OF NEURONS IN THE NUCLEUS A10 REGION OF THE RAT. M. Dalsass*, D.C. German and R.S. Kiser (Spon: H. Roffwarg). Depts. of Psychiat. and Physiol., U. of Texas Health Sci. Ctr., Dallas, TX 75235.

The dopamine (DA)-containing cells in the ventral tegmental area (nucleus A10) have been related to brain stimulation reward and locomotor mechanisms. In the present study the anatomical and electrophysiological properties of these neurons were examined in the rat. In the anatomical study, S^{35}-methionine (567-1065 Ci/mmole) was iontophoretically ejected into the A10 region, and the ascending projections of these cells were subsequently traced autoradiographically (survival = 1-4 days; emulsion exposure = 7 days). Labelled axons were traced through the medial forebrain bundle and entopeduncular nucleus and further rostrally into the cingulum bundle. Evidence of labelled axon terminals was found in the nucleus accumbens, olfactory tubercle, and anterior medial cortex of the frontal lobe.

Single unit activity was recorded from the A10 region in the chloral hydrate (400 mg/kg) anesthetized rat. Glass micropipettes filled with 2M NaCl and fast green dye were used for the recordings. Slowly-firing cells (1-9 Hz), with long action potential durations (> 2 msec.), and histologically localized within the A10 region had their firing rate transiently reduced to greater than 50% by an intravenous injection of the DA agonist, apomorphine (20-40 μg/kg). Cells with these electrophysiological and pharmacological characteristics are thought to represent DA-containing cells (Bunney, et al., 1973). These cells were antidromically activated, by stimulation in the nucleus accumbens area, as judged by either constant latency, or collision testing (latency - 10-24 msec., mean estimated conduction velocity = 0.43 m/sec.). This conduction velocity is similar to that reported for the DA neurons of the substantia nigra zona compacta (Guyenet & Aghajanian, 1977). These slowly-conducting fibers often had long refractory periods (e.g., about 1.8 msec.). Other cells in the A10 region were orthodromically activated at longer latencies than cells which were antidromically activated (latency = 25-30 msec.). The results of the present study indicate that, (1) nucleus A10 DA neurons can be antidromically activated from forebrain sites, (2) they have slow conduction velocities typical of small unmyelinated fibers, and (3) they can also be orthodromically influenced by descending fibers to nucleus A10. (Supported by USPHS grant MH-27574.)

1334 INHIBITORY EFFECTS OF TYPE A AND B MONOAMINE OXIDASE INHIBITORS ON THE SYNAPTOSOMAL ACCUMULATION OF ^{3}H-DOPAMINE. K.T. DEMAREST* AND A.J. AZZARO* (SPON: D.E. HAINES). WVU MED CTR, MORGANTOWN, WV 26506

Monamine oxidase (MAO) inhibitors were the first clinically useful antidepressant drugs. Previous studies have demonstrated a correlation between the relative potency of clinically used MAO inhibitors as inhibitors of the CNS catecholamine neuronal reuptake system and their clinical efficacy as antidepressant drugs (Nature 220: 1330, 1968). In other words, clinical efficacy seems more closely related to inhibition of catecholamine neuronal accumulation than inhibition of MAO. With the demonstration of multiple forms of MAO and the more recent development of selective inhibitors of these enzyme forms, it became of interest to us to examine the selective properties of various MAO inhibitors and to compare these properties to the ability of each agent to inhibit dopamine (DA) neuronal accumulation.

Accumulation of ^{3}H-DA was examined in rat brain synaptosomes. MAO activity was determined in osmotically lysed rat brain synaptosomes. ^{14}C-serotonin and ^{14}C-phenylethylamine deamination were used as an index of type A and B MAO activity, respectively. A wide concentration range (10^{-10} to 10^{-3}M) of each inhibitor was examined. ID_{50} values were obtained by probit analysis.

MAO INHIBITOR	ID_{50} VALUES OF DA ACCUMULATION	TYPE A MAO	TYPE B MAO
Clorgyline	34.0 μM	11.7 nM	88.3 μM
Lilly 51641	5.5 μM	40.5 nM	5.3 μM
Harmaline	12.9 μM	4.9 nM	0.3 μM
Deprenyl	23.3 μM	49.3 μM	49.7 nM
Pargyline	378.1 μM	5.0 μM	46.7 nM
Tranylcypromine	4.4 μM	1.6 μM	0.6 μM
Nialamide	3.1 mM	51.0 μM	70.1 μM

Both type A and B as well as common inhibitors of MAO produced a dose-related inhibition of ^{3}H-DA accumulation. All agents were quite potent in this regard with the exception of nialamide. No clear relationship was observed between specific MAO inhibitors and their ability to alter DA accumulation. However, each group of selective inhibitors was approximately 1000x more potent in inhibiting MAO than DA accumulation. Therefore with these newer agents it is possible to selectively inhibit type A or B MAO activity without altering the neuronal reuptake of DA. These results confirm the correlation between the clinical efficacy of such agents as tranylcypromine (highly effective), pargyline (moderately effective) or nialamide (ineffective) and their relative potencies to inhibit the neuronal reuptake of brain catecholamines. Since clorgyline, Lilly 51641, harmaline, and deprenyl are all more potent inhibitors of ^{3}H-DA accumulation than pargyline, these newer agents may prove beneficial in the treatment of depressions.

1335 THE EFFECT OF MORPHINE ON APOMORPHINE INDUCED STEREOTYPY IN RATS. J.J.I. Feigenbaum,* B.H. Moon*, and H.L. Klawans. Dept. Neurosci., Rush Presbyterian St. Lukes Med. Center, Chgo. Ill., 60612 (SPON: T. Pencek)

A number of recent reports have suggested that morphine (M) may exert an inhibitory effect on central dopaminergic(DA) mechanisms. Consistent with this proposal are experimental observations that M produces catalepsy in rodents; decreases DA neurotransmission in the rat due to a direct action on central DA neurons; produces denervation supersensitivity of striatal DA receptors (SDA_r) following chronic administration; and inhibits the stereotyped behavior induced by the direct DA agonist apomorphine (AISB). However, despite this and other evidence that M directly blocks the SDA_r, it has recently been found that highly specific SDA_r blocking agents could not be displaced by doses of M as high as 10^{-5}M. Furthermore, reports concerning the effect of M on AISB have been inconsistent. While most findings indicate that M has an inhibitory effect on AISB, a recent report suggested that M has an excitatory effect on AISB, which would preclude a direct effect of M on blocking the SDA_r. To resolve this discrepancy, we studied the effect of doses doses of M ranging from .10 to 70 mg/kg on the stereotypy induced by 0.5 mg/kg apomorphine in Sprague-Dawley rats, us using 10 mg/kg increments of M. Doses of M ranging from .10 to 30 mg/kg produced a significant, sigmoidal enhancement of AISB relative to control animals, characterized by high intensity components of stereotypy (licking, and biting of the metal cage bars with very little jaw movement) accompanied at lower doses by head movement and prolonged rearing. Doses beyond 30 mg/kg (40-70 mg/kg) were progressively less effective in potentiating AISB, with 60 mg/kg significantly inhibiting AISB and 70 mg/kg M completely abolishing it. The discrepancy in findings reported previously regarding the effects of M on AISB may thus be due to differences in the dosage of M used. The inhibition of AISB seen after extremely high doses of M is probably due to the pronounced sedative effect of this drug at high doses. Since drugs which block the SDA_r inhibit rather than potentiate AISB, the enhancement of AISB by low to moderate doses of M suggests that M does not block the SDA_r. This effect could be explained by a pre-synaptic inhibition of striatal DA neurons however, as we and others have found that drugs diminishing the availability of striatal DA tend to enhance AISB.

1336 IONTOPHORESIS OF LSD: EFFECTS ON RESPONSES OF SINGLE CORTICAL NEURONS TO VISUAL STIMULATION. P. C. Fox and A. Dray*. Department of Physiology, Duke University Medical Center, Durham, North Carolina 27710.

Microelectrophoretic techniques were used to study the effects of LSD on single neurons in striate cortex of cats anesthetized with 75% nitrous oxide. Cells were driven by physiological stimuli and their receptive field characteristics classified.

The evoked activity of most cells was enhanced or depressed by LSD. Enhancement occurred with small ejections (0-10 nA); while depression was seen at 0-10 nA, but more frequently when currents >10 nA were used. Also inconsistant changes in unstimulated background firing were observed. In some neurons there was clear impairment of directional selectivity. Tolerance of LSD appeared in many cells after repeated administrations. Methysergide sometimes produced similar effects to LSD, on the same neurons, but generally these were weaker and required more prolonged administration with higher currents (30-40 nA). 2-Bromo-LSD (BOL) however, was generally inactive on cells which LSD had clearly influenced. These observations using direct administration of LSD to cortical neurons are similar to those seen in visual cortex after its systemic administration.

Overall, the responses of cells to physiological stimulation were exquisitely sensitive to LSD. Since the effects of LSD were mimicked less frequently with larger administrations of methysergide (a hallucinogen in large systemic doses) and not with BOL (the inactive analogue of LSD) we suggest that some of the changes are related to the hallucinogenic properties of LSD.

Supported by the following grants: NIDA #DA 01458; NIEHS 5-T32-E5007002; and NIMH 5-T01-MH-08394-13.

1337 COMPARISON OF DRUGS PURPORTED TO ANTAGONIZE SPECIFIC ETHANOL ACTIONS. G.D. Frye, R.A. Vogel, R.B. Mailman, G.R. Breese and R.A. Mueller. Biol. Sci. Res. Ctr., UNC Sch. Med., Chapel Hill, NC 27514.

The present studies compare the specificity of several putative "ethanol antagonists"--thyrotropin releasing hormone (TRH), fenmetozole (F), and apomorphine (A) against a spectrum of ethanol actions. These included ethanol-induced impairment of airborne righting reflex, increased locomotor activity, increased shock-punished drinking in water-deprived rats, decreased cerebellar guanosine 3',5'-monophosphate (cGMP), and physical dependence.

Ethanol (3 g/kg, ip) impaired motor coordination in an airborne righting reflex test. Treatment ip with TRH (20-80 mg/kg) or F (15-30 mg/kg) significantly reversed ethanol induced impairment, whereas A (3-10 mg/kg) was without effect. A proposed "stimulatory" action of ethanol (2.0 g/kg, ip) to increase spontaneous locomotor activity was observed in HA/ICR mice but not in Sprague-Dawley rats. In mice ip treatment with ethanol and TRH (20 mg/kg) produced the same stimulation of activity as ethanol alone. Both A (2.5 mg/kg) and F (15 mg/kg) significantly reduced the increased activity in mice. Ethanol (0.5-1.5 g/kg) increased shock-punished water consumption in water-deprived rats in a manner parallel with clinically used anxiolytic drugs. F (15 mg/kg, ip) completely reversed increased punished responding while TRH (20 mg/kg) potentiated this anxiolytic effect of ethanol. In rat cerebellum ethanol produced a dose dependent decrease in the content of the proposed second messenger, cGMP. TRH (20 mg/kg), but not A (5 mg/kg) or F (5-30 mg/kg), significantly reduced the fall in cGMP after ethanol, although all three drugs elevated cGMP when given alone. Physical dependence was induced by feeding of rats 6-8% w/v ethanol liquid diet for 12 days, as indicated by the occurrence of 70-80% audiogenic seizures following ethanol withdrawal. None of the compounds studied precipitated seizures or other signs of withdrawal when given to dependent rats maintaining sufficient blood ethanol levels to suppress withdrawal reactions.

The observed differences in interactions of purported "ethanol antagonists" compared across a spectrum of ethanol's actions indicate that cGMP levels in cerebellum do not correlate with the behavioral effects of ethanol. Furthermore, these data support the concept of a general action of ethanol on a large variety of neural systems. (Supported by HD-10570, AA-02334, MH-00013, MH-05636 and AA-05047.)

1338 NALOXONE AND THE EFFECTS OF SYSTEMICALLY ADMINISTERED KAINIC ACID. T. Fuller* and J.W. Olney, Washington University School of Medicine St. Louis, MO 63110

Kainic acid (KA) is a heterocyclic structural analog of glutamate (Glu) with both the neuroexcitatory and the neurotoxic properties like the putative neurotransmitter Glu, although more potent. When systemically administered to mice, KA showed much greater potency than Glu as a convulsant and neurotoxin specifically affecting neurons of the arcuate nucleus of the hypothalamus (AH). In subsequent studies, KA has demonstrated neurotoxic effects in many brain regions when introduced directly into the brain or the ventricles, and has induced both wet dog shakes (WDS) and convulsions in rats when introduced intraventricularly. It has been postulated that WDS, a behavior seen in rats undergoing morphine withdrawal, relates to an interaction of morphine with the Glu excitatory system. The WDS induced by KA and by intraventricular endorphins are reportedly blocked by the narcotic antagonist Naloxone (Nal).

Here we report that KA, when administered systemically to adult male rats, induces WDS, convulsions and brain damage with the latter following a different pattern of distribution than was originally described in mice. As is documented more fully elsewhere (Schwob et al., N.S. Abst., 1978) this pattern involves olfactory cortex, amygdala, lateral septum, hippocampus and several thalamic nuclei but spares AH. Both glial and neuronal elements are affected with lesions tending to have a bilaterally symmetrical pattern. Glial swelling and dendritic dilation are prominent early findings (4-6 hrs) while neuronal soma degenerate on a slightly more delayed schedule (6-36 hrs). The WDS and convulsions were dose related such that animals given 5 mg/kg KA had neither symptom (50%) or WDS without convulsions (50%), but 88% given 12 mg/kg KA had both WDS and convulsions. The occurrence of brain damage correlated positively with convulsions; virtually all animals manifesting significant convulsive activity sustained brain damage and those manifesting only WDS escaped damage.

Subcutaneous administration of Nal (2-4 mg/kg) 5 min prior to KA administration (12 mg/kg) reduced the incidence of convulsions from 88% to 50% and increased the percentage of animals sustaining only WDS or having neither symptom. Whether Nal modified KA-induced brain damage remains under study. The modification of KA neurotoxicity by Nal is of great interest because it suggests a specific interaction between a glumimetic agent and an opiate antagonist, the further study of which may shed light on both Glu neurotransmission and opiate action. NIH grants NS-09156, DA-00259, ES-07066 and R.S.D. Award MH-38894 (J.W.O.).

1339 IN VIVO DEMONSTRATION OF AN INTERACTION BETWEEN BENZODIAZEPINES AND GABA SYSTEMS IN THE CNS. Dorothy W. Gallager, John W. Thomas and John F. Tallman. Biological Psychiatry Branch, NIMH, Bethesda, MD 20014.

Many of the pharmacological actions of the benzodiazepines (BZs) can be attributed to their actions on γ-aminobutyric acid (GABA) systems in brain. In the present study we have demonstrated electrophysiologically a synergistic effect between GABA and the BZs using microiontophoretic and single-cell recording techniques. BZs do not affect the spontaneous firing rate of dorsal raphe (DR) cells. However, both the intravenous and iontophoretic administration of BZs were found to potentiate the inhibitory response produced by GABA on these neurons. Following pretreatment of rats with an inhibitor of GABA catabolism, aminooxyacetic acid (AOAA) or the GABA agonist, muscimol, BZs decreased the firing rate of DR neurons. This inhibition was reversed by the i.v. administration of the GABA antagonists picrotoxin or bicuculline. Since direct binding studies using [^{3}H]diazepam ([^{3}H]DZ) have indicated that a specific high affinity binding site may be relevant to the actions of BZs in brain, we have also examined the effects of AOAA and muscimol on [^{3}H]DZ binding. Five minutes after the intravenous injection of [^{3}H]DZ via a tail vein, [^{3}H]DZ bound specifically to high affinity binding sites was found in rat brain. Compatible with results obtained in electrophysiological studies, specific BZ binding in brain was enhanced by pretreatment of animals with AOAA and muscimol. This increase in binding in vivo produced by AOAA and muscimol is a result of an increased affinity of the binding site for BZ. An increase in binding site affinity was obtained in in vitro binding assays where the binding of [^{3}H]DZ to washed cortical membranes is enhanced in the presence of GABA and muscimol. This effect was blocked by bicuculline and involves an increase in the affinity but not number of high affinity binding sites on the membrane. Taken together this data suggests that the interaction between GABA and BZ in brain is relevant to a pharmacological action in vivo.

1340 EVIDENCE FOR SEROTONIN PARTICIPATION IN GUT WITHDRAWAL FROM OPIATES. Alan R. Gintzler, SPON: Taube P. Rothman. Department of Anatomy, Columbia University, P&S, NY, NY 10032.

The ileum of the guinea pig chronically exposed to opiates develops tolerance/dependence as does the central nervous system. Therefore this preparation may be used to study the processes that are involved in the manifestation of dependence. Tolerance/ dependence was induced by the subcutaneous implantation of five morphine pellets (each containing 75 mg morphine base) under light ether anaesthesia. On the fourth day following implantation, animals were killed by decapitation, and the terminal portion of the ileum was removed, washed and mounted in a 25 cc organ bath. All experiments were performed on pieces of whole ileum suspended in Krebs-bicarbonate buffer which was maintained at 37°C and bubbled with 95% O_2 and 5% CO_2. Naloxone (10^{-7} M) produced a well sustained contraction which was totally blocked by a 15 min pretreatment with tetrodotoxin (10^{-6} g/ml). This naloxone induced contraction was only partially blocked by a 15 min pretreatment with $2x10^{-6}$M atropine, a concentration sufficient to abolish contractile response to 10^{-6}M acetylcholine. To examine the possibility that 5-HT was involved in mediating the atropine resistant contraction the response to naloxone (10^{-7}M) was examined after the excitatory response to 5-HT was inhibited by a 1 hour incubation with 10^{-5}M 5-HT. Under these conditions the atropine-resistant component of the naloxone-induced contraction was reduced by about 80%. Washout of the 5-HT and a brief rest resulted in the reappearance of the excitatory response to 5-HT and the partial restoration of the atropine-resistant naloxone induced contraction. Therefore, 5-HT appears to mediate at least a portion of the naloxone-induced contraction observed in guinea-pig ileum made tolerant to morphine. These experiments indicate that in addition to acetylcholine, serotonin may be involved in the manifestation of gut dependence on morphine. Supported by DA01772.

1341 EFFECTS OF BLOCKING NIGROSTRIATAL IMPULSE FLOW ON AMPHETAMINE-INDUCED MOTILITY. Larry P. Gonzalez and Everett H. Ellinwood, Jr. Dept. Psychiat., Behav. Neuropharm. Sect., Duke Univ. Med. Ctr., Durham, NC 27710.

Release of dopamine in the caudate nucleus following systemic d-amphetamine has been suggested to depend upon impulse flow in the dopaminergic nigrostriatal pathway. Since this pathway has been implicated in the mediation of amphetamine-induced stereotyped behaviors, these behavioral effects of amphetamine might also depend upon impulse-coupled dopamine release. In the present study, impulse flow in the nigrostriatal pathway was inhibited through local injection of apomorphine, and its effects on amphetamine-induced motility were observed.

Forty male Sprague-Dawley rats received chronic bilateral implants of 25-guage guide cannulae in the substantia nigra pars compacta. Following recovery from surgery, the changes in movement frequency induced by systemic injection of d-amphetamine (0, 3, or 6 mg/kg) were observed and quantified with an electronic transducer. Thirty minutes after systemic amphetamine, apomorphine (0, 15, or 30 µg) was administered through a 32-guage injection cannula into the substantia nigra. Movement frequencies were observed for 60 minutes following intranigral apomorphine.

Amphetamine produced dose-dependent alterations in the distribution of movement frequencies. Intranigral injection of apomorphine did not significantly affect motility and did not alter the amphetamine-induced changes in motility. These results suggest that the behavioral effects of amphetamine occur independently of nigrostriatal impulse flow.

1342 EFFECTS OF ETHANOL ON THE SPONTANEOUS ACTIVITY OF SINGLE UNITS IN THE HIPPOCAMPUS OF THE AWAKE SEMI-RESTRAINED RAT. Larry A. Grupp. Department of Pharmacology, University of Toronto, Toronto, Canada, M5S 1A8 and Addiction Research Foundation, Toronto, Canada.

The spontaneous firing rates of single units in the dorsal hippocampus of semi-restrained rats chronically prepared with bundles of fine wire nichrome microelectrodes, were monitored during an ethanol challenge. Several doses were administered to all rats, each dose being given on a separate day with an inter-dose interval of at least 48 hrs. Each ethanol injection was preceded by two control recording periods: 1) baseline period to obtain a general picture of the rate and pattern of firing and 2) a saline injection period to control for injection and volume effects. The results indicated that single cells in the hippocampus are sensitive to ethanol, and that this sensitivity which is reflected by a depression in firing rate is dose dependent with larger doses producing greater degrees of depression. The simultaneously recorded EEG indicated a marked bias in frontal cortical activity towards high amplitude slow waves while the hippocampal activity showed less marked but more varied changes including a bias to high amplitude slow waves and to "theta" activity at higher doses, and a bias to low amplitude fast activity at the lowest doses. These findings suggest that the hippocampus is among those structures whose activity and function are particularly sensitive to ethanol. Supported by the Alcoholism and Drug Addiction Research Foundation of Ontario.

1343 EFFECTS OF BARBITURATES ON ASTROCYTIC FUNCTIONS. L. Hertz, E. Hertz and S. Fedoroff*, Dept. of Anatomy and B.R. Sastry*, Dept. of Physiology, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0 Canada.

During recent years a considerable amount of information has accumulated suggesting that astrocytes are involved in the homeostasis of putative amino acid transmitters and of potassium at the cellular level of the brain by accumulating these compounds (1-3). Barbiturates are known to inhibit the uptake of γ-amino butyric acid (GABA) into brain slices (4) and to prolong presynaptic inhibition, in which GABA has been implicated (5), as well as to counteract the potassium-induced stimulation of oxygen uptake in brain slices (1) and to delay removal of potassium from the extracellular space in vivo and prolong the metabolic manifestation of this process (6). Conceivably, some or all of these effects could be brought about if barbiturates exerted an action on uptake processes, and on the metabolic manifestations of these processes, in astrocytes. To investigate this possibility we studied the effect of pentobarbital upon GABA uptake and potassium-induced stimulation of oxygen uptake in mouse astrocytes in primary cultures.

GABA uptake was studied by incubating cultures briefly with ^{14}C labelled GABA in media with different concentrations of nonradioactive GABA and calculating the uptake per mg protein from the specific activities in the media and the accumulation of radioactivity in the tissues. 2 mM pentobarbital caused an inhibition in the GABA uptake of about 30% regardless whether the medium contained 5, 20, or 50 µM GABA, but lower concentrations of pentobarbital, i.e., 1 mM (which caused an inhibition of 15-30%) and 0.5 mM (which caused an inhibition of 10-15%) seemed to be most efficient at a GABA concentration of 5 µM.

Potassium effects on oxygen uptake were studied by aid of an oxygen electrode and using the closed culture flask as the respirometer chamber. In a medium with 5 mM potassium the rate of oxygen uptake by the cultured astrocytes was initially about 300 µmol/hr per g wet wt.; this value was almost doubled by 50 mM potassium which caused an initial stimulation but subsequently (i.e., after the first ½-1 hr) led to an increased rate of respiratory decline. In the presence of 1-2 mM pentobarbital the inital stimulation of respiration by excess potassium was almost completely abolished and also the increased rate of respiratory decline seemed to be counteracted.

Supported by the MRC of Canada (grant MT 5957 and MA 6193).

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1344 EFFECTS OF ETHANOL, MORPHINE, AND PENTOBARBITAL ON CALCIUM LOCALIZATION IN BRAIN. Wm. F. Hood* and R. Adron Harris, Dept. of Pharmacology, Univ. of Missouri, Columbia, MO 65212.

Whole brain homogenates from Sprague-Dawley rats or Swiss-Webster mice were fractionated as previously described by Harris et. al. (Life Sci. 20:501, 1977) and the effect of acute ethanol, morphine, or pentobarbital on calcium localization was studied. Other groups of mice were chronically fed a liquid diet of 7% ethanol or were implanted with a pentobarbital pellet for three days. The calcium and magnesium content of myelin, extra-synaptosomal mitochondria, synaptosomes, SPM-1, SPM-2, intra-synaptosomal mitochondria, microsomes, and microsomal supernatants were measured by atomic absorption spectroscopy.

Acute administration of morphine (25 mg/kg s.c.) decreased rat brain synaptosomal calcium content by 20% ($p<.01$) and acute pentobarbital (75 mg/kg i.p.) decreased mouse brain synaptosomal calcium by 17% ($p<.02$). Moreover, acute pentobarbital decreased the calcium content of myelin 27% ($p<.005$) while increasing that of extra-synaptosomal mitochondria 17% ($p<.01$). However, the acute administration of ethanol to rats (3 g/kg i.p.) or to mice (4 g/kg) failed to change the calcium content of brain synaptosomes or any other fraction. None of the fractions showed a change in the magnesium content following acute ethanol, morphine or pentobarbital. Subsequently, while attempting to correlate our in vivo results with earlier in vitro studies which had demonstrated that morphine (10^{-7} M; End & Dewey, Fed. Proc. 37:763, 1978) and pentobarbital (.15 mM; Blaustein & Ector, Mol. Pharm. 11:369, 1975) inhibited rat synaptosomal K^+ stimulated calcium uptake, we found that 25 mM and 50 mM ethanol did not affect this uptake but 100 mM ethanol significantly inhibited the uptake by 31% ($p<.02$).

Chronic pentobarbital decreased mouse brain SPM-1 magnesium 6% ($p<.02$). In addition, ethanol withdrawal produced a decrease in brain myelin magnesium of 10% ($p<.005$). Also, mice in withdrawal from ethanol showed a decrease in serum magnesium and an increase in free fatty acids in agreement with clinical studies of alcoholics. Mice in withdrawal from ethanol and mice chronically exposed to pentobarbital showed no change in calcium levels in any of the brain fractions.

The results from the acute and in vitro studies suggest that the lower calcium content of synaptosomes following acute pentobarbital and morphine may be related to the inhibition of depolarization stimulated calcium uptake. The data also imply that a decrease in synaptosomal calcium content may occur at higher acute doses of ethanol than were used in our in vivo studies.

Supported by grants from the Pharmaceutical Manufacturer's Association Foundation to R.A.H.

1345 THE EFFECTS OF ANTIDEPRESSANT DRUGS ON ^{3}H-MONOAMINE RELEASE FROM SYNAPTOSOMES. W. D. Horst and G. Bautz*. Hoffmann-La Roche Inc., Nutley, N. J. 07110

Crude synaptosomes were prepared from rat brain cortex and preloaded with either ^{3}H-5-hydroxytryptamine (5-HT), ^{3}H-L-norepinephrine (NE) or ^{3}H-dopamine (DA). ^{3}H-Amine release was determined from superfused synaptosomes according to Raiteri et al (Eur. J. Pharmacol. 25: 411, 1975). The ED_{50} value is the drug concentration required to increase release 50% during a 3 min superfusion. ED_{50}'s (x 10^{-5}M) were determined for desipramine (0.9), nortriptyline (1.7), amitriptyline (1.7), iprindole (1.5), mianserin (2.6) and imipramine (3.0) induced ^{3}H-5-HT release. None of these drugs at concentrations of 10^{-4}M influenced the release of ^{3}H-NE or ^{3}H-DA. These drugs are not releasing 5-HT from intraneuronal amine storage vesicles since the drug induced release of ^{3}H-5-HT was not calcium dependent and was demonstrated in synaptosomes derived from reserpinized brains. Low Na^+ concentrations increased the spontaneous release of ^{3}H-5-HT from synaptosomes but prevented the drug induced release, suggesting that these drugs are transported into the synaptosomes by an active transport process. If these drugs must be transported across the neuronal membrane in order to release 5-HT, the medium drug concentration required to cause this effect may be much higher than the drug concentration at the site of action. Iprindole and mianserin are antidepressants with weak amine uptake inhibiting properties. Their ability to release 5-HT suggests that this phenomenon deserves further study with respect to the mechanism of action of antidepressant drugs.

1346 ERGOT DRUGS INHIBIT SPIROPERIDOL AND SEROTONIN RECEPTOR BINDING. R.E. Hruska and E.K. Silbergeld. Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20014.

Bromocriptine is of therapeutic value in the treatment of Parkinson's disease because of its proposed dopamimetic effects. Other ergot drugs may also be beneficial; however, they have not been fully evaluated. Unfortunately, the ergot drugs have been reported to produce some undesirable psychiatric and vasomotor effects, which may be attributed to their serotonergic properties. In order to analyze the relative dopaminergic versus the possibly undesirable serotonergic effects of the ergot drugs, we have tested the effects of these drugs on synaptic receptors. We have measured the ability of the ergot drugs to inhibit the receptor binding of ^{3}H-spiroperidol (SPIRO), a compound reported to label dopamine receptors, and ^{3}H-serotonin (5HT), which has been used to label serotonin receptors. The binding of SPIRO, at a concentration of 40 pM, to homogenates of rat caudate nuclei is potently inhibited by the ergot drugs. The binding of 5HT, at a concentration of 7 nM, to homogenates of rat cerebral cortex was also inhibited by the ergot drugs. The estimated IC_{50} values are listed below in the table. In general, the ergot drugs were weaker inhibitors of 5HT binding than SPIRO binding. The ratio of the IC_{50} values for the inhibition of 5HT to that for the inhibition of SPIRO binding are also listed in the table. The magnitude of the ratio indicates that bromocriptine and lisuride are much more potent inhibitors of SPIRO than 5HT binding, while only 29-712 was a better inhibitor of 5HT than SPIRO binding.

Inhibitor	IC_{50} (nM) 5HT	IC_{50} (nM) SPIRO	Ratio $\frac{5HT}{SPIRO}$
Bromocriptine	49.1	1.6	30.69
Lisuride	39.7	1.8	22.06
25-397 (Sandoz)	115.7	11.2	10.33
29-717 (Sandoz)	110.9	17.7	6.27
Lergotrile	510.8	221.1	2.31
Metergoline	29.1	18.7	1.56
29-712 (Sandoz)	92.0	327.8	0.28

These results may be of interest in the prediction of the therapeutic value of the ergot drugs in Parkinson's disease. Bromocriptine has the highest ratio of effects on the dopaminergic system and is known to be of therapeutic value. These results suggest that in Parkinson's disease, lisuride may also be of therapeutic value. Additionally, metergoline, which was the most potent inhibitor of 5HT binding tested, has been successfully used in the treatment of migraine and has been suggested to be a 5HT antagonist. Despite the great structural similarity of the ergot drugs, small changes in the side-chain substitution may be responsible for the large differences which occur in their inhibition of receptor binding.

1347 EFFECTS OF APOMORPHINE AND RO 20-1724 ON CYCLIC AMP AND CYCLIC GMP LEVELS IN VIVO IN PITUITARY, CEREBELLUM, N. ACCUMBENS-O. TUBERCLE AND STRIATUM. G. Jean Kant, James L. Meyerhoff and R.H. Lenox. Dept. Medical Neurosciences, Division of Neuropsychiatry, Walter Reed Army Inst. of Research, Washington, DC 20012.

The primary sites of action of the drugs apomorphine and RO 20-1724 are different but cyclic nucleotides have been postulated to be involved in the mechanism of action of both drugs. Apomorphine, which is considered to be a dopamine agonist that interacts with the dopamine receptor and stimulates adenylate cyclase, has been shown to increase levels of cyclic AMP in vitro (Kebabian et al., Proc. Natl. Acad. Sci. 69, 2145-2149, 1972) and to increase striatal cyclic AMP and cerebellar GMP in vivo (Stratten and Aylott, Fed. Proceedings, 33, 493, 1974; Gumulka et al., N.S. Arch. Pharmacol. 295, 21-26, 1976). RO 20-1724, has been shown to be a potent inhibitor of phosphodiesterase in vitro (Sheppard et al., Advan. Cyclic Nucleotide Res. 1, 103-112, 1972) and to increase levels of cyclic AMP in brain slices in vitro (Schwabe et al., Mol. Pharmacol. 12, 900-912, 1976). We were interested in determining whether RO 20-1724 could serve as a pharmacological amplifier for in vivo effects of a neurotransmitter receptor agonist such as apomorphine.

We examined the effects of apomorphine and/or RO 20-1724 in vivo on cyclic AMP and cyclic GMP levels in pituitary, cerebellum, striatum and n. accumbens-o. tubercle. Rats were injected with vehicle or RO 20-1724 (30 mg/kg) 30 minutes prior to a second injection of saline or apomorphine hydrochloride (1 or 10 mg/kg). The animals were then sacrificed by microwave irradiation 7 minutes after the second injection. Cyclic AMP and cyclic GMP were determined by a modification of the radioimmunoassay described by Steiner et al., (J. Biol. Chem. 247, 1106-1113, 1972).

RO 20-1724 significantly increased cyclic AMP levels in all 4 regions tested but especially in the pituitary where cyclic AMP levels increased over 10 fold. RO 20-1724 significantly increased cyclic GMP levels in only 2 regions: n. accumbens-o. tubercle, and striatum. Cerebellar cyclic GMP was increased but the elevation was not statistically significant at $p<.05$.

Apomorphine significantly increased cyclic AMP levels only in the pituitary where there was also a significant apomorphine dose effect. Levels of cyclic GMP were increased after apomorphine in the cerebellum and n. accumbens-o. tubercle. RO 20-1724 failed to produce supra-additive effects with apomorphine.

The most responsive system to both drugs involved cyclic AMP in the pituitary. Levels of cyclic AMP increased approximately 10 fold after either apomorphine or RO 20-1724.

1348 SEROTONERGIC-DOPAMINERGIC EFFECTS OF ERGOT DRUGS. S. Kennedy*, R. Hruska & E. Silbergeld, NINCDS, NIH, Bethesda, MD 20014.

On the basis of behavioral assays, such as rotation in rats with unilateral nigral lesions, several new ergot derivatives are proposed to possess dopamimetic properties. However, their indole structures suggest serotonergic activity as well. We have studied the effects of the following ergot drugs: bromocriptine, lergotrile, lisuride, metergoline, and Sandoz 25-397, 29-712, and 29-717. The behaviors studied in rats were stereotypy, which is thought to express stimulation of dopamine (DA) pathways; the "serotonergic syndrome" (Jacobs, Life Sci. 19:777, 1976), which may reflect stimulation of serotonin (5-HT) receptors; and post-decapitation convulsions, a behavior affected by changes in both DA and 5-HT function (Thut & Myslinski, Life Sci. 19:1569, 1976).

Induction of stereotypy was measured by a relative rating scale based on intensity. Bromocriptine, lergotrile, 29-712 and 29-717 all induce stereotypy at doses of 5 mg/kg and greater. The effects of all 4 drugs can be blocked by pretreatment with haloperidol (HAL) but not, in all cases, by inhibition of DA synthesis. Lisuride, metergoline, and 25-397 do not produce stereotypy.

The "serotonergic syndrome" was produced by pretreating rats with pargyline and then 5-hydroxytryptophan methyl ester (5-HTP). The syndrome was rated by number of characteristic signs present. Bromocriptine and lergotrile, at 5 mg/kg, potentiated response to 5 and 10 mg/kg 5-HTP. These potentiating effects, like the syndrome itself, can be blocked by pretreatment with HAL. Lisuride, at doses as low as 0.1 mg/kg, produces the syndrome in the absence of pargyline-5-HTP pretreatment. The apparently direct 5-HT-like actions of lisuride are not blocked by HAL, methysergide, or PCPA. 25-397, 29-712, 29-717, and metergoline do not affect the syndrome.

Post-decapitation convulsions (PDC) were studied in rats at the termination of other behavioral studies. Lergotrile (5 mg/kg), metergoline (25 and 50 mg/kg) and 25-397 (20 mg/kg) increase the latency and decrease duration of PDC. At higher doses, lergotrile and metergoline block PDC completely. These effects are not reversed by HAL, PCPA, or methysergide. Bromocriptine, lisuride, and 29-712 have no effect on PDC.

The results suggest that the ergots possess significant but varied behavioral effects in systems thought to involve DA and 5-HT modulation. The separation of DA from 5-HT in producing these behavioral effects is difficult, and this complicates the definition of the ergot drugs as relatively dopaminergic or serotonergic. However, on the basis of their behavioral activity, 29-712 and 29-717 may be relatively more dopaminergic, while lisuride is relatively more serotonergic. Bromocriptine and lergotrile affect both stereotypy and the "serotonergic syndrome" and may thus possess mixed actions on DA and 5-HT pathways.

1349 EFFECTS OF SYSTEMICALLY APPLIED GABA-ANTAGONISTS AND GABA-MIMETICS ON WAVE-SPIKE EEG ACTIVITY IN RAT. Gary A. King* (Spon: H.C. Kwan). Dept. Pharmacol., Univ. of Toronto, Toronto, Ont., Canada.

Drugs that antagonize inhibitory effects of GABA in the CNS (picrotoxin, bicuculline & pentylenetetrazol), and drugs that mimic GABA's actions when applied directly onto neurons (muscimol & imidazole acetate), were used to investigate the role of GABA in wave-spike EEG activity. In one experiment, wave-spike afterdischarges triggered by light-flash stimuli were recorded from occipital cortex in freely moving rats. IP injection either of GABA-antagonists or of GABA-mimetics caused a dose dependent increase of flash-evoked afterdischarges. Spontaneously occurring wave-spike activity was seen with the highest doses of all the drugs. Strychnine, however, did not enhance wave-spike EEG activity. In a second experiment, picrotoxin, bicuculline & imidazole acetate were examined for their effects on the ongoing EEG and behavior of freely moving rats. Subconvulsant doses of all three drugs induced generalized wave-spike seizures that were accompanied by immobility and varying amounts of forearm and facial clonus. These results will be discussed in terms of a correlation between the ability of some drugs to induce wave-spike EEG activity and to also block presynaptic inhibition, and in terms of hypotheses to explain the paradoxical effect of the GABA-mimetics.

1350 ENHANCEMENT OF NUCLEUS A10 SELF-STIMULATION BY THE NON-AMPHETAMINE STIMULANT, AMFONELIC ACID. R. Sanford Kiser, D.C. German, C.A. Brown* and P.A. Shore. Depts. Psychiat., Physiol. and Pharm., U. of Texas Health Sci. Ctr., Dallas, TX 75235.

Amfonelic acid (AFA) is a potent nonamphetamine psychotogenic stimulant. Biochemical and electrophysiological studies (German, et al., 1978) have indicated that, although AFA and amphetamine (AMP) both potentiate the function of the dopamine (DA) neuron, they do so by different means. Whereas AMP increases the release of newly-synthesized DA, AFA's action is not dependent upon newly-synthesized DA. Instead AFA appears to act by potently blocking DA reuptake and causing impulse-dependent overflow from reserve DA pools. The purpose of the present study was to test, in a rat behavioral model, the hypotheses that AFA, like AMP, would potentiate intracranial self-stimulation (ICSS) of DA cell bodies (nucleus A10) and that AFA effects, unlike AMP effects, are not dependent upon the availability of newly-synthesized DA.

Rats were stereotaxically implanted with chronic bipolar stimulating electrodes in nucleus A10 and beginning one week later were shaped for stable ICSS behavior. Each bar press produced a 0.3 sec. train of 0.2 msec. duration cathodal shocks at 100 Hz. In the first study, AFA was found to increase bar pressing rates at a wide range of stimulation currents (10-120 μa, peak pulse). In the second study, dose-response curves were obtained for both AFA and d-AMP. AFA (0.05, 0.1, and 0.2 mg/kg) was considerably more potent than d-AMP (0.1, 0.25, and 0.5 mg/kg) in increasing bar-pressing rates. In the third study, animals were pretreated with the DA synthesis inhibitor, α-methyl-para-tyrosine (AMPT, 100 mg/kg) before receiving either AFA (0.2 mg/kg) or d-AMP (0.5 mg/kg). In the AMPT-pretreated rats, AFA, but not d-AMP, increased bar-pressing rates above baseline levels. Furthermore, the AFA potentiation of ICSS seen in the AMPT-pretreated rats equalled the AFA potentiation seen in the normal rats. These data thus suggest that the effects of AFA, unlike those of d-AMP, are not dependent upon the availability of newly-synthesized DA.

The results of the present study are consistent with previously observed electrophysiological and biochemical findings which suggest that AFA and AMP both enhance the function of DA systems, although by different mechanisms. (Research supported by USPHS grants MH-27574 and MH-05831.)

1351 STREPTOZOTOCIN-INDUCED IMPAIRMENT OF BRAIN UPTAKE AND HYPOTENSIVE ACTION OF ALDOMET IN THE SPONTANEOUSLY HYPERTENSIVE RAT. M.C. Klein*, J.J. Poulakos*, A. Saha* and J.H. Jacoby. Depts. of Pharmacology and Microbiology, N.J. Med. Sch. (CMDNJ) Newark, N.J. 07103.

Recent observations indicate that elevation of plasma large neutral amino acids (NAA) in response to either their pharmacologic injection or ingestion of a protein meal (Markovitz, D. & Fernstrom, J.D., Science, 197,1014,1977) can act to impair brain uptake of aldomet (α-methyldopa; a NAA), presumably as a result of increased competition at a common transport site along the blood-brain barrier. Furthermore, concurrent injection of these competitive NAA, also attenuates the hypotensive effect of aldomet administered to hypotensive rats (Zavisca, F.G. & Wurtman, R.J., J. Pharm. Pharmacol., 30,60,1978).

Such results indicate that a similar alteration of the central actions of aldomet may occur in response to hyperamino acidemias of varying etiology. To further investigate this possibility we have made male spontaneously hypertensive rats diabetic as a result of injection of the pancreatic β-cell cytotoxin streptozotocin (70 mg/kg, i.v.) and measured the brain entry and hypotensive action of aldomet 3 days later. Animals were fasted the night prior to study and sacrificed 3 hrs. after aldomet injection (50 mg/kg, i.p.). All plasma NAA except tryptophan (which decreased by 50%) increased markedly in diabetic animals compared to controls. Branched-chain NAA (i.e., leucine, isoleucine, valine) were especially elevated (7-9 fold). Associated with this diabetes-induced hyperamino acidemia was a greater than 50% reduction of brain aldomet levels. Systolic blood pressure (BP) (measured by a tail-cuff transducer) in preconditioned non-diabetic rats fell about 20% 3 hrs. after aldomet. Diabetic animals showed no significant aldomet-induced reduction of BP. Diabetic rats receiving insulin replacement therapy (4 I.U. Protamine Zinc Insulin daily; 0.5 I.U. Semi-lente on last day) showed improvement of aldomet brain entry and hypotensive action.

Apparently altered plasma NAA patterns in disease states such as diabetes are also associated with impaired brain entry of certain centrally acting drugs.

(Supported by NINCDS grant No. 1 RO NS 12876).

1352 PHYSIOLOGICAL CONCENTRATIONS OF CALCIUM ACTIVATE RAT BRAIN TRYPTOPHAN HYDROXYLASE VIA INCREASED Vmax. Suzanne Knapp. Dept. Psychiatry, Sch. Med., UCSD, La Jolla, CA 92093

It was demonstrated previously that tryptophan hydroxylase (TPOH) prepared from rat midbrain, an area rich in serotonergic cell bodies, is activated in vitro by calcium (Ca^{++}) at concentrations of 1.0 mM or greater (Knapp et al., Life Sci. 16: 1583, 1975; Boadle-Biber, Biochem. Pharmacol. 24: 1455, 1975). Activation by such high concentrations of Ca^{++} was achieved by an increase in enzyme affinity both for pterin cofactor, biopterin, and for substrate, tryptophan. Having used a fluorometric assay in which cofactor and substrate are saturating to determine TPOH activity, we now report that EGTA pretreatment of midbrain TPOH followed by dialysis for 16 hours resulted in a 25- to 30-fold increase in the sensitivity of TPOH to Ca^{++}. The elevated activity of this enzyme preparation in the presence of physiological Ca^{++} concentrations was achieved by an augmented V_{max}, without demonstrable changes in affinity for either cofactor or substrate. Similar patterns of activation (by increased V_{max}) and sensitivity to Ca^{++} were demonstrated using midbrain TPOH that had been either dialyzed for 16 hours without EGTA pretreatment or allowed to stand at 20°C for 1 to 2 hours. Subsequently we examined TPOH from corpus striatum, an area rich in serotonergic nerve endings, to determine its response and sensitivity to Ca^{++}. In response to intermediate Ca^{++} concentrations ($\sim$ 200 µM) this preparation (without EGTA pretreatment, dialysis, or incubation at 20°C) demonstrated increased activity resulting from augmented V_{max} and no change in affinity for cofactor or substrate.

TPOH activity has been reported to be elevated by increased V_{max} following an acute load of phenylalanine (Neckers et al., J. Pharmacol. Exp. Ther. 201: 110, 1977), and a "prolonged" activation of tyrosine hydroxylase (TOH) also by increased V_{max} has been reported following cholinergic stimulation by oxotremorine (Lewander et al., Nature 258: 440, 1975). In neither case could changes in the enzyme's affinity for cofactor or substrate be shown. Immunochemical experiments showed that the increase in TOH activity was by activation rather than an increase in enzyme molecules. The TPOH activation by physiological concentrations of Ca^{++} which we have demonstrated, i.e. an effect resulting from increased V_{max} rather than increased affinity for cofactor or substrate, might account for such changes in neurotransmitter biosynthetic enzymes.

This work is supported by DA-00265-07.

1353 EFFECTS OF GABA AND STRUCTURALLY-RELATED COMPOUNDS ON CONDUCTANCE OF CRAYFISH ABDOMINAL STRETCH RECEPTOR. Diana N. Krause*, Kazuo Ikeda and Eugene Roberts. Division of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010.

The interaction of potential agonists with the postsynaptic γ-aminobutyric acid (GABA) receptor was investigated physiologically and quantitatively in an isolated crustacean neuron. The crayfish slowly-adapting, abdominal stretch receptor neuron, which receives direct GABAergic inhibitory innervation, was mounted in a constant perfusion chamber and impaled with two intracellular electrodes for monitoring membrane input conductance. Increase in conductance, the postsynaptic response to GABA, was measured for bath-applied GABA and compared with the effect of various structurally-related compounds. Non-cumulative log concentration vs. conductance change (Δg) plots were obtained, and in each case the concentration producing the half-maximal response was determined. The following compounds, listed in order of decreasing potency, produced a reversible increase in input conductance: muscimol> GABA>isoguvacine>(-)γ-amino-β-hydroxybutyric acid>3-aminopropane sulfonate>(+)γ-amino-β-hydroxybutyric acid>isonipecotic acid. All agonists acted in a dose-dependent manner with no apparent receptor desensitization and appeared capable of producing the same maximal response as GABA. When the dose-conductance data were analyzed with Hill plots [log(Δg/Lg$_{max}$-Δg) vs. log concentration], a steeper Hill slope was found for GABA (n≈3) than for the other agonists (n≈2). Nipecotic acid, guvacine, and L-α,β-diaminopropionic acid (L-DAPA), which are all thought to be blockers of GABA uptake processes, had essentially no effect by themselves on the membrane input conductance at concentrations up to $5x10^{-3}$M. However, when applied at 10^{-3}M simultaneously with GABA, nipecotic acid and L-DAPA significantly shifted the GABA dose-conductance curve to the left (towards lower concentrations) without altering the maximal response. In addition, the steepness of the GABA curve was reduced, i.e. the apparent cooperativity of the GABA response as assessed by the Hill slope was changed. The muscimol dose-conductance curve, on the other hand, was not affected in any way by nipecotic acid. β-(4-chlorophenyl)GABA (Lioresal) at concentrations up to 10^{-2}M had no significant effect on either the resting membrane conductance or the GABA response. (Supported by USPHS grants NS-12116 and 1615).

1354 NOREPINEPHRINE AND CYCLIC AMP: EVIDENCE AGAINST SECOND MESSENGER CONCEPT IN CENTRAL NERVOUS SYSTEM (CNS), SPINAL CORD (SC) AND PERIPHERAL NERVOUS SYSTEM (PNS). Barry J. Kraynack, Linda L. Kraynack* and Major L. Cohn. U.S. Naval Base, Guantanimo, Cuba and Univ. of Pgh. Sch. Med., Pittsburgh, PA 15213.

Based on the findings that norepinephrine (NE) and epinephrine (E) are potent adenyl cyclase stimulants, it is generally assumed that all the actions of NE and E are mimicked intracellularly by the second messenger adenosine 3':5'-monophosphate (cAMP). However, our in-vivo studies increasingly demonstrate that cAMP does not mimic NE and E in neural tissues. In the CNS, we showed that administered centrally to rats anesthetized with amobarbital, dbcAMP dose-relatedly shortens while NE prolongs narcosis. Here, we studied the actions of dbcAMP and E in SC and PNS. Adult mongrel cats were permanently implanted with epidural catheters. To control cats, 30 mg of lidocaine in 7.5% glucose was injected through catheter. To the same lidocaine preparation was added either 1.5 or 3 mg of dbcAMP or 10 µg of E. Each cat served as its own control. DbcAMP dose-relatedly shortened epidural blocks by 30-40 and 55 to 59% while E prolonged blocks by 29-66%. Next, we tested whether dbcAMP and E actions are the result of altered vascular blood flow, metabolism or displacement of lidocaine, by studying clearance of ^{14}C lidocaine from cerebrospinal fluid (CSF) of rhesus monkeys. Lidocaine 30 mg/7.5% glucose was administered through first catheter implanted in subarachnoid space between L_2 and L_3 either with ^{14}C lidocaine or ^{14}C lidocaine to which was added either 3 mg of dbcAMP or 5 µg of E. Through a second catheter implanted between T_{11}-T_{12} samples of CSF were withdrawn at 0, 5, 10, 15, 30, 45, 60, 90, 120, 150 and 180 minute intervals. DbcAMP significantly shortened while E potentiated the blocks but neither dbcAMP nor E altered CSF ^{14}C lidocaine concentrations. Next, in the PNS we investigated the interactions of dbcAMP and E with lidocaine, procaine, mepivacaine, tetracaine, bupivacaine, benzocaine, amobarbital, meperidine, naloxone, ketamine, E, ephedrine and tetrodotoxin. DbcAMP shortened and E prolonged the sciatic nerve blocks produced in rats by all 13 structurally different local anesthetics. Based on our findings that dbcAMP dose-relatedly shortens sciatic nerve blocks produced by the specific channel blocker tetrodotoxin, we propose that the nucleotide may regulate the duration of conduction blocks by overcoming local anesthetic's inhibition of sodium ion flux through sodium channel. Finally E (30 µg) injected alone produces sciatic nerve blocks for 131 minutes; 2 mg of dbcAMP shortens the blocks by 92%, suggesting that E-induced potentiation is the result of a direct neural blocking action. Our findings that in the CNS, SC and PNS dbcAMP shortens while NE and E potentiate anesthesia, suggest that in the 3 neural systems, cAMP is not the second messenger of NE or E. (NIDA 00605)

1355 THE K^+ LIQUID ION EXCHANGE ELECTRODE: RESPONSES TO DRUGS AND NEUROTRANSMITTERS. Taketeru Kuramoto*, David H. Shine and Bernard Haber. The Marine Biomedical Institute, and Dept. of Human Biological Chemistry & Genetics and Dept. of Neurology, The University of Texas Medical Branch, Galveston, Texas, 77550.

Neuronal activity is associated with transient potassium fluxes, and the accumulation of that ion in the extracellular space appears possible. The changes in extracellular K^+ may in turn modulate the behavior of both neurons and glia. The development of liquid cation exchange resins with a large selectivity for K^+ over Na^+, the major extracellular cation, has made possible the utilization of such resins for K^+ specific microelectrodes, to measure both intracellular and extracellular K^+ changes in a variety of preparations. As a result of neuronal activity, extracellular changes of many organic molecules as well as ions may occur. Therefore, neurotransmitters or their precursors may well contribute to the potential change recorded by the K^+ ion specific electrodes, which are interpreted as specific changes in extracellular K^+ concentration. In fact, such electrodes have been shown to respond to acetylcholine (Ach) at 10^{-3}M suggesting that responses might be obtained at lower and more physiological concentration of Ach. The paucity of data on the chemosensitivity of the K^+ ion exchange electrode to organic compounds of physiological significance prompted the present study.

K^+ sensitive liquid ion exchange micro electrodes respond with a significant potential change to acetylcholine, choline, anticholinergic drugs, such as curare, biogenic amines, l-propanolol in most cases it is at extremely low concentrations (10^{-7}- 10^{-5}M). The direction of the potential change varies, but in most instances is additive to that produced by external K^+. The K^+ electrode is further sensitive to decreasing pH, within a narrow and possible physiological range of pH (7-5.5). These findings suggest that small measured changes in extracellular K^+ are biased by the chemosensitivity of the ion exchange resin to some drugs and neurotransmitters at physiologically relevant concentrations.

Supported by PHS Grant NS 11255 Welch Foundation Grant H-504 and the Marine Biomedical Institute Visiting Scientist Program.
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1356 ETHANOL AND ACETALDEHYDE EFFECTS ON THE COUPLING OF CEREBRAL METABOLISM AND FUNCTIONAL ACTIVITY. Joseph C. LaManna, Roger L. Novack*, B. Wyatt Younts* and Myron Rosenthal. Depts. Neurol. and Physiol/Biophys. Univ. Miami Med. School, Miami, Florida 33156

Electrical and metabolic responses to direct cortical stimulation were measured in cats administered ethanol and acetaldehyde i.v. Stimuli were presented in pulse trains of 1-2 sec at 20 Hz which produced a shift of steady potential up to 6mV and a decrease in the ratios of reduction/oxidation of the mitochondrial respiratory chain components, NAD and cytochrome a,a_3. The latter were measured noninvasively by reflection microfluorometry and spectrophotometry. The rates of oxidation and re-reduction of each of these were slowed by ethanol and acetaldehyde. The slowing of oxidation is similar to the effect of ouabain when microinjected under the cortical surface and probably reflects an inhibition of $Na,^+K^+$-ATPase in the in situ tissue. The slowing of the rate of re-reduction following stimulation is reminiscent of the effect of barbiturate and could be due to direct drug effects in the respiratory chain or to a prolonged utilization of energy due to a slower rate of reestablishment of ion gradients altered during increased tissue activity. Under control conditions, the amplitude of the stimulus-evoked shift in steady potential is proportional to the oxidation of cytochrome a,a_3 or NADH. However, ethanol or acetaldehyde each produced characteristic alteration in this relationship. Low ethanol doses (up to approx 1g/kg) decreased the NAD response relative to the electrical response. Above approx 2g/kg, ethanol had an opposite effect. Acetaldehyde, from its lowest effective dose (approx 20 mg/kg) to 200 mg/kg always showed this latter effect, i.e. a larger metabolic response with respect to the SP shift. Both electrical and metabolic signals were decreased in amplitude as toxic dose levels of each drug were approached. It appears that dose levels which result in large metabolic amplitudes for give SP shift responses are producing an uncoupling of the function-metabolism relationship suggestive of an "inefficiency" in the capability of the metabolic apparatus to meet metabolic demand. (Supported in part by PHS grants NS 14319 and NS 14325).

1357 INHIBITION OF CATECHOLAMINE UPTAKE BY NATURALLY-OCCURRING TETRAHYDROISOQUINOLINES AND ITS POSSIBLE RELEVANCE TO BOTH L-DOPA CHEMOTHERAPY IN PARKINSONISM AND TO PHENYLKETONURIA. John M. Lasala* and Carmine J. Coscia*, Dept. of Biochem., St. Louis Univ. Sch. of Med. and Theodore J. Cicero, Dept. of Anat. and Neurobiol., Washington Univ. Sch. of Med., St. Louis, MO 63110.

A class of naturally occurring tetrahydroisoquinoline derivatives, norlaudanosolinecarboxylic acids (NLCAs), were found to be competitive inhibitors of dopamine and norepinephrine uptake into ouabain-sensitive synaptosomes. Using a 1000 g supernatant from whole rat brain, (±)-NLCA (K_i 6.30×10^{-6} M), blocked [1-^{14}C] dopamine uptake (K_m 8.48×10^{-8}M) into synaptosomes. NLCA was about two-thirds as potent as (+)-amphetamine in blocking dopamines uptake. In rat striatal tissue the K_i of (±)-NLCA decreased to 5.10×10^{-6} M ($r \geq 0.997$) because of the higher affinity for dopamine (K_m 6.4×10^{-8} M) in this dopamine-rich region. Two trace constituents of normal brain, 3',4'-desoxynorlaudanosolinecarboxylic acid (DNLCA) and 3'-O-methylnorlaudanosolinecarboxylic acid (MNLCA) also restricted dopamine uptake into both whole brain (K_i 2.11×10^{-5} M) and striatal (K_i 1.06×10^{-5} M) synaptosomes. Interestingly the NLCAs also blocked noradrenergic uptake in whole brain: (±)-norepinephrine K_m 2.30×10^{-7}M, (±)-NLCA 4.93×10^{-6} M, (±) DNLCA and (±) MNLCA 6.55×10^{-6} M, (±)-amphetamine, 3.22×10^{-6} M. In addition to competing with catecholamines for uptake, [^{3}H]NLCAs exhibited saturation kinetics (K_m 1.59×10^{-6} M and 1.51×10^{-5} M for NLCA and DNLCA, respectively) suggesting that they may be taken up and stored in synaptic vesicles. In other studies the NLCAs did not inhibit the binding of ^{3}H-spiroperidol to striatal synaptic membranes suggesting that they do not occupy post-synaptic receptor sites. These results are consistent with the hypothesis that the NLCAs interfere with catecholaminergic transport mechanisms in presynaptic terminals. The NLCAs described above are formed by condensation of α-keto acids with dopamine which can occur spontaneously under physiological conditions. MNLCA can be derived from NLCA in vivo by enzymatic methylation, while NLCA and DNLCA are the condensation products of dopamine with 3,4-dihydroxyphenylpyruvate and phenylpyruvate, respectively. MNLCA and DNLCA have been detected by computerized mass fragmentography in regions of normal human and rat brain at concentrations ranging from 10-200 ng/g of tissue. After L-dopa administration, MNLCA levels are elevated several-fold (C.J. Coscia et al., Nature 269, 617, 1977), whereas in experimentally induced hyperphenylalaminemia DNLCA rises 10-fold. Therefore, NLCAs can reach concentrations in certain brain regions which are within the range of K_i values obtained in this study. These data suggest that it is possible that NLCAs may account for certain therapeutic effects observed in L-dopa chemotherapy, beyond that attributable to dopamine replenishment alone.

1358 APOMORPHINE INHIBITION OF AUTOMATIC SWALLOWING IN THE RAT: EVIDENCE FOR MEDULLARY DOPAMINE RECEPTORS. Jon Levine* and Detlef Bieger. Neural and Behavioral Biology Program and Sch. Basic Med. Sci., Univ. Illinois, Urbana, IL 61801.

Previous experiments have shown that automatic swallowing (AS) in the urethane-anesthetized rat is a sensitive indicator of dopaminergic and serotoninergic activity in the brain (Bieger et al., 1977, Neuropharm., 16:245). The dopaminomimetic apomorphine (APO) exerts both an excitatory as well as inhibitory effect on AS, the former being dependent, the latter being independent of the integrity of certain forebrain structures. The present investigation provides further evidence for a locus of action within the lower brain stem. Thus, APO activation of AS was eliminated, but inhibition was unaffected in animals subjected to premammillary complete or partial transection or electrothermic coagulation (acute or subacute). This inhibition was manifest with AS driven by the serotonin agonist quipazine as well as rebound AS following acute knife cuts. Pimozide (1.5 mg/kg, i.p. or i.v.) and haloperidol (1 mg/kg, i.v.) blocked APO inhibition, this antagonism being surmountable. However, recovery was not evident even after periods up to 9 hours.

APO inhibition and its blockade by pimozide and haloperidol could also be demonstrated when these drugs were administered via the left vertebral artery at 10-fold lower concentrations. When injected onto the area postrema, APO and dopamine inhibited AS after a latency of approximately one minute. However, this effect persisted after coagulation of the area postrema. Interestingly, the inhibition of AS was accompanied by an increase in respiratory frequency. Animals pretreated with massive intracerebroventricular doses of 6-hydroxydopamine (400 µg) failed to show activation of AS by moderate doses of APO (100-200 µg/kg) and displayed lowered threshold and greater intensity of APO inhibition, compared to controls.

Our observations provide suggestive, if circumstantial, evidence for medullary dopamine receptors located in the immediate vicinity of the area postrema which modulate bulbar motor functions. These receptors do not appear to be prejunctional with respect to local catecholamine neurons, as postulated by other workers in reference to blood pressure and respiration (Bolme et al.., 1977, Adv. Bioch. Psychopharm., 16:281). The possible significance of these dopamine receptors in the production of involuntary movements by neuroleptic drugs calls for careful examination.

1359 EVIDENCE FOR CENTRAL MEDIATION OF ANTICONVULSANT ACTIONS OF MUSCIMOL. W. D. Matthews, A. P. Intoccia* and G. McCafferty*. Dept. Biol. Res., Smith Kline & French Labs, Philadelphia, PA 19101.

Muscimol (MCL) is a potent in vivo agonist of γ-aminobutyric acid (GABA) when tested iontophoretically (Krogsgaard-Larsen et al., J. Neurochem. 25:803, 1975). MCL also binds to central GABA receptors in vitro (Enna et al., Brain Res. 124:185, 1977). However, passage of MCL into brain after systemic administration has not been demonstrated. A study of MCL actions on seizures induced by drugs which impair GABA neurotransmission was performed in the rat. To ascertain that intravenously injected MCL penetrates brain tissue, whole brain levels of radioactivity were determined at various intervals after ^{14}C-muscimol and compared to the time-course of anticonvulsant activity. MCL delayed the onset of isoniazid and picrotoxin-induced convulsions; doses which produced a significant ($p<0.05$) increase in seizure onset in 50% of animals tested were 0.4 and 0.9 mg/kg, respectively. Bicuculline (BIC) treated rats exhibit clonic-tonic seizures within 5 secs of i.v. injection. The tonic forelimb extension component of BIC seizures was abolished by MCL (ED_{50}=1.0 mg/kg). Inhibition of forelimb extension was chosen as an end-point for comparison of other antiepileptic drugs with MCL. The order of potency against this component of BIC seizures was diazepam>MCL>> phenobarbital>diphenylhydantoin. MCL had no effect on strychnine-induced convulsions in doses up to 8 mg/kg i.v., however tonic metrazol seizures were prevented by MCL (ED_{50}=0.5 mg/kg). The time course of anticonvulsant activity of MCL (2 mg/kg, i.v.) against BIC convulsions was determined. Maximal protection (92% of rats tested) occurred 15-30 min after MCL and gradually declined over 300 min. ^{14}C-Muscimol experiments reveal that intravenously administered drug (2 mg/kg; 66.4 µc/kg) rapidly enters brain tissue. Ninety percent of maximum brain radioactivity was present 5 min after injection. Peak brain ^{14}C concentration (0.18±0.02 µg/g tissue) occurred 30 min after ^{14}C-MCL. Radioactivity decreased very slowly over the next 360 min to 58% of maximum. Twenty-four hrs post injection 0.046±0.001 µg/g of radiolabel remain in the brain. Thus peak brain concentration of radioactivity occurred simultaneously with maximum protection against BIC seizures. Further studies in progress will determine amounts of parent compound and metabolite(s) present with time. These results suggest that systemically administered MCL readily penetrates rat brain and parent compound and/or metabolite antagonizes BIC blockade of GABA receptors.

1360 LITHIUM TREATMENT EFFECTS ON CHOLINE TRANSPORT AT THE BLOOD-BRAIN BARRIER. A. L. McCall,* W. R. Millington, L. J. Botticelli and R. J. Wurtman. MIT, Cambridge, MA 02139.(Spon:J.Fernstrom)

We have characterized lithium chloride's (LiCl) effects on choline transport into the brain using the Brain Uptake Index (BUI) technique described by Oldendorf. This technique measures the percent extraction of a test compound (e.g., choline) from the blood (carotid artery) into the brain by comparing the carrier-mediated transport of a ^{14}C-labeled test substance to the passive diffusion of $^{3}H_2O$.

We observed that: 1) Single injections of LiCl (50 mg/kg-200 mg/kg) decrease the carrier-mediated transport of choline into the brain in a dose-dependent fashion (Table 1) without affecting the transports of glucose, tyrosine, glutamate or adenosine; 2) Addition of LiCl to the solution injected into the carotid artery (in doses of 5mM and 10mM) also decreases blood-brain barrier choline transport (from 6.71 ± 0.46% extraction for controls to 5.85 ± 0.42% and 4.56 ± 0.68%, respectively, for 5mM and 10mM); 3) Chronically administered LiCl, given in the rats' diets, elevates serum lithium levels and decreases choline transport into the brain; 4) Other group IA elements, cesium and rubidium, also decrease the barrier transport of choline.

TABLE 1. Acute Effects of LiCl Injection on Serum Lithium Levels and Choline Transport into the Brain

Dose of LiCl	% Choline Extraction 30 min later	Serum Lithium meq/liter
Control	6.71 ± 0.46*	-
50 mg/kg	4.94 ± 0.45	1.69 ± 0.61
100 mg/kg	4.63 ± 0.53	2.45 ± 0.48
200 mg/kg	3.57 ± 0.18	6.71 ± 0.78

*All values expressed as means ± SEM.

Addition of 20 mmoles of LiCl per kg dry weight to an 18% casein diet given to rats for 5 weeks produced no change in the choline transport into the brain. However, addition of 50 mmoles LiCl per kg dry weight decreased brain choline extraction by about 57%. Serum lithium levels were 0.34 ± 0.03 and 0.56 ± 0.04 respectively in these two groups of animals. Cesium or rubidium (10mM as the chloride salt), added to the carotid artery injection solution, each decreased the extraction of choline by 32%.

Our observation that acute or chronic lithium administration decreases choline transport into the brain raises the possibility that lithium also alters central cholinergic neurotransmission.

1361 ACUTE MORPHINE ADMINISTRATION INCREASES INCORPORATION OF ^{3}H-THYMIDINE INTO BRAIN STRIATAL DNA. Rita B. Messing, Jack C. Waymire*, Gary S. Lynch, Sam A. Deadwyler and Cort Flinchbaugh*. Dept. of Psychobiol., Univ. of Calif., Irvine, CA 92717.

The extremely long time-course of some morphine effects suggests that opiates may induce a permanent anatomical alteration in brain. One possibility is that morphine induces changes in glial cell proliferation. For this reason we undertook a study of tritium uptake into DNA fractions of rat brain regions following in vivo administration of [methyl-^{3}H] thymidine and morphine. The spleen was also examined as a control tissue. In acute experiments adolescent male rats were treated with 10 mg/kg of morphine SO_4 or vehicle and 1 mg/kg of naloxone HCl or vehicle 30 and 45 min, respectively, prior to administration of 800-900 μCi/kg of ^{3}H-thymidine. In chronic experiments rats were given 3 injections at 72 hr intervals of 150, 300 and 450 mg/kg of a slow-release preparation of morphine or vehicle. On the tenth day following the first injection, some rats were given naloxone 15 min prior to ^{3}H-thymidine. Rats were sacrificed one hr after thymidine injections and DNA fractions were isolated from tissues Acute morphine administration increased incorporation of ^{3}H-thymidine into DNA of rat striatum to 150% of control. This effect was antagonized by naloxone. No effect of morphine on thymidine incorporation was observed in the diencephalon or midbrain. In contrast, in the spleens of morphine-injected rats incorporation into DNA dropped to 45% of the control value. This effect was also antagonized by naloxone. No change in ^{3}H-thymidine incorporation into DNA was observed in any area of the brains of morphine-addicted rats or in rats undergoing withdrawal. In spleens, however, ^{3}H-thymidine incorporation was decreased to 60% of control in rats undergoing naloxone-precipitated withdrawal. To see if the observed changes in incorporation of ^{3}H-thymidine into DNA in striatum and spleen could be accounted for by differences in the local availability of the label in morphinized rats, amounts of tritium in DNA were correlated with amounts in supernatants of the tissue homogenates. Again, thymidine incorporation was higher in striatal DNA of morphinized rats, even though there was less tritium in supernatants. There was again less label in DNA in spleens, but this was correlated (r = 0.92) with lesser amounts of label in the supernatants from morphine-injected rats. The apparent decrease in mitosis in the spleen is therefore probably due to a decrease in the availability of the label, but the increased incorporation of ^{3}H into striatal DNA cannot be a consequence of greater access of thymidine to the striatum. The increase of thymidine incorporation into DNA in the striatum probably reflects an increase in glial cell proliferation induced by morphine. Supported by grant DA 01685.

1362 MORPHINE DOES NOT PRODUCE CATALEPSY. B. H. Moon*, J. J. I. Feigenbaum*, W. J. Weiner, and H. L. Klawans (SPON: C. Schauf). Dept. Neurosci., Rush Presbyterian St. Lukes Med. Center, Chicago, Ill., 60612

The administration of morphine (M) produces a dose-dependent catalepsy (C) in the rat which is regarded as one of the primary behavioral features of this drug. A few investigators have objected to the classification of M as a cataleptic because it produces skeletal muscle rigidity. Such objections are not relevant to low doses of M, however, which induce C that is indistinguishable in appearance from that produced by drugs which induce C without rigidity (e.g. neuroleptics: Costall and Naylor, 1974).

Among the several most commonly used test models of C are those measuring the length of time an animal will rest its forepaws on an elevated bar (1); or one of its hindlegs on an elevated surface (2); the number of times an animal inverted by its tail will pass through a cylinder (3); and the length of time an animal requires to right itself from a prone position (4). All four tests were used to determine their relative sensitivity, reliability, and specificity in measuring C in Sprague-Dawley rats injected with M (20-70 mg/kg) or haloperidol (H)(1-10 mg/kg). Of the tests used, (1) proved the most sensitive and reliable, and (4) the least sensitive and reliable. However, all four tests proved to have poor specificity. While measuring the ability to initiate movement, they failed to measure the ability to sustain exogenously stimulated movement as they provided little or no external stimulation. To measure this important but widely ignored parameter of immobility, a fifth test of C providing external stimulation was administered to M or H injected rats. Each animal was flipped by its tail into the air to determine if it could right itself (i.e. land on its feet). Throughout the entire dose range of H, none of the animals could properly right themselves and were inert after landing. By contrast, the M injected animals all righted themselves irrespective of the dose used, and exhibited a dose-dependent locomotor hyperactivity upon landing. Apparently, M inhibits only the initiation of movement, but once external stimulation is provided, M can stimulate and sustain movement in a dose-dependent manner. We propose that any definition of C should include: 1) an inability to initiate movement, 2) an inability to respond to external stimulation with movement, 3) an absence of somnolence or paralysis.

1363 LIDOCAINE MAY BE A CENTRAL STIMULANT. P. A. Nausieda, J. J. I. Feigenbaum*, H. L. Klawans, Dept. Neurlogical Sci., Rush Presbyterian St. Lukes Med. Center, Chgo. Ill., 60612

Lidocaine (L) is a synthetic local anaesthetic related to cocaine and widely used in the treatment of cardiac arrhythmias. Though sharing the nerve blocking effect of cocaine, L is thought to be devoid of central stimulant activity. A number of observations, however, suggest that this concept is erroneous. L is known to demonstrate reverse tachyphylaxis relative to seizure threshold when chronically administered, and to induce compulsive stereotyped behavior (SB) in sub-human primates, properties which suggest a similarity to the actions of cocaine centrally. The ability of L to induce SB in rodents was studied. The induction of SB in the rat is a well accepted model of central stimulant activity which is felt to represent dopaminergic activity at the level of the corpus striatum. L was administered s.c. to Sprague-Dawley rats at doses of 5-20 mg/kg. SB was rated at 5 minute intervals using a previously described scale. At low doses of L a clear-cut SB was induced. At intermediate doses, SB was seen initially, but was inhibited by the induction of sedation, only to reappear as the apparent sedative effect cleared. At doses above 10 mg/kg, the induction of sedation was of shorter latency and longer duration, and at 20 mg/kg, only an initial sedative effect was observed. The effect of L on the SB induced by amphetamine (5 mg/kg) or apomorphine (.5 mg/kg) was also studied. L showed mild potentiation of amphetamine induced SB at low dosages. Amphetamine at this dosage did not appear to reverse L induced sedation. Apomorphine (Ap) .5 mg/kg appeared to shorten the latency of onset of sedation induced by L and it enhanced the sedative effect at all dosages. Low doses of Ap were employed and inhibition of Ap induced SB was observed when low doses of L were concomitantly administered. L was unable to reverse reserpine induced cataplexy, and could not induce SB in animals pretreated with α methyl-para-tyrosine. Haloperidol (H) completely blocked L induced SB. Our data clearly demonstrate that L possesses central stimulant-like activity in this animal model. In addition, it appears that L possesses a sedative effect which predominates when higher doses are employed and may mask the central stimulant effect of this agent. Finally, the blockade of L induced SB by H, reserpine, and α methyl-para-tyrosine suggests that L induces SB via a central dopaminergic mechanism.

1364 DIFFERENTIAL EFFECTS OF CARBON MONOXIDE ON DOPAMINE AND NOREPINEPHRINE TURNOVER IN RAT BRAIN. M.B. Newby*, R.J. Roberts*, and R.K. Bhatnagar, (SPON: R. Roskowski, Jr.) The Toxicology Ctr., Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

Carbon monoxide (CO) poisoning produces neurological disorders which may involve damage to catecholaminergic neurons. Exposure to CO produces a reduction in turnover of dopamine (DA) in rat striatum as indicated by the decline of DA levels subsequent to synthesis inhibition by α-methyl-p-tyrosine (AMPT) (Newby, et al. JPET, in press). We now report the specificity of the CO effect on catecholamines and the dependence of the effect on DA turnover on the concentration of CO. Adult rats were injected with AMPT (250 mg/kg) or saline. This dose of AMPT blocks the conversion of ^{3}H-tyrosine to ^{3}H-DA or -norepinephrine (NE) for at least 5 hr in the presence of air or CO. One hr after injection the rats were exposed to CO (1700 ppm), hypoxia (7.5% O_2) or air and killed after 3 hr of exposure. Various regions of the brain were dissected for assay of DA and NE levels. Both CO and hypoxia exposures significantly decreased the DA turnover in the caudate nuclei and olfactory tubercles but were without effect on NE depletion in the hippocampus and olfactory tubercles. In contrast, NE turnover in the hypothalamus was increased by both CO and hypoxia.

In separate experiments the effect of varying the concentration of CO and duration of CO exposure on DA turnover were studied. AMPT was injected as above and 1 hr later rats were exposed to CO. After 3 hr of exposure, DA levels in the striata were elevated 12, 24, and *64% above air controls in rats exposed to 500, 1000, and 1500 ppm CO, respectively (*P<.05). Other groups of rats were given a second injection of AMPT (175 mg/kg) and exposures to 500 or 1000 ppm CO were continued for an additional 3 hr. The turnover of DA was reduced in the striata of rats subjected to 7 hr of 1000 ppm CO; 500 ppm CO did not produce this effect. In addition, steady-state DA levels in saline treated rats exposed to 1000 or 1500 ppm CO were significantly elevated above levels in the saline treated air controls; this effect was absent in the rats exposed to 500 ppm CO.

In conclusion, the effects of CO and hypoxia on NE turnover differ from their effect on DA turnover. Moreover, the effect of CO on DA turnover is concentration-dependent. The reasons for the differential sensitivity of DA and NE neurons are not known but likely involve alterations in the catabolism or release of the transmitters. (Supported in part by PHS Grant 5T32GM07069-03).

1365 ELECTROPHYSIOLOGY OF ETHANOL WITHDRAWAL COMPARED WITH THE BARBITURATE WITHDRAWAL IN THE SPINAL CORD. Masako Nozaki, and Michiko Okamoto, Cornell Univ. Med. Coll., New York, N.Y. 10021.

The segmental reflex system for the low spinal cord has long been an important model system for studying drug effects on the CNS. This system has been effectively utilized to uncover the synaptic alterations produced during barbiturate withdrawal (Rosenberg & Okamoto, JPET, 1976). Although electrophysiological changes in the spinal cord produced by a single acute dose of ethanol are known (Kolmodin, Acta Physiol. Scand., 1953; Miyahara, et al., JPET, 1966; Eidelberg & Wooley, Archiv. Int. Pharmacol., 1970; Meyer-Lohmann, Arch. Pharmacol., 1972; Lathers & Smith, JPET, 1976), no studies have been reported on ethanol withdrawal. Therefore, the present study was conducted in ethanol dependent animals (1) to uncover the neuronal alteration produced during withdrawal in the spinal cord (2) to compare the results of ethanol and barbiturate withdrawals (3) to correlate as much as possible these findings with behavioral withdrawal.

Cats were made physically dependent on ethanol administered twice daily via intragastric route for 30 days. All animals treated this way exhibited signs of severe dependence, including spontaneous grand mal type convulsions. 24 hours after abrupt withdrawal of ethanol, and when the animals display near peak intensity of withdrawal signs, electrophysiological measurements of spinal cord activity were made. The method used in the present study has been described for barbiturate withdrawal (Rosenberg & Okamoto, JPET, 1976, 1978). The excitatory functions measured by the amplitude of 2N spikes, polysynaptic discharge pattern, 2N discharge zone, frequency response curve for post-tetanic potentiation, and synaptic recovery time have been little affected by ethanol withdrawal compared to that produced by barbiturate withdrawal. On the other hand, the inhibitory functions treated by "direct" (post-synaptic) and pre-synaptic inhibitions were markedly attenuated.

These findings indicate that the primary role in alcohol withdrawal is a loss of functions in inhibitory pathways while excitatory functions are little affected. These may contribute to the general hyperexcitation (Kalant, et al., Pharm. Rev. 1971) and behavioral characteristics produced during alcohol withdrawal (Okamoto et al., Comm. Probl. Drug. Depend. 1978). (Supported by NIDA Grant DA-00591).

1366 ANTIDEPRESSANT RECEPTOR BINDING IN BRAIN. R. A. O'Brien*, N. M. Spirt* and W. D. Horst (SPON: E. B. Sigg). Hoffmann-La Roche Inc., Nutley, N.J. 07110

Antidepressant drugs bind specifically to rat brain synaptosome fractions. The binding, using [^{3}H]-imipramine as the radioligand, is rapid, saturable, reversible and of low affinity; half-maximal saturation occurs at 8-16 μM. Specificity of the interaction of antidepressants with receptor sites correlates with clinical efficacy but not with the inhibition of monoamine uptake. Thus, the clinically-active antidepressants, iprindole and mianserin, which are active in inhibiting [^{3}H]-imipramine binding (IC_{50}'s of 7 and 48 μM, respectively) are inactive in standard antidepressant evaluations such as inhibition of monoamine uptake and prevention of tetrabenazine effects in vivo. Amphetamines and a number of monoamine oxidase inhibitors are inactive or have only a slight effect on imipramine binding. Binding does not correlate with an effect on dopaminergic, cholinergic, histaminergic or other recently described aminergic receptor systems. Chronic treatment of animals with imipramine for three weeks (15 mg/kg i.p. twice daily) causes a 20-30% diminution in the amount of [^{3}H]-imipramine bound. In contrast to other types of specific drug receptor binding (e.g. benzodiazepine) which are reported to be inhibited only by structural analogs, imipramine binding is blocked by structures other than the classical tricyclics This binding phenomenon may be a valuable tool for investigations on the mechanism and site of action of antidepressants and the search for endogenous antidepressant- or depressant-like compounds in brain.

1367 CONTRALATERAL TURNING AFTER INTRANIGRAL KAINIC ACID IS INDEPENDENT OF THE TELENCEPHALON, INCLUDING THE STRIATUM. Georges Papadopoulos+, Joseph P. Huston. Inst. Psychol., Lab. Comp. & Physiol. Psychol., Univ. Düsseldorf, D-4000 Düsseldorf, West Germany.

Rats, under Equithesin anesthesia, were injected unilaterally into the substantia nigra with kainic acid (0.5 - 1 μg in a volume of 0.5 μl), and at the same time the whole telencephalon (including cortex, hippocampus striatum, globus pallidus and septum) was removed by suction, resulting in the so-called "thalamic rat" preparation. 24 hours after the operation the rats exhibited contralateral turning to various degrees. In some cases it was very intense, while in animals suffering movement difficulties, it could only be induced by tactile stimulation or had the form of contralateral asymmetry. In a "control" group of rats only kainic acid was injected unilaterally in the same dosage. All these animals showed intense spontaneous contralateral turning 24 hours after the operation in accordance with previous reports. However, in the first twelve hours after the operation ipsilateral turning was sometimes present for intervals up to several hours duration. The animals of the latter group showed an unusually strong sensitivity of tactile stimuli, to which they reacted with a very vigorous and persistent biting of any available object, so that handling of these rats was virtually impossible. A possibly comparable form of "supersensitivity" to tactile stimuli was sometimes observed in the thalamic animals with kainic acid lesion in the substantia nigra, who reacted with unusually and intense opening and closing of the mouth when grasped.

The above findings show that contralateral turning after unilateral nigral lesion with kainic acid is not dependent on the presence of telencephalic structures, including the striatum. This is possibly also true of other behavioral consequences of such lesions.

1368 A COMPARISON OF ETHYLKETOCYCLAZOCINE, KETAMINE AND PHENCYCLIDINE ON EEG AND BEHAVIOR IN THE DOG. Wallace B. Pickworth and Lawrence G. Sharpe. NIDA Addiction Research Center, Lexington, Kentucky 40583

We have found that ethylketocyclazocine (Win 35,197-2), an opiate antagonist of the κ type, has a pharmacological spectrum of activity similar to that reported for the disassociative anesthetics. In this study we compared the effects of Win 35,197-2 with those of ketamine and phencyclidine, two chemically related disassociative anesthetics with growing abuse potential. The compounds were studied in unrestrained adult beagle dogs with chronic electrodes implanted for recording cortical and hippocampal EEG. During a 2 hr experiment, the animals were placed in a dimly lit sound-attenuated chamber while their behavior was observed on a videomonitor. Intravenous drug injections and EEG records were made remotely. Ketamine (4 mg/kg) and Win 35,197-2 (0.05, 0.1 and 0.2 mg/kg) caused EEG-behavioral dissociation in that the dogs displayed cortical synchrony in non-sleeping positions. Both drugs decreased total sleep, abolished paradoxical sleep, and produced ataxia and hyperthermia. Ketamine, but not Win 35,197-2, caused stereotypic head bobbing. Phencyclidine (0.1, 0.25 and 1.0 mg/kg) caused stereotypic head bobbing, licking, EEG and behavioral activation at the lower doses, but grand mal seizures (in one animal) after the highest dose. No EEG-behavioral dissociation followed phencyclidine administration. Instead, sleep was abolished and cortical EEG desynchronized, accompanied by hippocampal theta activity. These results indicate that, in the dog, ketamine has agonistic activities similar to those of the κ agonists and that phencyclidine has opposite effects which are more amphetamine-like.

1369 MORPHINE IS NOT A SUBSTANCE P RECEPTOR ANTAGONIST. M. F. Piercey, R. P. Hollister*, L. A. Schroeder*, P. J. K. Dobry and F. J. Einspahr*. The Upjohn Company, CNS Research, Kalamazoo, MI 49001.

Morphine is reported to depress nociceptive neurons in the spinal cord dorsal horn. Since substance P (SP) is currently the leading candidate for the neurotransmitter released by primary pain afferents, morphine might depress dorsal horn neurons by antagonizing SP. In order to test the hypothesis that morphine is an SP receptor antagonist, we examined morphine's capacity to modify SP's effects on a variety of assays. Morphine concentrations as high as 9×10^{-4}M did not alter SP-induced contractions of the in vitro guinea pig ileum. Nevertheless, 3×10^{-6}M depressed serotonin-induced contractions by 50%. Morphine was also incapable of antagonizing in vivo effects of SP. Thus, concentrations as high as 10 mg/kg (i.v.) did not depress the SP induced hypotensive effects in urethane-anesthetized rabbits nor the sialagogic effects observed in pentobarbital-anesthetized rats. Finally, in a direct test of the hypothesis, morphine was found not to antagonize the discharges evoked in dorsal horn neurons by iontophoretically released SP. In these experiments, unanesthetized decerebrate cats with low (L1) spinal sections were used. Conventional 5 and 7 barrelled microelectrodes were used to eject drugs onto L6 and L7 dorsal horn neurons whose firing rates were monitored via the central recording barrels. Dorsal horn locations were verified by histological recovery of pontamine sky blue spots electrophoresed during the experiments. Morphine, 50-100 nA, did not antagonize SP-induced discharges for any of the cells studied. For some cells, morphine actually potentiated the responses. This effect was not specific for SP since morphine also sometimes potentiated responses to noxious cutaneous heat and iontophoretic glutamate. These weak excitatory effects of morphine, which were often accompanied by small increases in spontaneous activity, are considered to be due to the accumulation of supratherapeutic doses of morphine at the electrode tip. In confirmation of this theory, iontophoretic naloxone did not antagonize the excitatory actions of morphine. It is concluded that morphine is not an SP antagonist. Thus any spinal antinociceptive effects of morphine must be due to some other mechanism [e.g. decrease in SP release, Jessel and Iversen, Nature (1977) 268:549].

1370 IBOTENIC ACID: EXCITATORY AND INHIBITORY ACTIONS IN CEREBRAL CORTEX. E. Puil and K. Krnjević, Dept. of Anaesthesia Research, McGill University, 3655 Drummond St., Montreal, P.Q. Canada. H3G 1Y6

Ibotenic acid is generally considered as an excitatory agent, closely related to L-glutamic acid. In our experiments on neurons in the cat's cerebral cortex, when DL-ibotenic acid was applied microiontophoretically, we indeed observed a strong excitation but with a much slower time course than is seen when glutamate is applied (a long latency of onset and prolonged after-discharge was more prominent in cats under Dial than under methoxyflurane anaesthesia). Even minute, subthreshold amounts of ibotenic acid could strongly potentiate responses evoked by L-glutamate, applied either simultaneously or several seconds later. However, this potentiation could be prevented by a concurrent iontophoretic application of methohexital.

An even more impressive effect of ibotenic acid was a powerful and very prolonged depression of glutamate-evoked activity. This was produced by releasing ibotenic acid in conjunction with regular applications of constant amounts of glutamate: this would typically first enhance the glutamate responses, and then lead to ongoing firing; after the end of the release of ibotenic acid, there would be a 4-5 min period of silence before the continuing regular applications of glutamate started to become effective again. It is unlikely that the silent period was caused by a depolarizing type of block, since responses could be temporarily restored by increasing the iontophoretic dose of glutamate, or even by a short application of ibotenic acid. It is also unlikely that ibotenic acid antagonized specifically the action of glutamate, since responses evoked by acetylcholine were blocked just as effectively (and took even longer to recover). These observations confirm previous findings in the spinal cord (MacDonald & Nistri, 1978, J. Physiol., 275, 449). They are consistent with the possibility that ibotenic acid has a dual action on neuronal receptors: a relatively quickly reversible one on receptors to glutamate, leading to excitation, and a much more prolonged interaction with inhibitory receptors. One possible explanation for the inhibitory effect is a very slowly reversible combination of this close relative of muscimol with GABA receptors.

Supported by the Canadian Medical Research Council.

1371 CHANGES IN NEOSTRIATAL AND MESOLIMBIC NEURONAL ACTIVITY PRODUCED BY HALOPERIDOL AND CLOZAPINE. George V. Rebec. Dept. Psychol., Indiana Univ., Bloomington, IN 47401

Some recent behavioral and biochemical evidence has indicated that whereas the "classical" neuroleptics (e.g., haloperidol) may be acting equieffectively at neostriatal and mesolimbic dopamine (DA) sites, the "atypical" neuroleptics (e.g., clozapine) may exert selective effects on the mesolimbic DA system (see, Anden, J. Psychiat. Res., 1974, 11: 97). Other studies, however, have not supported this notion (see, Stanley and Wilk, Eur. J. Pharmacol., 1977, 44: 293).

In order to further elucidate the possible mechanism of action of these drugs, spontaneous neuronal activity in the neostriatum and accumbens nucleus of immobilized, locally anesthetized rats (350-450g) was recorded in response to intraperitoneal (i.p.) injections of haloperidol (1-2 mg/kg) or clozapine (20-80 mg/kg). Both drugs generally produced a prolonged dose-dependent increase in firing rate in the neostriatum which ranged in magnitude for individual neurons from 200-800 percent. Comparable results were obtained in the accumbens nucleus. In a separate group of animals, i.p. administration of 2.5 mg/kg d-amphetamine sulfate or 1.0 mg/kg apomorphine produced a prolonged depression of unit activity in the neostriatum and accumbens nucleus, and in each instance this response was rapidly reversed by either haloperidol or clozapine. The results of these experiments, which suggest that the neostriatal and mesolimbic DA systems are not differentially sensitive to the actions of haloperidol and clozapine, may have important implications for the behavioral pharmacology of the neuroleptics and related drugs.

This research was supported, in part, by Biomedical Research Support Grant #46-314-02 from Indiana University.

1372 MICROIONTOPHORESIS OF INHIBITORY AMINO ACIDS IN THE MEDIAL HYPOTHALAMUS : EVIDENCE FOR GABA AS AN INHIBITORY HYPOTHALAMIC NEUROTRANSMITTER. Leo P. Renaud, Quentin J. Pittman and Howard W. Blume. Division of Neurology, Montreal General Hospital and McGill University, Montreal, Canada, H3G 1A4

Recent electrophysiological studies on the connections of medial hypothalamic neurons have indicated the presence of a prominent postsynaptic inhibition presumably mediated by local inhibitory interneurons activated through recurrent or afferent pathways. This report details our preliminary observations on the neuropharmacology of these hypothalamic inhibitory pathways, with emphasis on the possible contribution of the inhibitory amino acids.

Experiments were conducted on pentobarbital anaesthetized male Sprague Dawley rats implanted with stimulation electrodes in several extrahypothalamic areas known to be connected to the medial hypothalamus. Using a transpharyngeal approach, extracellular recordings were obtained from 181 medial hypothalamic neurons. The excitability of these neurons was tested with the microiontophoretic application from multibarrel micropipettes of several test compounds. L-glutamate and L-aspartate enhanced the excitability of the majority of tested neurons. Both the glutamate evoked and spontaneous activity of these cells could be depressed in a dose dependent manner by application of GABA, glycine and related amino acids according to the following order of potency : β-guanidinopropionic acid (β-GP)> GABA > β-alanine > δ-aminovaleric acid (δ-AVA) > glycine. Simultaneous microiontophoretic application of picrotoxin or bicuculline appeared to selectively antagonize depressions evoked by β-GP, GABA and δ-AVA, whereas strychnine appeared to cause a selective antagonism of the actions of glycine and β-alanine.

Picrotoxin, bicuculline and strychnine were also tested for possible antagonistic effects on postsynaptic inhibition evoked by stimulation of extrahypothalamic sites. The microiontophoretic and intravenous administration of picrotoxin and bicuculline, but not strychnine, could be shown to partially or completely antagonize postsynaptic inhibition. However, with intravenous administration, this action usually required administration of antagonists in convulsive doses.

These observations suggest that hypothalamic neurons have both GABA and glycine receptors, but that GABA is the more likely candidate as a hypothalamic inhibitory neurotransmitter. (Supported by the MRC).

1373 DIFFERENTIAL EFFECTS OF ANESTHETIC-LIKE DRUGS ON ISOLATED NEURON PREPARATION. Sheldon H. Roth and Bruce M. MacIver*. Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

The isolated abdominal muscle receptor organ (MRO) of the crayfish (Procambarus clarkii) provides an excellent neuronal model system for a study of the effects of anesthetics and various other depressants on cellular activity. It is generally accepted that the mechanism of action of all anesthetics is similar at the molecular (membrane) level, and the basic mechanism of action is purely a physicochemical interaction of a lipid soluble substance with a hydrophobic region. We propose that all anesthetics do not act via a common mechanism, but can demonstrate a spectrum of activity (e.g. depression and excitation) at the cellular level which suggests a selectivity of effect. Several neurophysiological parameters of the MRO are monitored simultaneously, using both intra and extracellular techniques, in the absence and presence of drugs. Input stimulus (i.e. stretch) is varied and quantitatively measured via a sensitive strain gauge while simultaneously recording neuronal activity. The preparation is sensitive to relatively low concentrations of depressant drugs, such as ethanol, pentobarbital, methoyflurane and harmaline. All the agents are capable of depressing the characteristic output activity of the MRO in a dose and time related fashion. However, in addition to the general depressant activity, most of the agents elicit an initial excitatory effect, and some are capable of producing a unique alteration of the temporal pattern of discharge.

Harmaline, at concentrations of the order of 10^{-5}M changes the characteristic single action potential output to a rhythmical doublet or paired activity. Ethanol is effective at concentrations as low as 0.1mM, and at 10mM produces a periodic discharge of high frequency bursts, consisting of between 7 to 15 spikes/burst. Other burst patterns and lack of burst patterns have been observed with various other drugs. These distinct changes in action potential discharge are both drug and dose dependent, and may result from direct interactions with neuronal membranes in a selective manner.

Supported by Medical Research Council of Canada.

1374 THE EFFECT OF MONOACYLATED DIAMINES ON THE SLEEP-WAKING BEHAVIOR OF THE RAT. Steven K. Salzman* and Matej Stepita-Klauco, Dept. Biobehavioral Sci., Univ. of Conn., Storrs, Conn. 06268.

Cadaverine (1,5-diaminopentane) and its acylated derivatives occur in mammalian brain (Dolezalova et al., 1974, 1977) but a physiological function unique to the nervous system has not been ascribed to these compounds. Cadaverine has been shown to be taken up by brain slices (Picolli, 1972) and along with its acyl derivatives (acetyl and propionylcadaverine) is purportedly elevated in the urine and blood of schizophrenics (Perry, 1967, Dolezalova, 1977). Single cell microiontophoresis studies by our group showed that while cadaverine had no effect on the membrane potential of molluscan neurons its acyl derivatives caused a long-lasting, slowly decrementing depolarization (Miller, 1977). It was suggested that masking of one of the amine groups of cadaverine by acylation rendered a pharmacologically active monoamine. The purpose of this study was to test if the same pharmacological profile could be seen in a mammalian system. Black-hooded rats were chronically implanted with screw electrodes for recording both the EEG and EMG. One week was allowed for recovery, then the rat was connected to a recording cable and habituated for two days in a temperature controlled chamber before records were taken. After a 4-5 day period acetylcadaverine was administered. Drug delivery was done with an osmotic minipump providing continuous doses at 100ng/hr, 10ug/hr and 100ug/hr, respectively. EEG power spectra were calculated for each behavioral stage. Acetylcadaverine caused a biphasic change in the average duration of NREM sleep and the average NREM to waking interval. At the low dose (100 ng/hr), the average duration of sleep, NREM, and the NREM to waking interval were all decreased. With the middle dose (10 ug/hr) these parameters were all increased. At the highest dose (100 ug/hr), only NREM duration was increased, with an increased variance of values for other parameters. Interesting day-night differences also emerged. With the lowest dose, the average NREM epoch was shorter only at night, while the middle dose had the same effect at night as it had during the day. Spectral analysis of the EEG signal revealed an increase in its power in the bandwith between 15-18 Hz during NREM with no change in either the waking or REM corticogram. Studies are in progress on the effects of chronic intracerebral administration of cadaverine, putrescine and their acyl derivatives on sleep-waking behavior and the EEG spectral components.

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1375 ELECTROPHYSIOLOGICAL EVIDENCE FOR A DIFFERENCE IN THE NOREPINEPHRINE (NE) AND DOPAMINE (DA) NEUROTRANSMITTER STORAGE MECHANISMS. M.K. Sanghera*, D.C. German, R.S. Kiser and P.A. Shore. Depts. of Physiol., Psychiat. and Pharmacol., U. of Texas Health Sci. Ctr., Dallas, TX 75235.

Biochemical studies provide evidence for the existence of a fundamental difference between the NE and the DA neurotransmitter storage systems. For example, rapid equilibration occurs between stored and releasable NE pools (McMillen & Shore, 1977), whereas the rate of movement of stored DA to an impulse-releasable site occurs only slowly (Shore & Dorris, 1975). The slow equilibration between the stored and releasable (newly-synthesized) DA pools is consistent with the electrophysiological finding that inhibition of tyrosine hydroxylase with α-methyl-para-tyrosine (AMPT) blocks or reverses the d-amphetamine (d-AMP)-induced depression of DA unit activity; the d-AMP action being dependent upon the presence of a newly-synthesized transmitter pool (cf., German, et al., 1978).

The aim of the present study was to examine, by electrophysiological means, the hypothesis that the NE and DA storage systems differ. If the stored and releasable NE pools are in rapid equilibrium, then AMPT should not influence the inhibitory action of d-AMP on NE neuronal impulse flow. In the chloral hydrate-anesthetized rat, recordings were made from single cells in the locus coeruleus (LC). The identification of these neurons was based upon electrophysiological characteristics as reported by Cedarbaum & Aghajanian (1977), and subsequent histological verification. All drugs were given via a jugular catheter. Animals were pretreated with AMPT (50 mg/kg), 20-60 min. prior to the administration of d-AMP. All LC neuron firing rates were reduced to below 50% of their basal level with 0.5-1.0 mg/kg of d-AMP. The d-AMP-induced suppression in LC unit activity was subsequently increased to or above basal levels by an injection of the α-adrenoreceptor-blocking agent, dibozane. These electrophysiological data are consistent with the above-mentioned biochemical and electrophysiological studies which provide evidence for a difference between the NE and DA neurotransmitter storage mechanisms. (Supported by USPHS grants MH-27574 and MH-05831.)

1376 ALTERATIONS OF CENTRAL AUTONOMIC CARDIOVASCULAR RESPONSES BY Δ^9-TETRAHYDROCANNABINOL. William T. Schmeling* and Michael J. Hosko. Department of Pharmacology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226.

Δ^9-Tetrahydrocannabinol, the primary psychoactive component of marijuana, has a pronounced ability to induce hypotension and bradycardia in experimental animals. This hypotensive effect and induced bradycardia have been postulated to occur partially through reduction of sympathetic tone to the cardiovascular system. The ability of Δ^9-THC to induce hypotension is not compromised by transection of the neuraxis at the midcollicular level but is abolished by high cervical section. Experiments were conducted in rats and cats to determine the effect of Δ^9-THC on carotid sinus nerve (CSN) baroreceptors. Cats and rats were anesthetized with a combination of α-chloralose (50-60 mg/kg) and urethane (500-600 mg/kg) or transected at midcollicular levels. Bipolar tungsten hook electrodes were placed on the stumps of proximal (PV) and distal vagus (DV) in those animals in which the vagi were sectioned. Square wave pulse trains of 10 second duration (16-80 Hz, 0.1-0.2 msec, 0.25-4.0 ma) were delivered to the CSN, PV and DV. Stimulus current was selected to produce "threshold," two, and four times threshold stimuli to the PV and CSN. A single maximal stimulus was delivered to DV. Stimulus induced alterations in blood pressure (BP) and heart rate (HR) were recorded.

In both vagotomized, nonvagotomized and midcollicular rats and cats Δ^9-THC (2.0 mg/kg, iv) essentially abolished the pressor response to carotid occlusion and significantly attenuated (0.0-30.0% of control) the depressor response to CSN stimulation within 5 min of administration. In some animals, Δ^9-THC reversed the blood pressure response to CSN stimulation. PV stimulations graded to produce pressor responses were augmented after Δ^9-THC, while those producing depressor responses were attenuated. The response to DV stimulation was unchanged. These results would be consistent with altered central neuronal processing of cardiovascular afferent information following administration of Δ^9-THC. These findings are consistent with the hypothesis that Δ^9-THC induces significant disruption of sympathetic and parasympathetic tonic homeostatic cardiovascular control mechanisms. (Supported by USPHS NIH Grant DA00124.)

1377 EFFECTS OF DIETARY AND PARENTERAL CHOLINE ADMINISTRATION ON AMBULATORY ACTIVITY IN RATS. Dennis E. Schmidt*, Robert J. Barrett* and Lynn Wecker (SPON: D.M. Buxbaum). Dept. Pharmacol., Vanderbilt Univ. Sch. Med., Nashville, TN 37232.

Parenteral administration of choline (Ch) to rats has been shown to alter the sensitivity of discrete regions of the brain to biochemical manipulation by pharmacological agents (Sci.199:86, 1978;Fed.Proc.37:820,1978). In light of clinical findings that Ch loading may be beneficial in alleviating mood or movement disorders, we have extended our biochemical studies and investigated the effects of dietary and parenteral Ch administration on behavior. Male Sprague-Dawley rats (145g) were maintained for 2 weeks, with water available ad libitum, on a dietary regimen consisting of: a) a Ch free (CF) diet, b) a "normal" (N) rat diet, 1.6g free Ch/kg chow or c) a high Ch (HC) diet, 11.4g free Ch/kg chow. At the end of the 2 week period, rats were injected (ip) with either saline, ChI (60 mg/kg free base), atropine sulfate (20 mg/kg) or Ch followed by atropine at 60 min. Rats were placed in a symmetrical Y-maze and ambulatory activity was recorded automatically for a 30 min period following drug administration. Basal activity (saline injected) did not differ between rats in the N and CF groups, whereas, rats on the HC diet exhibited a basal activity 186% of the activity of rats on the N diet. Ch administration did not affect ambulatory movement in any of the dietary groups,while atropine significantly (P<.05) increased the activity of animals in both the N and CF groups to 171% and 159% of basal activities, respectively.The activity of rats on the HC diet was not different from basal HC group activity after atropine administration, but was 249% of basal N group activity.When Ch was administered 60 min prior to atropine, the atropine-induced increase in activity in N rats was prevented (P<.05) and activity was not different from basal activity for rats in the N group. In the CF group, Ch pretreatment potentiated the actions of atropine to 263% of basal activity (148% of the atropine responses). Ch pretreatment did not alter the responses to atropine in rats on the HC diet. Therefore, it is apparent that dietary levels of Ch may be involved in the mechanisms regulating locomotor activity in rats.In addition, we have extended our previous observation on the modification of the central actions of atropine by Ch pretreatment to include behavioral as well as biochemical parameters.Therefore, the nutritional status of an animal,i.e.,Ch availability,may be significant in determining the efficacy, both behavioral and biochemical, of pharmacological agents affecting cholinergic function in the central nervous system.Further studies will hopefully elucidate the specific mechanisms involved in this interaction. (Supported by a Biomedical Research Support Grant RR-05424-16 and an NIMH Grant MH-29182.)

1378 A TONIC MUSCARINIC INHIBITORY INPUT TO PUPILLOCONSTRICTOR NEURONS IN THE EDINGER-WESTPHAL NUCLEUS OF THE DOG. Lawrence G. Sharpe and Wallace B. Pickworth. NIDA Addiction Research Center, Lexington, Kentucky 40583

Male and female beagle-type dogs had indwelling guide cannulae (19 ga) implanted with the tips located above the Edinger-Westphal (EW) nucleus. The animals were acclimated to sling restraints, and during experimentation a concentric chemitrode (30 ga inner, 23 ga outer, insulated except at the tips) was lowered through the guide cannula into a site where electrical stimulation (20 Hz, 0.5 msec, 2-6 V) had produced marked miosis without ocular movements. Drugs dissolved in sterile saline were injected in a 0.5 µl volume over 1 min into this site. The injection cannulae were located within the EW nucleus as verified by standard histological procedures. Pupil diameter was measured photographically. Carbachol (0.05 to 0.25 µg) and physostigmine (5 to 10 µg) produced a dose-dependent pupillodilatation. Pretreatment with microinjections of methylatropine nitrate (2.73 nmol, in 1.0 µl) completely antagonized the mydriatic activity of carbachol (0.1 µg) and physostigmine (5 µg). Microinjections of the nicotinic antagonists mecamylamine (2.73 nmol) and hexamethonium (2.73 nmol) did not block the carbachol-induced mydriasis. Methylatropine, but not mecamylamine and hexamethonium, produced miosis. Microinjections of carbachol (0.1 µg) into sites 2 mm above or below the site that yielded stimulation-produced miosis caused either no change or a delayed mydriasis. The mydriatic activity of the cholinomimetics was unrelated to peripheral sympathetic activation. These data suggest that the tonic inhibitory input to the EW nucleus is muscarinic. Drugs that act centrally to produce miosis (morphine) and mydriasis (amphetamine) may act on this proposed cholinergic inhibitory pathway.

1379 EFFECTS OF CHRONIC FLUPHENAZINE ON STRIATAL CHOLINERGIC AND DOPAMINERGIC MECHANISMS: A NEUROCHEMICAL AND BEHAVIORAL ASSESSMENT. Kathleen A. Sherman,* Ann L. Acheson,* Michael J. Zigmond, and Israel Hanin. Departments of Psychology, Biological Sciences and Psychiatry (WPIC), University of Pittsburgh, Pittsburgh, PA 15260.

Tolerance is known to develop to the clinically observed extrapyramidal effects of neuroleptics such as fluphenazine. Since these drugs are believed to act in part by blocking striatal dopamine (DA) receptors, we have compared some of the effects of acute and chronic fluphenazine treatment (0.5 mg/kg, s.c.) on two indices of the post-synaptic response to DA. First, we examined the effect of such treatments on striatal acetylcholine (ACh)-containing interneurons, cells which are known to receive an inhibitory DA innervation. We had previously shown that a single injection of fluphenazine decreases striatal ACh concentration without altering high affinity choline (Ch) uptake, the rate-limiting step in ACh synthesis. This is consistent with reports that such treatments increase ACh release. In the present studies rats were injected daily with fluphenazine or saline, and striatal ACh concentration was determined 60 min after the last injection. After a single injection of fluphenazine, striatal ACh concentration was reduced by 40%. Striatal ACh content was reduced by 30% after 5 daily injections, but was unchanged after the tenth day of injection. High affinity Ch uptake was unchanged throughout these studies. These data suggest that, after 10 daily repeated injections, ACh release is no longer increased by fluphenazine, perhaps because the drug is no longer effective in blocking the inhibitory action of DA. In the second series of experiments we examined the ability of fluphenazine to block the behavioral effects of apomorphine (.05-32 mg/kg, s.c.), a dopaminergic agonist. A method of scoring was used which permitted a detailed analysis of the drug-induced behavior. A single dose of fluphenazine completely blocked the effects of apomorphine at all but the highest doses tested (>16 mg/kg). In contrast, the tenth daily injection of fluphenazine was approximately 16-fold less effective in blocking these effects (although significantly more effective than saline). These neurochemical and behavioral studies both suggest that the ability of fluphenazine to inhibit the post-synaptic effect of striatal DA is considerably reduced after chronic pretreatment with this drug.
(Supported, in part, by USPHS grants MH20620, MH00055, and MH 26320.)

1380 IN VIVO SYNTHESIS RATE OF SEROTONIN (5-HT) AND CATECHOLAMINES (CA) IN BRAIN AND SPINAL CORD OF YOUNG SPONTANEOUSLY HYPERTENSIVE (SH) RATS. M. L. Smith*, R. A. Browning and J. H. Myers* (SPON: D. G. King). Southern Illinois University, School of Medicine, Carbondale, Ill. 62901.

Recent work in several laboratories, including ours (Browning et al., Fed. Proc. 36, 4059, 1977), has led to considerable controversy regarding the role of central serotonergic neurons in the development and maintenance of hypertension. Moreover, several laboratories have implicated centrally occurring CA in the regulation of blood pressure in the SH rat. Accordingly, we have now examined the simultaneous in vivo synthesis rates of 5-HT and the CA in hypothalamus (HYP), pons-medulla (P-M) and spinal cord (SC) of 4-week-old (prior to the development of hypertension) and 8-week-old SH and normotensive (NT) rats. Synthesis rates of 5-HT and CA were obtained by measuring the accumulation of 5-hydroxytryptophan (5-HTP) and DOPA following inhibition of aromatic amino acid decarboxylase with Ro4-4602. Blood pressure was recorded in all rats using the indirect tail cuff method on the day prior to decarboxylase inhibition and sacrifice. Animals were sacrificed at 0 or 30 min following pretreatment with Ro4-4602 (800 $mg \cdot Kg^{-1}$; i.p.). 5-HTP and DOPA in tissue samples were measured fluorometrically after separation on Dowex-50 W columns. No differences in CA synthesis rates between SH and NT rats could be detected at 4 or 8 weeks of age. However, as shown in the table below, we did find a significant increase in the rate of synthesis of 5-HT in P-M and SC of pre-hypertensive, 4-week-old SH rats. This difference was not detected in the 8-week-old SH rats with established hypertension. These findings show a transient increase in 5-HT turnover during the development of hypertension which is not continued during the maintenance phase.

5-HTP Accumulation ±SEM (ng/g/hr)

	4-wk-old		8-wk-old	
	NT	SH	NT	SH
HYP	1387±305	1337±221	1076±249	853±188
P-M	614± 47	1133±138*	716± 81	679±128
SC	242± 22	435± 25**	203± 31	185± 43

*p<0.01 compared to NT control; **p<0.001 compared to NT control.

(Supported by a grant from the Illinois Heart Association.)

1381 6-METHOXY-1,2,3,4-TETRAHYDRO-β-CARBOLINE: A SPECIFIC MONOAMINE OXIDASE-A INHIBITOR IN CF1 MICE. David L. Sparks* and Neil S. Buckholtz., Dept. Biochem. and Dept. Psychiat. Behav. Sci., Med. Univ. S.C. Charleston, S.C. 29403.

Buckholtz and Boggan (Biochem. Pharmac. 26, 1991, 1977) reported a time course of inhibition of brain monoamine oxidase-A (MAO-A) by 6-Methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THβC; 100 mg/kg ip) with a maximal inhibition (22%) by 1 hr. They also showed an inhibition (13%) of MAO-B. This was done in whole brain homogenates of CF1 mice, using a column method for metabolite extraction. Meller et al. (J. Neurochem 28, 995, 1977) showed an inhibition (55%) of MAO-A and no inhibition of MAO-B by MeO-THβC (100 mg/kg) in rat hypothalamus, using an organic metabolite extraction. They also reported an IC50 of 3.7 μM for 6-MeO-THβC in vitro. The present study was done to determine if difference in amount of inhibition by 6-MeO-THβC was due to strain difference or extraction technique, and also to characterize further the MAO inhibition produced.

These studies were done using Meller et al.'s extraction procedure. First, the lowest concentration of substrates giving linear MAO activity over a 30 min period was determined. These were 1.5 μM serotonin (5-HT) for MAO-A and 4.0 μM β-Phenylethylamine (PEA) for MAO-B. It was then determined that there were no differences in inhibition patterns seen whether using [H^3] or [C^{14}]-5-HT as substrate for MAO-A. 6-MeO-THβC had an IC50 of 1.6 μM, in vitro, using whole brain homogenate. A time course of apparent in vivo MAO-A inhibition using 6-MeOTHβC (100 mg/kg) showed a maximal inhibition by 1 hr (56%), a decline to 30% at 12 hr, and no inhibition at 24 hr. There was a maximal inhibition of MAO-B by 1 hr (16%), a decline to 5% at 12 hr, and no inhibition at 24 hr. A dose study done at 1 hr. after injection showed 6-MeO-THβC to inhibit MAO-A by 25% at 25 mg/kg, 43% at 50 mg/kg, and 58% at 100 mg/kg; for MAO-B there was no inhibition at 25 and 50 mg/kg and 15% inhibition at 100 mg/kg. In vivo kinetic studies showed MAO-A to have a Vm of 0.19 μM product/hr/μg protein and a Km of 61.2 μM, with 6-MeO-THβC showing competitive inhibition. MAO-B had a Vm of 0.15 μM product/hr/μg protein and a Km of 11.4 μM, with 6-MeO-THβC showing non-competitive inhibition.

These data indicate that more accurate MAO values can be obtained by using organic extraction of metabolites. Any strain and/or brain region differences in MAO inhibition seem to be in the B form. The data also indicate that, at doses ≤ 50 mg/kg, 6-MeO-THβC is a specific MAO-A inhibitor. The kinetic data confirm the existence of two distinct MAO enzyme forms, due to differential inhibition patterns caused by 6-MeO-THBC. Supported in part by P.H.S. grant MH-26712.

1382 SPECIFIC BINDING OF ^{3}H-DIPHENYLHYDANTOIN TO RAT WHOLE-BRAIN HOMOGENATE. L. Spero* and M. Burnham. Dept. Pharmacol., Fac. Med., University of Toronto, Toronto, Ont., Canada.

Radioreceptor assays have been conducted on rat whole-brain homogenate using ^{3}H-diphenylhydantoin (^{3}H-DPH) and a modified filter-assay technique. These studies indicate the existence of a high-affinity, saturable binding site. Spirodilantin A, an active anticonvulsant enantiomer, competes for ^{3}H-DPH binding whereas Spirodilantin B, the inactive stereoisomer, does not. The anticonvulsant barbiturates also show significant competition for the high-affinity binding site, but the anti-petit mal drugs, trimethadione and ethosuximide, do not. These results suggest that the anticonvulsant drugs effective against grand mal seizures may act via a specific receptor system in the brain. Research in progress will attempt to determine the regional distribution of the specific binding sites, and to establish a correlation between binding potencies and clinical effectiveness within a large series of anticonvulsant drugs, including some with stereoisomer pairs.

(This research was supported by grant MT 5611 from the Medical Research Council of Canada.)

1383 BENZODIAZEPINE BINDING IN THE RAT BRAIN: EFFECT OF VARYING TEMPERATURE. Speth, R.C. and Yamamura, H.I.: Dept. of Pharmacology, University of Arizona Health Sciences Center, Tucson AZ 85724.

Several investigators have measured [^{3}H]diazepam binding to mammalian brain between 0° and 37° and have shown decreased binding with increased temperature. Therefore in routine studies of [^{3}H]diazepam binding, the incubation medium has been chilled to 4° prior to separation of the bound from free [^{3}H]ligand. The binding kinetics of [^{3}H]diazepam at 37° have not yet been determined, presumably because the rate of dissociation of the diazepam-benzodiazepine binding site complex is too rapid. [^{3}H]Flunitrazepam has a higher affinity for the benzodiazepine binding site than does diazepam, in addition, its rate of association with the benzodoazepine binding site is slower than that of diazepam. Consequently the rate of dissociation of [^{3}H]flunitrazepam with the benzodiazepine binding site is considerably slower than diazepam. We have taken advantage of this property of [^{3}H]flunitrazepam to determine its binding kinetics at varying temperatures.

In our initial studies, we observed an activation (34%) of [^{3}H]flunitrazepam binding when incubations were carried out at 37° prior to cooling at 4° as compared to incubations done at 4° alone. To ascertain whether the increase in [^{3}H]flunitrazepam binding occurring at 37° was due to an increase in the number of binding sites or an affinity change, saturation studies were performed at several temperatures and the results analyzed by Scatchard analysis. [^{3}H]Flunitrazepam binding was examined under 5 different conditions: 0° with prior 37° incubation, 0°, 12°, 25°, and 37°. The results of our studies showed a systematic decrease in [^{3}H]-flunitrazepam's affinity for its binding sites with increasing temperature. The K_D values obtained at the various temperatures were 0.7 nM, 1.3 nM, 2.1 nM, 2.0 nM and 5.7 nM, respectively. There appeared to be little alterations in maximal [^{3}H]flunitrazepam binding with increasing temperatures. These data suggest that benzodiazepine binding sites may undergo conformational changes with increasing temperatures or perhaps an endogenous substance is liberated in varying amounts as a function of increasing temperature. Supported by USPHS grants, a Postdoctoral Clinical Pharmacology Training Grant (GM-07533) and a Research Scientist Development Award from the NIMH (MH-00095).

1384 LOW CALCIUM LEVELS ENHANCE ETHANOL-INDUCED MULTIPLE FIRING AT THE CRAYFISH NEUROMUSCULAR JUNCTION. Allan L. Staiman and Juan Acosta-Urquidi. Dept. Pharmacology and Toxicology, Univ. of Mississippi Medical Center, Jackson, Miss., Dept. Zoology, Univ. of Toronto, Toronto, Ont., Can. and Addiction Res. Fdn., Toronto, Ontario, Canada.

The effects of ethanol were examined on the leg opener-neuromuscular junction preparation of the crayfish. The excitatory junctional potential (EJP) mediated by glutamate was studied intracellularly using selective stimulation of the excitor axon. Ethanol in the concentration range of 0.4M to 1M with a mean of 0.8M produced hyperexitability of the nerve terminals on stimulation of the excitor axon. One stimulus to the axon would produce an EJP followed by 1 to 10 other EJPs. The frequency of the multiple firing averaged approximately 80 hertz. Spontaneous multiple firing was not observed unless a stimulus was previously applied. The technique of focal extracellular recording was used to examine the origin of the ethanol-induced multiple EJPs. It was observed that each EJP was preceeded by an action potential and therefore the multiple EJPs observed were spike-triggered events. In order to examine whether ethanols action was via a depolarizing action, 15 mM potassium was applied to the preparation. The size of the EJP was slightly depressed and there was no sign of multiple spiking after stimulation in the presence of high potassium. The effect of calcium ion on the ethanol-induced multiple spiking was examined. In most experiments, raising the normal Ca^{++} concentration 2-fold abolished ethanol induced multiple firing. When the concentration of ethanol was slightly below the threshold concentration for induction of multiple spiking and the Ca^{++} concentration was reduced by 2-fold, still in the presence of the ethanol, multiple release occurred on stimulation of the excitor axon. In some experiments, concentrations as low as 0.2 M ethanol could produce multiple spiking when the Ca^{++} concentration was lowered by 2- to 4- fold. It is suggested that ethanol-induced multiple spiking may be mediated through ethanols action on a Ca^{++} pool and that this action may be of significance in the production of part of ethanols central pharmacological and toxicological actions. The observation that ethanol can produce seizure-like activity in a low Ca^{++} environment may be of significance clinically.

(supported by the Addiction Research Foundation of Ontario and University of Mississippi Medical Center BRSG grant)

1385 LACK OF TOLERANCE DEVELOPMENT IN THE RAT SUBSTANTIA NIGRA FOLLOWING LONG-TERM AMPHETAMINE ADMINISTRATION. David A. Staunton*, Steven J. Young*, and Philip M. Groves. Dept. of Psych., Univ. of Colorado, Boulder, CO 80309.

There is substantial evidence to suggest that amphetamine acts in part to enhance the release of catecholamines from central catecholaminergic neurons. While several of the physiological and behavioral effects of amphetamine show tolerance development following long-term administration of the drug, a number of such effects do not. Indeed, several of the behavioral effects of amphetamine show a progressive augmentation following administration of low doses for periods of several days or more. In the experiments reported here, rats were given daily intraperitoneal d-amphetamine sulfate injections for periods of 8 (2.5 mg/kg twice daily, n=5; or 5.0 mg/kg twice daily, n=9) or 16 days (2.5 mg/kg twice daily, n=8) or equivalent injections of saline then prepared for extracellular recording of single neuron activity utilizing chloral hydrate anesthesia. After an initial baseline period during which spontaneous activity was recorded, 0.25 mg/kg d-amphetamine sulfate was injected intravenously every four minutes until a 50% reduction in neuronal firing rate was achieved. The cumulative dose necessary to produce such a criterion inhibition of neuronal firing was not altered following any of the above amphetamine pretreatment regimens when compared to saline control values. There were also no significant differences in mean baseline firing rates between any of the pretreatment groups. Such evidence is consistent with the view that for many of the physiological and behavioral effects of amphetamine, tolerance does not develop following long-term administration of the drug. (Supported in part by grant DA 01467 from the National Institute on Drug Abuse and Research Scientist Development Award K02 MH 70706 from the National Institute of Mental Health. Data reported here will be used to satisfy, in part, the requirements for the Ph.D. degree in Pharmacology from the University of Colorado Medical Center by DAS.)

1386 BRAIN NORADRENERGIC SYSTEMS AS A PREREQUISITE FOR DEVELOPMENT OF BARBITURATE TOLERANCE. Boris Tabakoff, R.F. Ritzmann*, Gary Oltmans* and Paula L. Hoffman*. Dept. of Physiology & Biophysics, Univ. of Ill. Med. Ctr. and Dept. Pharmacol., Chicago Medical School, Chicago, Illinois 60612.

Chronic exposure of the CNS to barbiturates results in the development of functional tolerance to these drugs. Mice, fed laboratory chow containing phenobarbital (Ph), and rats, fitted with Alza Minipumps^{R} for intraventricular delivery of Ph, were shown to develop tolerance to the hypothermic and hypnotic effects of barbiturates. In our studies, the CNS "functional" tolerance was distinguished from the metabolic tolerance which develops with oral administration of barbiturates. After withdrawal of the animals from the chronic treatment regimen, the animals were challenged with an acute hypnotic dose of barbiturate. Brain levels of barbiturate at the time of regaining of righting reflex were found to be significantly higher in animals fed or infused with Ph compared with brain barbiturate levels in control animals. In trying to ascertain which neuronal systems were important in development of functional tolerance, we injected 6-OHDA into the lateral ventricle of mice prior to chronic feeding with Ph. The dose of 6-OHDA was chosen to deplete brain NE levels ~50% and to produce little change in other neurotransmitter systems of brain. This partial destruction of NE neurons resulted in a blockade of development of functional tolerance to barbiturates. To more closely determine the anatomical distribution of the NE systems responsible for tolerance development, ascending dorsal and ventral NE bundles were selectively lesioned in rat brain. Destruction of either the dorsal or ventral bundle and subsequent lesion-specific depletion of brainstem and forebrain NE levels resulted in a block in development of functional barbiturate tolerance. Since depletion of hypothalamic NE was common to both lesions, the results of our experiments will be discussed with consideration of the importance of hypothalamic/hypophyseal hormones in development and maintenance of tolerance to sedative hypnotics.

This work was supported by grants from the National Institute on Alcohol Abuse and Alcoholism, AA 2696-03; the State of Illinois Department of Mental Health and Developmental Disabilities, 720-03 and the National Science Foundation, NSF BNS 76-11779.

1387 6-HYDROXYDOPA DEPLETES BOTH BRAIN EPINEPHRINE AND NOREPINEPHRINE: INTERACTIONS WITH ANTIDEPRESSANTS. Philip F. VonVoigtlander and Elizabeth G. Losey*. The Upjohn Company, Kalamazoo, MI 49001.

The neurotoxic agent, 6-hydroxydopamine, and its precursor 6-hydroxydopa, have been widely used to selectively destroy neurons containing the catecholamines, dopamine and norepinephrine. These effects have been particularly useful in the study of the function of catecholaminergic neurons. The basis of the specificity of 6-hydroxydopamine is its ability to be accumulated into these neurons via the dopamine and norepinephrine uptake systems. Once within the cell it exerts cytotoxic effects leading to disruption of the neuron and subsequent loss of dopamine and norepinephrine from the tissue. Certain tricyclic antidepressant drugs (imipramine, protriptyline) that are known to inhibit the membrane norepinephrine uptake system, block the neurotoxic effects of 6-hydroxydopamine at norepinephrine-containing neurons. Thus these compounds have been used to enhance the specificity of 6-hydroxydopamine; after pretreatment with protriptyline, 6-hydroxydopamine depletes dopamine but not norepinephrine. The converse may be achieved by the administration of 6-hydroxydopa after pretreatment with a monoamine oxidase inhibitor; this results in norepinephrine but not dopamine depletion. The ability of antidepressant drugs to antagonize 6-hydroxydopamine and 6-hydroxydopa provides a useful assay for identifying antidepressant drugs.

We have recently found that intravenous administration of the neurotoxic agent, 6-hydroxydopa, to mice treated with pargyline lowered both epinephrine and norepinephrine concentrations in the brainstem. Pretreatment with the tricyclic antidepressant drugs (imipramine, iprindole and protriptyline) differentially blocked these depletions. Imipramine and protriptyline blocked the effects on both epinephrine and norepinephrine, although higher doses were required to protect epinephrine. Iprindole selectively blocked epinephrine depletions.

Thus previous studies which involved the use of 6-hydroxydopa or 6-hydroxydopamine to lesion noradrenergic and/or dopaminergic neurons may well have also resulted in degeneration of adrenergic (epinephrine-containing) neurons. Our results indicate that pretreatment with iprindole would have eliminated this potentially confounding effect. The ability of antidepressant drugs to block the effects of 6-hydroxydopa upon noradrenergic neurons is related to their ability to block norepinephrine uptake. We assume that blockade of epinephrine depletion, demonstrated here, involves a similar mechanism. Thus we interpret these results to indicate that iprindole, protriptyline and imipramine block the catecholamine uptake system of adrenergic (epinephrine) neurons. This action of antidepressants may play a role in their clinical effects, particularly in those of iprindole which does not block the uptake of other biogenic amines.

1388 BRAIN TRYPTAMINE : EVIDENCE FOR EXTRACEREBRAL ORIGIN. Jerry J. Warsh, Donald V. Coscina, Peter W. Chan* and Damodar D. Godse*. Depts. of Neurochemistry and Biopsychology, Clarke Institute of Psychiatry, University of Toronto, Toronto, Canada, M5T 1R8.

The presence of tryptamine (TA) in rat brain has been unequivocally demonstrated by mass spectrometric (MS) and gas chromatographic-MS methods (Philips et al., Can. J. Biochem. 52; 447, 1974; Warsh et al., Biochem. Med. 18; 10, 1977). As TA penetrates the blood-brain barrier (Oldendorf and Braun, Brain Res. 113; 219, 1976), brain TA may derive from peripheral tryptophan (TP) decarboxylation, particularly after monoamine oxidase inhibition.

Two groups of male Wistar rats (190-240g) received pargyline (75 mg/kg) or pargyline (75 mg/kg) plus MK-486 (100 mg/kg) i.p. in an acid saline vehicle (ph = 1.4). The animals were sacrificed by cervical decapitation 2 hours later and whole brains removed for determination of brain TA, 5-HT and TP. Two additional groups of rats received pargyline or pargyline plus MK-486, as above, followed one hour later by TP (100 mg/kg) i.p. Rats were sacrificed 1 hour later and brains removed for assay. Brain TA and 5-HT were determined by GC-MS and brain TP by spectrophotofluorometry.

Administration of the selective peripheral decarboxylase inhibitor MK-486 produced a 40-50% reduction in brain TA accumulation after pargyline or pargyline plus TP. A slight (8%) but significant decrease also occurred in brain 5-HT accumulation. Co-administration of MK-486 did not affect brain TP levels.

TREATMENT	BRAIN INDOLES			
	TA (ng/g)	5-HT (µg/g)	TP (ng/g)	N
pargyline	36.8 ± 9.0	1.22 ± 0.02	5.91 ± 0.24	6
pargyline + MK-486	18.7 ± 1.3*	1.12 ± 0.02**	6.29 ± 0.20	6
pargyline + TP	120 ± 5.9	1.70 ± 0.03	44.5 ± 6.3	4
pargyline + MK-486 + TP	75.1 ± 3.1**	1.67 ± 0.04	54.1 ± 1.9	6

* $p < 0.05$ and ** $p < 0.01$ compared to non-MK-486 treated rats

These data indicate that following MAO inhibition at least 40% of brain TA originates from the periphery and suggest that a substantial fraction of endogenous brain TA may also be derived from the decarboxylation of TP in extracerebral pools.

1389 **EFFECTS OF MUSCIMOL UPON THE ACTIVITY OF SUBSTANTIA NIGRA PARS RETICULATA NEURONS.** **Barbara L. Waszczak, Joan M. Lakoski and Judith R. Walters. NIH, NINCDS, Bethesda, Md. 20014.**

GABAergic neurons, which originate in the striatum and globus pallidus and terminate in the substantia nigra pars reticulata, have been postulated to tonicly inhibit the activity of the nigral (pars compacta) dopamine (DA) neurons. However, recent studies have shown that muscimol, a potent GABA agonist both in vivo and in vitro, did not inhibit the firing rate of nigral DA cells (Walters and Lakoski, Europ. J. Pharmacol. 47: 469,1978). Furthermore, i.p. administration of 3.5 mg/kg muscimol produced increases in the firing rate of 64% of DA neurons recorded in gallamine-paralysed rats. Since substantia nigra pars reticulata neurons might also be considered possible sites of termination of the striato-nigral GABA pathway, the effects of muscimol (i.p. and i.v.) upon the extracellular, single-unit activity of these cells in chloral hydrate-anesthetized rats was investigated. The reticulata cells do not show consistent (dose-dependent) responses to drugs which alter the rate of firing of DA neurons by interacting with DA receptors. However, i.p. administration of 3.5 mg/kg muscimol caused a complete inhibition of firing of 90% of the reticulata cells recorded (n=10). The average time required to inhibit firing was approximately 25 minutes (range: 5-60 min). I.v. administration of successively increasing doses of muscimol caused a dose-dependent decrease in activity of all of the cells recorded (n=6). The amount of drug required to produce the first significant inhibition of firing was variable over a wide range of doses. In a separate series of experiments, administration of a single i.v. dose of muscimol (1.6 mg/kg) produced significant decreases in activity in 79% of cells recorded, increases in 14%, and no change in rate in 7% of cells studied (n=14). The average effect of muscimol upon the firing rate of reticulata cells in these experiments was inhibition to 70% of the control rate. The onset of the inhibitory response could be observed as rapidly as 20-30 sec after a single i.v. dose of 1.6 mg/kg, and the rate remained depressed for more than 30 minutes. In several experiments, picrotoxin was administered after the muscimol-induced inhibition of firing, and the activity of these cells was restored to control levels. Although non-specific or peripheral actions of muscimol at sites other than nigral GABA receptors remain to be considered, these studies suggest that a population of cells in the substantia nigra pars reticulata is sensitive to inhibition by muscimol (i.p. or i.v.) at doses which do not alter the firing rate of the nigro-striatal, pars compacta DA neurons. In addition, these findings are consistent with the observation that the reticulata cells are more sensitive to iontophoresed GABA than the nigral DA cells.

1390 PHARMACOLOGICAL MANIPULATION OF BRAIN ACETYLCHOLINE:DEPENDENCE ON DIETARY CHOLINE CONCENTRATION. Lynn Wecker and Dennis E. Schmidt*. Dept. Pharmacol., Vanderbilt Univ. Sch. Med., Nashville, TN 37232.

A discrepancy exists concerning the effect of acute choline (Ch) administration on brain acetylcholine (ACh) levels and it has been suggested that these results may be due to variations in the Ch concentration of rat diets used by various investigators. Furthermore, acute administration of Ch to rats has been shown to modify the biochemical effects of atropine and it was postulated that the nutritional status (i.e., Ch availability) of an animal may be important in determining the responsiveness of cholinergic neurons to pharmacological manipulation (Sci.199:86,1978). This study was designed to measure the effects of diets containing varying levels of Ch on the efficacy of atropine and acute Ch administration in altering regional ACh levels in rat brain. Male Sprague-Dawley rats (145g) were maintained for 2 weeks, with water available ad libitum, on a dietary regimen consisting of: a) a Ch free (CF) diet, b) a "normal" (N) rat diet, 1.6g free Ch/kg chow or c) a high Ch (HC) diet, 11.4g free Ch/kg chow. At the end of the 2 week period, rats were injected (ip) with saline, ChI (60 mg/kg free base) or atropine sulfate (20 mg/kg). Rats were sacrificed by head-focused microwave irradiation and the concentration of ACh was quantitated by pyrolysis-gas chromatography. No significant differences in ACh levels were measured in the hippocampus of saline injected rats in any of the dietary groups. The ACh level in the striatum of N and HC rats did not differ, whereas, the ACh level in the striatum of CF rats was reduced to 85% of control N rats. Acute Ch administration (40 min) also failed to alter hippocampal ACh levels in any dietary group. In the striatum, no changes were noted in ACh levels in the N and HC groups while in CF rats, acute Ch administration increased ACh levels in striata to 133% of CF controls, but these levels were not significantly greater than ACh levels in striata of N controls. Atropine (30 min) caused a 30% decrease in ACh levels in both the striatum and hippocampus of N and CF rats. In HC rats, however, atropine reduced ACh levels in the striatum and hippocampus by 52% and 43%, respectively. From these results, it is concluded that acute or dietary modification of Ch availability does not alter hippocampal ACh levels.Similarly in striata, a 10-fold increase in dietary Ch does not cause either an increase in ACh levels or a response to acute Ch administration. The CF diet does, however, cause a modest reduction in striatal ACh levels which is reversed by acute Ch administration. The efficacy of atropine in depleting ACh levels does appear to be Ch related, for animals maintained on a HC diet exhibited a greater sensitivity to ACh depletion by atropine than animals on a N or CF diet. Hence, dietary modification of Ch levels does influence the responsiveness of cholinergic neurons to pharmacological manipulation. (Supported by BRSG RR-05424-16 and NIMH MH-29182.)

1391 STUDIES ON THE ANTINOCICEPTIVE EFFECTS OF INTRATHECAL BACLOFEN IN THE RAT AND CAT. Peter R. Wilson* and Tony L. Yaksh (SPON: F.W.L. Kerr). Mayo Foundation, Rochester, MN 55901.

Baclofen, a GABA-like compound noted clinically for its antispasticity actions has been shown by several workers to exert an antinociceptive effect which can be distinguished from its effects upon motor function when administered systemically. Though its mode of action is not clear, physiological experiments have indicated that it exerts a direct effect upon spinal function. To determine whether its antinociceptive effects are mediated by this spinal action, rats and cats were prepared with polyethylene (PE-10) catheters chronically placed within the lumbar spinal subarachnoid space. The L- and D- isomers of baclofen were injected and their effect upon the hot plate and tail flick response of the rat and the escape response to a thermal probe applied to depilated regions in the cat was assessed. In the rat, L-baclofen (0.01 to 1.0 µg) produced a dose dependent block of the hot plate and tail flick response. At the 1.0 µg dose a complete block of these measures was noted though less than 25% of the animals tested showed any detectable signs of flaccidity. Lower doses produced a significant antinociception but no sign of flaccidity. Higher doses (10-100 µg) produced both a block of the responses as well as debilitating amounts of flaccidity. In the cat, over the dose range employed, (4-25 µg) no signs of motor flaccidity were noted, but a significant increase in the escape response latency to the thermal probe was readily observed. In both the rat and the cat, the regions rendered non-responsive to the thermal probe or to forceps pinch were limited to the caudal regions of the body in those dermatomes associated with the spinal segments affected by the intrathecal baclofen. In contrast to L-baclofen, the D-isomer failed to show any antinociceptive effect at doses up to 100 times the effective dose of the L-isomer, though a significant degree of flaccidity was in fact noted. Neither the antinociceptive or flaccidity effects were antagonized by naloxone (1 mg/kg, i.p.). Similarly, rats made tolerant to the antinociceptive effects of morphine (20 mg/kg, i.p. twice each day) showed no change in the dose response effects of intrathecal L-baclofen either in terms of antinociception or flaccidity. These data suggest that baclofen is not acting directly upon an opiate receptor or indirectly through an opiate sensitive link. The present experiments demonstrate a focal site of action of baclofen in the cord. Importantly, this demonstration of dose dependency and stereospecificity suggest an action upon a specific, though as yet undetermined receptor system, within the spinal cord. Importantly, as with the opiates, the functional characteristics of this spinal system affected by baclofen at these doses appears closely associated with the throughput of nociceptive stimuli. This work was supported by funds from the Mayo Foundation.

1392 THE SYNERGISTIC INTERACTION OF THREE PHARMACOLOGICALLY DISTINCT SPINAL SYSTEMS MEDIATING ANTINOCICEPTION: THE INTRATHECAL ACTION OF MORPHINE, SEROTONIN AND BACLOFEN. Tony L. Yaksh. Mayo Foundation, Rochester, MN 55901.

It has been shown that morphine (MOR) with an action limited to the spinal cord can produce elevations in the nociceptive threshold. More recently, both serotonin (5-HT) and baclofen (BAC) have also been shown to produce a significant elevation in the nociceptive threshold of the rat and cat when given intrathecally. To demonstrate that these three drugs were exerting their effects through pharmacologically independent spinal systems, it was shown in the present experiments that while naloxone (0.5 mg/kg, i.p.) antagonized MOR, it had no effect upon the antinociceptive effects of intrathecal 5-HT, but failed to have any effect upon intrathecally injected MOR or BAC. To examine the interaction of these three spinal systems, dose response curves in rats on the hot plate and tail flick were obtained for intrathecally administered MOR either alone or injected together with doses of BAC (0.01 µg) of 5-HT (50 µg) which alone were insufficient to produce any antinociceptive effects. Similarly, dose response curves for intrathecal BAC were obtained in the presence of doses of MOR (1 µg) or 5-HT (50 µg) which alone had no detectable effect. Finally, such curves were obtained for 5-HT, again with a dose of MOR (1 µg) or BAC (0.01 µg) which was ineffective. These experiments revealed that each drug produced a multiplicative potentiation of the antinociceptive effects of the other. Thus dose response curves were shifted such that MOR (1 µg) and 5-HT (50 µg) or MOR (1 µg) and BAC (0.01 µg) for example produced a 98 and 80% elevation in the nociceptive thresholds, respectively. As with each agent alone, the antinociceptive effects of the combined doses were not associated with any signs of motor dysfunction or flaccidity. Importantly, for all drug conditions, during the periods when the tail flick and hot plate thresholds were elevated, the response to forcep pinches applied to the caudal, but not rostral portions of the body were blocked. This indicates a local action on the spinal cord. It is suggested that this multiplicative interaction evidence through the action of these agents on these three pharmacologically distinct spinal systems is not due to altered metabolism or clearance but may represent a common model for the functional interaction of spinal modulatory systems controlling sensory throughput. This work was supported by the Mayo Foundation.

1393 CENTRALLY MEDIATED RESPIRATORY ARREST FOLLOWING INFUSION OF THE LOCAL ANESTHETIC AND OPIATE ANTINOCICEPTIVE AGENTS, LIDOCAINE, COCAINE, CHLORDIMEFORM, AND MORPHINE. G.K.W. Yim, W.R. Pfister*, J. Rolley* and M.L. Holsapple*. Dept. Pharmacol. and Toxicol., Purdue Univ. West Lafayette, IN 47907.

We have recently reported that the local anesthetic agents cocaine, chlordimeform (CDM), and lidocaine possess antinociceptive activity on the rat tail flick test (Pfister and Yim, Pharmacologist 19(2): 216, 1977). Since local anesthetics and CDM (Wang and Narahashi, Pest. Biochem. Physiol. 5: 119, 1975) depress neuromuscular transmission, these studies were initiated to identify whether respiratory depression was of central or peripheral origin. Rats were lightly anesthetized with urethane (1.2 g/Kg, i.p.), and the agents were infused over a period of 20-30 min via the jugular vein until respiratory arrest. The decreasing order of lethality and dose resulting in respiratory arrest were: cocaine (35.3 ± 11 mg/Kg), lidocaine (35.4 ± 9.6 mg/Kg), CDM (62.3 ± 6.0 mg/Kg), and morphine (93.5 ± 11.0 mg/Kg). In lidocaine treated rats, the amplitude and rate of diaphramatic movements and of phrenic nerve bursts gradually decreased until respiratory arrest. In contrast, respiratory rate was initially increased by cocaine and CDM. Diaphramatic contractile force and the amplitude of phrenic nerve bursts remained near control values, but abruptly disappeared upon respiratory arrest. The profile of morphine on the phrenic nerve activity was unique in that continuous inter-burst discharge preceded the abrupt decrease in phrenic nerve amplitude and respiratory arrest. Naloxone reversed both actions of morphine but did not reverse the respiratory depression induced by the other agents. Following respiratory arrest produced by all of these agents, the diaphram and gastrocnemius muscle still contracted following electral stimulation of the phrenic and sciatic nerves, respectively. These results indicate that respiratory arrest induced by these agents is central in origin. Supported in part by grants from NIH (NS 12077) and EPA (5-803965).

1394 PRENATAL MATERNAL PHENOBARBITAL REDUCES CONVERSION OF TYROSINE TO CATECHOLAMINES IN BRAINS OF YOUNG OFFSPRING. John W. Zemp, Lawrence D. Middaugh, Thomas A. Grover, and Larry W. Simpson*. Dept. Biochem., Med. Univ. of S.C., Charleston, S.C. 29403.

We have previously reported (Zemp & Middaugh, Perinatal Addiction, Harbison, R.D., ed. p. 307-331, Spectrum, N.Y. 1976) that phenobarbital injected into mice for the last third of pregnancy, causes a dose related increase in neonatal mortality and decrease in brain growth of surviving offspring. The effects of early exposure to phenobarbital appear to be long lasting since offspring of animals injected with the drug differ from control animals in a number of behavioral tasks after maturity (Middaugh et al., Devel. Psychobiol. 18, 305-313, 1975; Middaugh et al., Pharmac. Biochem. Behav. 3, 1137-1139, 1975; Zemp & Middaugh, Perinatal Addiction, Harbison, R.D., ed. p. 307-331, Spectrum, N.Y. 1976). Some of the behavioral abnormalities noted have also been reported in animals with manipulations of the central catecholaminergic systems (Antelmann & Caggiula, Sci. 195, 646-653, 1977.). The purpose of this experiment was to determine if maternal injections of phenobarbital for the last third of pregnancy would alter catecholamine metabolism in the brains of offspring. We have examined concentrations and the turnover of radioactive tyrosine (TYR), dopamine (DA), and norepinephrine (NE) in the brains of offspring of C57 BL/6J mice injected daily with phenobarbital for the last 6 and 7 days of pregnancy. Brain concentration of TYR, DA, and NE were not altered by prenatal drug exposure; however, one hour after injection of ^{3}H-TYR the specific activity of ^{3}H-TYR was significantly increased by 15-23% in the brains of 21-day-old mice exposed prenatally to the drug. The conversion of ^{3}H-TYR to DA and NE in 21-day-old offspring was also reduced in the drug treated mice in a dose dependent manner when compared to controls (Table 1.)

Table 1. Prenatal injections of phenobarbital reduces the conversion of ^{3}H-TYR to DA and NE in brains of 21-day-old offspring.

Treatment	Dopamine (nmole/g/hr) $\bar{X}$ ± SEM(N)	Norepinephrine (nmole/g/hr) $\bar{X}$ ± SEM(N)
Saline	7.55 ± .46(11)	2.95 ± .23(11)
Phenobarbital(20mg/kg)	5.41 ± .43(12)	2.03 ± .18(12)
Phenobarbital(40mg/kg)	4.74 ± .37(14)	1.73 ± .13(14)

The results suggest that neural systems using catecholamines as transmitters are altered by prenatal exposure to phenobarbital.

(Supported by grant #DA01624 from the National Institute for Drug Abuse.)

NEUROTRANSMITTERS

1395 THE ^{3}H NOREPINEPHRINE UPTAKE AND FATE IN THE ISOLATED CEREBRAL CAPILLARIES. T. Abe*, K. Abe*, M. E. Swink*, I. Klatzo and M. Spatz. Lab. Neuropath. & Neuroanat. Sci., NINCDS, NIH, Bethesda, MD 20014.

In vivo studies had shown that norepinephrine doesn't cross the blood-brain barrier (Weil-Malherbe, Whitby and Axelrod, J. Neurol. 8, 55-64, 1961). In order to elucidate the mechanism responsible for the reported observations the uptake of ^{3}H norepinephrine was investigated in isolated capillaries which were previously proven to be metabolically active and suitable for such studies (Mrsulja, Mrsulja, Fujimoto, Klatzo and Spatz, Brain Res. 110, 361-365, 1976).

The isolated capillaries took up the ^{3}H norepinephrine and the labeled substance increased with the duration of incubation (2-60 minutes). The uptake of ^{3}H norepinephrine in the capillaries was found to be saturable since it was inhibited by increasing concentrations of unlabeled (cold) norepinephrine when it was added to the incubating media containing the labeled substrate. The capillary ^{3}H uptake of norepinephrine was also cross inhibited by addition of cold L-dopa, dopamine, epinephrine and metaraminol but not by normetanephrine or metanephrine in concentrations of 1-2 mM. Pyrogallol, the known inhibitor of catechol-O-methyl transferase competitively inhibited the uptake of ^{3}H norepinephrine in the isolated capillaries. Moreover the preincubation of the capillaries with pargyline, the inhibitor of monoamine oxidase (MAO) led to a decreased level of ^{3}H labeled substance in the capillaries.

Preliminary investigations of the accumulated substances in the capillaries were so far found to be the methylated metabolites of norepinephrine namely normetanephrine and metanephrine.

These results suggest that the uptake of norepinephrine takes place by carrier mediated process (which may be shared by other catecholamines) but the norepinephrine is not accumulated as such since it is metabolized by the catechol-O-methyl transferase and MAO present in the capillaries. These findings also indicate that the capillaries are probably unable to retain the norepinephrine after the inhibition of the enzymes since the inhibition of methyl transferase and MAO inhibited also the "uptake' of norepinephrine. Therefore the cerebral capillaries are the site of enzymatic barrier which prevents the intact norepinephrine to enter or leave the brain.

1396 GABA AND GLYCINE ACTIVATE CHANNELS WITH DIFFERENT PROPERTIES ON CULTURED SPINAL NEURONS. J.L. Barker and R.N. McBurney, LNP, NINCDS, NIH, Bethesda, Md. 20014.

Analysis of conductance fluctuations induced on peripheral vertebrate and central invertebrate post-synaptic membranes by application of putative neurotransmitters has revealed the dimensions of ion channels. We have applied fluctuation (or "noise") analysis to amino acid responses of dissociated spinal neurons derived from mouse embryos and grown in tissue culture. Intracellular recordings with two independent electrodes were made from spinal neurons or dorsal root ganglion (DRG) cells. γ-aminobutyric acid (GABA) and glycine were applied extracellularly by microiontophoresis. $MgCl_2$ was added to reduce spontaneous synaptic activity and allow clearer study of the post-synaptic events. The cells were voltage-clamped and amino acid-induced membrane current responses recorded on magnetic tape for off-line analysis. Both amino acids caused dose-dependent increases in membrane current which were associated with increases in current fluctuations. The fluctuations were analyzed on the assumption that they derive from the statistical variation in the number of open ion channels around a mean level of open channels. The amplitude of the single channel conductance, γ, can be calculated knowing the variance in current, mean current and the driving force. Spectral analysis of the fluctuations gave power density spectra which were generally approximated by a single Lorentzian and allowed estimate of the average channel lifetime, τ. $\bar{\gamma}$ and $\bar{\tau}$ were 18 pS and 20 msec for GABA-activated channels and 30 pS and 5 msec for glycine-activated channels (n= 342 observations on 13 spinal neurons). GABA-activated channels on DRG cells had dimensions similar to those activated by GABA on spinal neurons. The values obtained on spinal neurons are significantly different from each other and indicate that the amino acids activate Cl^- channels with different dimensions.

However, clear interactions between GABA and glycine current responses suggest that the amino acids may utilize the same channel. The amplitudes of glycine responses superimposed on GABA responses which could be desensitized almost completely were only slightly diminished relative to control values, while the amplitudes of GABA responses superimposed on glycine responses which could only be partially desensitized were markedly depressed relative to control. The antagonism was not due to a change in the driving force acting on the Cl^- conductance. One explanation for these results is that desensitization occurs at the receptor step and that GABA and glycine share a common Cl^- conductance mechanism.

1397 LIGHT STIMULATED INCREASE OF CHOLINE UPTAKE AND ACETYLCHOLINE SYNTHESIS IN THE TURTLE RETINA IS ASSOCIATED WITH AN INCREASE IN THE V_{max} OF HIGH-AFFINITY CHOLINE UPTAKE. Robert W. Baughman and Daniel Y. Tso*. Department of Neurobiology, Harvard Medical School, Boston, MA 02115

Acetylcholine (ACh) appears to be a neurotransmitter in the inner plexiform layer of many vertebrate retinas (Masland and Ames, 1976; Baughman and Bader, 1977). The present study concerns the effects of light stimulation on choline transport and ACh synthesis in an isolated, perfused turtle eyecup preparation. The ERG was monitored before and after the period of stimulation to confirm the viability of each eyecup. To control for variability in the perfusion, caused by vitreous remaining in the eyecup, the uptake of ^{3}H choline (1 µM) was measured relative to uptake of ^{14}C leucine (400 µM), which itself was unaffected by stimulation. In constant light the uptake of choline increased slightly, but with flashing light uptake was increased substantially, reaching a level 70% greater than the level seen with constant dark. The increase in choline uptake was accompanied by an increase in ACh synthesis; the ratio of ^{3}H ACh to ^{3}H choline after exposure to flashing light, constant light and constant dark was 0.41, 0.25 and 0.20, respectively. When the flashing light experiment was repeated with 1.8 mM Co^{2+} in the perfusate, which should block synaptic transmission and which in fact eliminated the ERG b-wave, choline uptake and ACh synthesis were no longer enhanced. In a flashing light experiment with 5 µM hemicholinium-3, which should block high-affinity choline uptake and which caused some reduction of the ERG b-wave, choline uptake was reduced to a level below that seen with constant dark, and ACh synthesis was essentially blocked. To determine the changes in kinetic parameters underlying the increased choline uptake, choline uptake was measured in synaptosomal preparations made from retinas previously exposed to either constant dark or flashing light. Relative to values seen after constant dark, with flashing light the low-affinity K_m and V_{max} and the high-affinity K_m were unchanged, but the high-affinity V_{max} was increased more than twofold. Thus the light-stimulated increase in choline uptake, which is accompanied by an increase in ACh synthesis, is associated with an increase in the V_{max} of the high-affinity choline uptake system. (Supported by NIH Grants EY01995 and EY00082.)

1398 IMMUNOCYTOCHEMICAL LOCALIZATION OF L-GLUTAMATE DECARBOXYLASE IN THE RABBIT RETINA. C. Brandon*, J.-Y. Wu and Dominic M. K. Lam* (Spon: G. F. Ayala) Dept. of Cell Biology and Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030

Biochemical and physiological studies indicate that GABA is probably a neurotransmitter in the vertebrate retina. The sites of the GABA synapses in mammalian retinas have, however, not been precisely localized. Recent studies by Cadwell and coworkers (J. Physiol. 276, 277) showed that in the rabbit retina, picrotoxin alters certain specific properties of retinal ganglion cells. These findings, together with light microscopic autoradiographic studies of GABA uptake, suggest that some amacrine cells in the rabbit retina may be GABA neurons. With the availability of an antibody against L-glutamate decarboxylase (GAD) from mammalian brains (Wu, J.-Y. "GABA in Nervous System Function", Roberts, E., Chase, T. and Tower, D., ed., Raven Press, NY, 1976), we have begun to precisely localize the GABA synapses and their connections in the rabbit retina by immunocytochemical methods at light and EM levels. Rabbits were perfused through the heart with buffered 4% paraformaldehyde. After enucleation, the anterior chamber and vitreous humor were removed and each eye cup containing the retina was cut radially into strips, embedded in agar and sectioned with a tissue chopper. Sections were incubated in rabbit-anti-mouse GAD serum, washed and treated with a conjugate of horseradish peroxidase (HRP) and protein A (Dubois-Dalcq et al., J Histochem. Cytochem. 25, 1201-1206 (1977)). After washing, the sections were incubated in diaminobenzidine and hydrogen peroxide and the HRP reaction products were visualized by differential interference microscopy. Specific HRP reaction products in the rabbit retina were limited to the half of the inner plexiform layer proximal to the ganglion cell layer cell bodies, and the outer plexiform layer contained little such products. These results indicate that similar to the goldfish retina,(R. Marc et al., J. Comp. Neurol., in press) the GABA synapses in the inner plexiform layer of the rabbit retina may be localized to sublamina b.

Supported by grants from the Retina Research Foundation of Houston, the Huntington Chorea Foundation and the NIH (EY 02423 and NS 13224).

1399 SOME ADENOSINE ANTAGONISTS AND ADENYLATE CYCLASE INHIBITORS. Robert F. Bruns* (SPON: C.E. Spooner). Dept. Neurosci. Sch. Med., UC-San Diego, La Jolla, CA 92093.

Adenosine (ado) causes a 50-fold increase in intracellular cyclic AMP in VA13 human fibroblast cells. Studies with analogs of ado show that the structure-activity relationship (SAR) of this response is similar to the SAR reported for a similar response in guinea pig cerebral cortex. VA13 cells may thus provide a simple model for the brain ado receptor and its linkage with adenylate cyclase.

128 nucleosides were tested as ado agonists and antagonists. 11 compounds, all previously unreported, were competitive antagonists. The only commercially available competitive antagonist was 5'-deoxy-5'methylthio-ado, which had a K_i of 8 μM. Results with rigid analogs indicate that ado binds to the receptor in the "anti" conformation. Three nucleosides were noncompetitive antagonists. They blocked responses to isoproterenol and prostaglandin E_1 (PGE_1) as well as to 10 μM and 1 mM ado and were presumably adenylate cyclase inhibitors. The best cyclase inhibitor, 2', 5'-dideoxyadenosine, was a "partial inhibitor", since it only inhibited the response to isoproterenol by 68% even at very high concentrations.

110 purine, pyrimidine, and pteridine bases were tested as ado antagonists. Three distinct classes of competitive antagonists were found: methylxanthines (such as theophylline), benzo[g]pteridines (such as alloxazine), and adenine derivatives (such as 9-methyladenine). None of the bases were cyclase inhibitors. K_i values for some important xanthine derivatives were:

	K_i (μM)
caffeine (1, 3, 7-trimethylxanthine)	13
theophylline (1, 3-dimethylxanthine)	5
3-isobutyl-1-methylxanthine (IBMX)	4
7-(2-chloroethyl)theophylline	1.0
1, 3-dipropylxanthine	.7
8-(p-bromophenyl)theophylline	.05

Theophylline, caffeine, and IBMX were all much better ado blockers than phosphodiesterase (PDE) inhibitors. For this and other reasons the methylxanthines cannot be considered specific PDE inhibitors. Methylxanthines known to be CNS stimulants were good ado blockers, while non-stimulants tended to be poor blockers. This suggests that ado may have a sedative role in vivo.

(Supported by NIMH DA-00265 and PHS RR05665.)

1400 ACTION OF MICROIONTOPHORETIC ADMINISTRATION OF DOPAMINE ON SUBTHALAMIC NUCLEUS NEURONS IN THE RAT. Gerald A. Campbell* and Forrest F. Weight. Laboratory of Neuropharmacology, NIMH, St. Elizabeths Hosp., Wash., D.C.

In recent investigations using glucose utilization determined by the ^{14}C-deoxy-D-glucose technique as an index of neuronal activity, both apomorphine (L. I. Wolfson and L. Brown, Society for Neuroscience Abstracts, 1976, Vol. II, Pt. I, p510) and d-amphetamine (L. R. Wechler, H. Savaki, C. Kennedy, and L. Sokoloff, Society for Neuroscience Abstracts, 1977, Vol. III, p325 produced greater increases in activity in the subthalamic nucleus than in other areas of the brain. In view of the roles of amphetamine in enhancing release and reuptake of catecholamines at synapses and of apomorphine as a dopamine agonist, these results raised the possibility that there may be dopamine receptors on neurons in this nucleus. The effect of dopamine on the electrical activity of neurons in the subthalamic nucleus was therefore tested using microiontophoretic administration. Sprague Dawley rats weighing 140-160 gm, anesthetized with 25% urethane in saline (6 ml/kg), were used in these experiments. In order to ensure accurate stereotaxic placement of the five-barrel microelectrode, the cortex was sucked away around the horizontal coordinates of the insertion site down to the dorsal surface of the hippocampus, and the depth of penetration was measured from that landmark. Glutamate was used to induce firing in some cells, which were not spontaneously active. One of the side barrels of the five-barrel microelectrode was filled with 3M NaCl and used to pass an automatically generated balance current, equal and opposite in polarity to the sum of the remaining three barrels. At the end of each experiment, horseradish peroxidase (Sigma, Type VI) was microiontophoretically injected from the center, recording barrel to mark the point of maximal penetration. Cell positions were then referenced to the known depth of the injection, visualized in the frozen section by a tetramethylbenzidene reaction. Both increases and decreases in spontaneous firing rates in response to dopamine were observed in the vicinity of the subthalamic nucleus. Responses of both types have been obtained in individual animals with the same microelectrode.

1401 BEHAVIORAL AND BIOCHEMICAL PROPERTIES OF PARA-CHLOROPHENYLETHYLAMINE. Eunyong Chung Hwang* and M.H. Van Woert* (SPON: J. Schwartz). Dept. of Neurol. and Pharmacol., Mt. Sinai Sch. of Med., New York, N.Y. 10029.

p-chlorophenylalanine (pCPA) depletes brain serotonin levels by inhibiting tryptophan hydroxylase. The maximum reduction in brain serotonin occurs between 1 and 3 days after 316 mg/kg of pCPA i.p.. However, several behavioral studies have suggested that pCPA has a serotonin agonist-like action during the first 6 hours after injection. In an attempt to explain these paradoxical observations, behavioral and biochemical actions of pCPA metabolites were investigated.

p-chlorophenylethylamine (pCPEA) (50 mg/kg), a metabolite of pCPA, was found to produce in mice head weaving, tremor, hypertonus, Straub tail, hind leg abduction and salivation; a syndrome characteristic of serotonin receptor stimulation (Jacobs, B.L., Life Sci. 19, 777, 1976). The intensity and duration of this pCPEA-induced "serotonin" syndrome was enhanced by the monoamine oxidase inhibitor, pargyline (75 mg/kg) and partially counteracted by methiothepine (1 mg/kg), a serotonin receptor blocker, pCPA (400 mg/kg, 24 hours prior to pCPEA) and fluoxetine (10 mg/kg) and Org 6582 (10 mg/kg), which are serotonin uptake inhibitors. However, reserpine (10 mg/kg) and alpha-methyl-p-tyrosine (250 mg/kg) had no effect. The same pretreatment with fluoxetine or pCPA entirely prevented p-chloroamphetamine-induced "serotonin" syndrome.

A single injection of pCPEA (50 mg/kg) reduced mouse brain serotonin by 15% ($p < 0.01$) and increased 5-hydroxyindoleacetic acid (5HIAA) by 30% ($p < 0.01$). Pretreatment with fluoxetine (10 mg/kg) completely prevented pCPEA-induced changes in brain serotonin and 5HIAA.

The biochemical data suggest that pCPEA is taken up by the same neuronal transport process as serotonin and causes endogenous serotonin to be released. However, pCPEA is probably also a direct serotonin receptor agonist, since it can produce the "serotonin" syndrome even after pretreatment with fluoxetine, Org 6582 or pCPA. The previously reported paradoxical early effects of pCPA may be due to the action of its metabolite pCPEA.

Supported by USPHS grants NS 12341-03, NS 05802 and NS 11631-05.

1402 EFFECTS OF KAINIC ACID ON THE NEURONS RECEIVING OLFACTORY NERVE FIBERS IN THE RAT OLFACTORY BULB. Jody Patricia Corey* and Garl K. Rieke. Department of Anatomy, Hahnemann Medical College, Philadelphia, Pennsylvania 19102.

The collected data on glutamate suggests that this naturally occurring amino acid may be the excitatory neurotransmitter for the main afferent pathways in the central nervous system (Curtis and Johnson, 1974). Schwarcz and Coyle (1977) have demonstrated that microinjection of nanomolar amounts of kainic acid, a potent neuroexcitatory analogue of glutamate, produces selective degeneration of central neurons with glutamate receptors. Most recently, this "excito-toxic" effect of kainic acid has been used by Bird et al. (1978) to demonstrate glutamate's role as a primary afferent neurotransmitter in the rostral AVCN of the cochlear nucleus. It has been suggested by Nicoll (1971) that glutamate and/or aspartate may be excitatory transmitters for certain neurons in the olfactory bulb. To test this possibility, the effects of kainic acid on the neurons of the olfactory bulb were examined.

Two micrograms of kainic acid in 2 microliters of phosphate buffer (pH 7.4) were injected into the olfactory bulb. The bulbs of animals injected 24-48 hours prior to fixation were studied with light microscopy. At 24 hours, selective destruction of those neurons receiving synaptic input from primary olfactory axons, i.e. mitral, tufted, and periglomerular cells, was observed. The remainder of the intrinsic neurons within the bulb were not affected, suggesting that the kainate lesions were specific. Injection of phosphate buffer without kainic acid into the contralateral bulb caused no loss of neurons.

Each of the neuronal types that are sensitive to the toxicity of kainic acid receives synaptic input from primary olfactory nerve fibers; the selective loss of these cells supports the suggestion that glutamate is a primary afferent neurotransmitter in the olfactory bulb.

1403 OPIATE, BENZODIAZEPINE AND MUSCARINIC RECEPTORS IN DIFFERENT AREAS OF THE CEREBRAL CORTEX IN MONKEYS. Ivan Divac and Claus Braestrup*. Institute of Neurophysiology, University of Copenhagen and Skt. Hans Hospital, 4000 Roskilde, Denmark.

Twenty areas were taken from each of six hemispheres of three adolescent specimens of Cercopithecus aetiops. Aliquots of each sample were used to study specific binding of 3H-naloxone, 3H-diazepam and 3H-quinuclidinyl benzilate (QNB). Statistical analysis of results revealed that the studied receptors are distributed independently from one another. The steepest gradient was found for naloxone binding, and the flatest for QNB. The gradient for diazepam binding was in between. The highest values for naloxone binding were found in some limbic areas, the next highest in the medial and orbital prefrontal cortex, and the low values in the parietal, inferotemporal, and particularly visual cortex. Diazepam binding gave the highest values in the visual cortex and the frontal eye field, whereas the lowest values were found in the motor cortex and limbic areas. The only significant difference in QNB binding was between a high value in the visual cortex and a low value in the motor cortex.

1404 PUTATIVE NEUROTRANSMITTERS DECREASE ACTION POTENTIAL DURATION OF EMBRYONIC CHICK DORSAL ROOT GANGLION NEURONS. K. Dunlap* and G.D. Fischbach (SPON: M.L. Karnovsky). Dept. of Pharmacology, Harvard Medical School, Boston, Mass. 02115.

The soma membrane of embryonic dorsal root ganglion neurons in vitro generates both a fast Na spike and a slower Ca spike which produces a prolonged plateau on the falling phase of the action potential (Dichter and Fischbach, J.Physiol., 280:281, 1977). Several putative neurotransmitters were shown to effect a decrease in action potential duration in the absence of changes in resting membrane potential or input conductance. Known concentrations (10^{-7} to 10^{-4} M) of each drug were applied to individual neurons via pressure ejection from a 3-5μ tip micropipet positioned 100-200μ away. The control spike duration in 5.4 mM Ca^{++} was 8 msec. Gamma-aminobutyric acid (CABA), nor-epinephrine, serotonin, and glutamate produce dose-dependent decreases in spike duration with a maximum decrease of ca. 50% at 10^{-4} M. The time course of action was prolonged, lasting several minutes at higher concentrations. Acetylcholine and glycine (10^{-4} M) were without effect. In addition to their action on spike duration, these drugs decreased the rate of rise and peak potential of the Ca spike (recorded in 10^{-7} g/ml TTX) without affecting the Na component of the action potential. The nor-epinephrine response was blocked by the α-adrenergic antagonist phentolamine but not by the β-antagonist propranolol. The non-competitive GABA antagonists, bicuculline and picrotoxin, which block chloride-mediated responses, show no inhibition of the action of GABA on the spike. Several peptides were also tested. Enkephalin and somatostatin decreased spike duration (Mudge, Leeman and Fischbach, ms in prep.) while neurotensin, bradykinin and thyrotropin releasing hormone were without effect at 10^{-5}M. The action potential duration was unaffected by prolonged application of either dibutyryl cGMP or dibutyryl cAMP in bath concentrations as high as 1 mM.

These results imply that the effectors interfere with a voltage-sensitive ion conductance mechanism which ultimately produces a decrease in inward Ca current. Whether this results from a direct action on Ca channels or reflects an increase in outward current is presently unknown. The decrease in rate of rise and peak potential of the Ca spike suggests the former. A voltage clamp analysis to distinguish between these possibilities is in progress. The Ca currents recorded from the DRG soma may provide a useful model both for the study of presynaptic membrane Ca currents involved in transmitter release and ultimately for investigations into the neuromodulatory role of transmitter function. (Supported by USPHS Grant #NS 11160)

1405 MORPHINE SUPPRESSION OF SPONTANEOUS CAUDATE NEURONAL ACTIVITIES IN THE RAT: THE PARTICIPATION OF A CAUDATO-NIGRAL FEEDBACK MECHANISM. Edward P. Finnerty and Samuel H.H. Chan. Dept Life Sci., Indiana State Univ., Terre Haute, IN 47809

Morphine has been reported to inhibit the spontaneous unitary activities in the caudate nucleus (CN). Such suppression has been attributed to a possible direct excitatory action of the opiate on the nigro-striatal neurons in the substantia nigra (SN), and promoting a release of dopamine (DA) at their terminals in the CN (Lee, Wong and Chan, Neuropharmacol. 16:571, 1977). At the same time, a caudato-nigral inhibitory feedback loop has been described neurochemically, which functions to control the DA content in the CN. This study attempts to investigate the role of this loop as another possible mechanism in the morphine suppression of CN units.

Adult male Sprague-Dawley rats (300-800 g) were used in the present study. Under light sodium pentobarbital anesthesia (50mg/kg, i.p.), the trachea and left jugular vein were routinely cannulated. The head of the animal was then placed on a stereotaxic apparatus and appropriate craniotomy was performed to expose the cortex overlying the CN and SN. Spontaneous, single-unit activities from the SN were recorded by means of stereotaxically positioned tungsten microelectrodes which were further advanced by a hydraulic micro-drive. Local injections to the CN at a volume of 1 μl were made by means of a stereotaxically placed 27-gauge syringe needle attached to a microinjection-device. Systemic injections were made via the cannulated jugular vein.

In the first series of experiments, spontaneous SN units were found to respond to a systemic injection of morphine (5 mg/kg) with an almost complete inhibition of activity, lasting beyond 10 min. Subsequent microinjection of DA (50 μg/kg) into the CN resulted in essentially no change in the depressed activity. Naloxone (0.5 mg/kg i.v.), however, was able to reverse the suppression and allow the return of the SN spikes to the control rate.

In the second series of experiments, the SN units responded to the microinjection of DA (50 μg/kg) to the CN with an increase in discharge rate. Subsequent systemic injection of morphine (5 mg/kg) resulted in a twofold effect. There was an initial further enhancement of spike activity lasting 2-3 min, to be followed by a profound suppression of activity. Naloxone (0.5 mg/kg, i.v.) was relatively effective in reversing this depression.

It is concluded that the morphine suppression of caudate spontaneous unit activity may also involve the caudato-nigral feedback mechanism.

(We acknowledge the generous supply of morphine sulfate by Eli Lilly & Co. and Naloxone HCl by Endo Laboratories used in the present study.)

1406 EXCITATORY H1- AND INHIBITORY H2-HISTAMINE RECEPTORS ON NEURONS IN TUBERAL HYPOTHALAMIC TISSUE CULTURES. H.M. Geller. Dept. Pharmacol., Rutgers Med. Sch., Piscataway, NJ 08854.

Histamine was iontophoretically applied to spontaneously active neurons in tissue cultures of new born rat tuberal hypothalamus. Both excitations and depressions of unit activity were observed on different cells. These effects persisted in Ca^{++}-free/4.6 mM Mg^{++} media, suggesting the presence of post-synaptic histamine receptors on tuberal hypothalamic neurons. In order to classify the types of receptors involved, the histamine H1-receptor blocking drugs diphenhydramine and promethazine and the H2-receptor blocking drug metiamide were perfused through the culture chamber during iontophoresis of histamine. Diphenhydramine (10^{-4} M) blocked histamine-elicited excitations, but did so at concentrations at which local anesthetic actions were observed. Promethazine, a phenothiazine antihistamine, (10^{-5} M), also blocked histamine-elicited excitations and had no effect on inhibitions at concentrations below which local anesthetic effects were observed. Metiamide, (10^{-6} M) selectively blocked histamine-elicited depressions of activity, and had no effect on excitations. Further evidence for two independent receptors was obtained with the iontophoretic application of the H1-specific agonist 2-(2-pyridyl) ethylamine (PEA) and the H2-specific agonist S-[3-(n, n-dimethylamino) propyl] isothiourea (Dimaprit). PEA was generally excitatory while Dimaprit was uniformly depressant to unit activity. Evidence that cyclic AMP may be involved in the H2-mediated inhibitions was obtained by perfusing either of the cyclic nucleotide phosphodiesterase inhibitors isobutyl methyl xanthine (IBMX, 10^{-3} M) or RO 20-1724 (3×10^{-5} M). Both of these agents prolonged the depressant actions of H2-agonists.

These results suggest that there exist two populations of histamine receptors on tuberal hypothalamic neurons, with H1-receptors mediating excitatory and H2-receptors mediating depressant actions of histamine. Furthermore, these experiments are consistent with the hypothesis that hypothalamic neurons possess a histamine-sensitive adenylate cyclase coupled to the H2-receptor.

(Supported by NSF grant BNS 77-09241. PEA and Dimaprit were kindly supplied by SKF and RO 20-1724 was supplied by Hoffmann-LaRoche.)

1407 STUDIES OF THE ACETYL-DONOR FOR ACETYLCHOLINE SYNTHESIS. Gary E. Gibson and Adriana Vasil*. Neuropsychiatric Inst., UCLA, Los Angeles, CA 90024.

It is widely accepted that the acetyl-coenzyme A used for acetylcholine synthesis in the cytoplasm is derived from the oxidation of pyruvate or ketones in the intramitochondrial space, but the mechanism of transfer of the active acetyl units across the mitochondrial membranes is a matter of controversy. Quastel (Cholinergic Mechanisms and Psychopharmacology) and Lefresne, et. al. (Biochemie 59, 197) have suggested that the active acetyl groups for acetylcholine synthesis may be generated in the cytoplasm. These suggestions led us to reinvestigate a possible role of acetylphosphate, which is a product of cytoplasmic pyruvate oxidation in bacteria. We have examined its role in acetylcholine synthesis by synaptosomes incubated in a high potassium (31 mM) Krebs-Ringer phosphate buffer with 1.25 mM glucose, 50 μM choline and 40 μM paraoxon.

In the first series of experiments, the mass of acetylcholine and the incorporation of [U-^{14}C]glucose into acetylcholine were determined. Thus, we could determine the specific activity (DPM/nanomole) of the synthesized acetylcholine. Addition of K^+L^+-acetylphosphate (10, 50 or 100 mM) reduced the specific activity of acetylcholine to 82.9 ± 54; 55.6 ± 5.2; and 38.5 ± 3.4 of control, respectively. This is consistent with acetylcholine acting as a donor of acetyl groups for acetylcholine synthesis. Although synthesis of acetylcholine as measured by GC-MS was also inhibited (70.6 ± 2.6; 24.8 ± 1.9; 8.2 ± 1.3 % of control) this was shown to be in part due to the addition of the excess K^+ and L^+ which caused inhibition when added as the chloride salts (31.8 ± 2.2% of control with 100 mM). The specific activity was increased by their addition (128.7 ± 15.2%).

In order to determine if there was a direct transfer of acetyl groups from acetylphosphate to acetylcholine, synaptosomes were incubated with [^{3}H]acetylphosphate synthesized from [^{3}H]acetic anhydride. The purity was determined by enzymatic assay to be 89.9%; IR spectrometry showed it to be identical to commercial acetylphosphate. Although large amounts (38 X 10^6 CPM) of [^{3}H]-acetylphosphate were incubated with synaptosomes, no radioactivity above non-incubated control values were found in acetylcholine (4 counts). The lack of incorporation was not due to hydrolysis of added acetylphosphate, since greater than 90% of the acetylphosphate remained at the end of the incubation. Acetate (10 mM) itself had no effect on acetylcholine specific activity (97.8 ± 2.7% of control). Furthermore, in the absence of glucose, acetylphosphate was unable to stimulate synthesis above non-incubated controls. Thus, acetylphosphate does not appear to be an intermediate in acetylcholine synthesis.

1408 PERMEABILITY OF THE BLOOD-BRAIN BARRIER (BBB) TO NOREPINEPHRINE (NE) IN EXPERIMENTAL CEREBRAL ISCHEMIA. H. Hervonen*, O. Steinwall*, K. Nishimoto*, M. Spatz and I. Klatzo. Lab. Neuropath, & Neuroanat. Sci., NINCDS, NIH, Bethesda, MD 20014.

Observations on the behavior of BBB to NE and other tracers were carried out in Mongolian gerbils subjected for one hour to occlusion of the left common carotid artery and sacrificed at various time intervals following release of the occlusion. Evans blue dye and ^{3}H sucrose served as other than NE tracers. Only symptom-positive animals were used in this study. The abnormal passage of sucrose was observed in the ischemic hemisphere earlier than leakage of other tracers and it persisted for one week. Extravasation of Evans blue was seen only during 10-15 hour interval following release of occlusion. Quantitative measurements of ^{3}H NE and ^{14}C sucrose revealed that abnormally high passage of sucrose into the ischemic hemisphere was evident at all periods studied whereas abnormal elevation of ^{3}H tracer in the ischemic hemisphere was moderate at earlier periods (5 and 12 hours) and it rose to higher levels at 72 hours after release. At that time histofluorescent observations in animals which were also injected with reserpine and pargyline showed in the vicinity of ischemic lesions abnormal noradrenergic structures resembling thickened nerve fibers. The green fluorescence, specific for NE, was also observed in an accentuated fashion in the neurons and arterioles of the basal ganglia. The abnormal passage of NE or its metabolites could be surmised also from the radioautographs showing dark areas in the ischemic hemisphere, especially conspicuous at 72 hour interval following release of occlusion.

1409 TARDIVE DYSKINESIA: SUPPRESSION BY LECITHIN INGESTION. Madelyn J. Hirsch, John H. Growdon* and Richard J. Wurtman. M.I.T., Cambridge, MA 02139 and Tufts Sch. of Med. Boston, MA

We have previously shown that choline consumption raises serum choline (Ch), brain Ch, and brain acetylcholine (ACh) in rats, and serum and CSF Ch levels in humans. Choline has already been used by several investigators to treat a human disease associated with deficient cholinergic tone, tardive dyskinesia (TD); we found it effective in suppressing buccal-lingual dyskinetic movements (TD) that had been refractory to other therapies in 9 of 20 patients (none of whom responded to placebo).

The naturally-occurring dietary Ch source is lecithin (Lec) (phosphatidylcholine). Its ingestion also elevates serum and brain Ch and brain ACh contents in rats; it produces a greater and more prolonged rise in serum choline levels of humans than an equimolar quantity of choline salts. To test lecithin's therapeutic utility in TD, we gave Lec granules (Sigma Chem.Co; approximately 20% pure) to 2 subjects whose abnormal movements had previously responded to Ch therapy, and a partially-purified Lec preparation (Phospholipon-80, Nattermann Co; approx. 80% pure) to 1 additional subject (#3) who had not taken Ch. Lec both increased serum Ch levels and suppressed choreic movements in all 3 subjects (Table 1).

Table 1

Subject	Lecithin Dose (g/day)	Choline Content (g/day)	Movement	Percent Improvement	Serum Choline (nmol/ml) Before	During
1	60	1.8	jaw tremor	90	12.2	21.8
2	80	2.4	facial grimace	75	7.5	29.4
3	40	4.8	tongue twitch	86	10.8	20.0

Lec was as effective as Ch in reducing choreic movements, but, fortunately, did not produce the fishy odor that accompanys Ch ingestion. Lec may be used to treat those diseases where clinicians desire to enhance central cholinergic neurotransmission. (These studies were supported by grants from ADAMHA and NASA.)

1410 DIFFERENTIAL EFFECTS OF DIPROPYLACETATE AND AMINO-OXYACETIC ACID ON GABA LEVELS IN DISCRETE AREAS OF RAT BRAIN. Michael J. Iadarola, Arthur Raines and Karen Gale*. Dept. Pharmacology, Georgetown Univ., Schools of Medicine and Dentistry, Wash., D.C. 20007

The effects of two inhibitors of γ-aminobutyric acid (GABA) transaminase were examined in three regions of rat brain, each of which contained distinctly different concentrations of GABA (nmoles/mg protein); substantia nigra (SN): 102, superior colliculus (SC): 40, frontal cortex (CTX): 18. Brain areas were rapidly dissected out at 4° C and frozen on dry ice; GABA was measured by the method of Odaka, *et al.* (Exp. Br. Res. 13: 514, 1971). Time course of the dipropylacetate (DPA)-induced increase in GABA levels was determined by decapitating animals at 15, 30 and 45 min after i.p. injection of 400 mg/kg. Peak effect in all areas was reached at 30 min and began to decline by 45 min. At 15 min, SC was the only area to show an increase in GABA level (122% of control). When DPA was administered i.p. 30 min prior to sacrifice in doses of 200, 300 and 400 mg/kg, all three areas showed maximum increases with 300 mg/kg. No significant additional increases were achieved with higher doses. Of the three regions examined, SC showed the largest response to DPA and this differential effect was most evident at 200 mg/kg, a dose which produced insignificant increases in GABA levels in CTX and SN (see table). In contrast, amino-oxyacetic acid (AOAA) appeared to affect GABA levels in CTX to a greater degree than in SN or SC. Ninety min after AOAA (40 mg/kg, i.p.), GABA levels in SN and SC were 150% of control, whereas cortical GABA increased to over 250% of control. These results are being verified in rats sacrificed by microwave irradiation. The different GABA profiles may reflect different mechanisms of action of the two compounds, or a difference in ability of the two drugs to interfere with the synthesis of GABA. Further comparisons with other brain regions are currently being made.

% OF SALINE-TREATED CONTROL (CONTROL=100%)

DPA (mg/kg)	SN	SC	CTX
200	109%	143%*	116%
300	151%*	158%*	131%*
400	140%*	157%*	141%*

*Doses where GABA concentration was significantly ($p<0.05$) greater than control.

Supported by NINCDS Grants 10667 and 12566.

1411 THE INTERNAL PH OF ISOLATED PLATELET SEROTONIN GRANULES. R.G. Johnson, A. Scarpa, and L. Salganicoff. Dept. Biochem.&Biophys. Sch. Med., University of Pa. 19104 and Dept. Pharm. and Specialized Center Throm. Res., Sch. Med., Temple Univ., Phila., Pa.19140

The internal pH of isolated serotonin granules was measured by [^{14}C]-methylamine distribution. The serotonin granules were isolated from pig platelets by a procedure based upon the incubation of the platelets with a proteolytic enzyme to soften the membrane, cell disruption by a French cell press, and fractionation on a sucrose-Ficoll-D_2O gradient in order to preserve isotonicity. When [^{14}C]-methylamine was added to a granular preparation suspended in 0.3 M sucrose-30 mM Tris-Maleate, a ΔpH of 1.16 was measured with the internal pH being found acidic (pH 5.74). The ΔpH could be predictably perturbed by compounds known to transport H^+ across biological membranes. When nigericin, an ionophore known to exchange K^+ for H^+, was added to a suspension of serotonin granules containing 10 mM K^+, the ΔpH decreased to 0.42 pH units due to the alkalinization of the intragranular space. The addition of 400 µM $CaCl_2$ and A23187, an ionophore which transports Ca^{++} in exchange for protons in a ratio of 1:2, resulted in a ΔpH of o.65. Addition of 30 mM NH_4Cl which is thought to permeate biological membranes as NH_3, resulted in a decrease to 0.35 pH units. The internal pH was found to be independent of the external pH. When the granules were suspended in a medium at pH 5.95, the ΔpH was 0.19. Increasing the external pH resulted in a parallel increase in the pH such that at pH 7.71, the ΔpH was 1.48. The internal pH was also constant when the granules were suspended in media consisting of various ions. Choline chloride, KCl, NaCl, and sucrose media were utilized with the result suggesting the the pH is not due to the establishment of a Donnan equilibrium. The [^{14}C]-methylamine distribution method was also used to monitor the intragranular pH after the addition of various concentrations of serotonin. The addition of 1 mM serotonin decreased the ΔpH from 1.1 to 1.0 pH units; the addition of 10 mM serotonin to 0.6 pH units; and the addition of 33 mM serotonin to 0.25 pH units. When serotonin granule membranes formed by hypoosmotic lysis of the granules were utilized, the ΔpH was found to be o.18 pH units. These results which suggest that a large ΔpH exists across the membrane of the platelet serotonin granule, that the proton permeability of the membrane is low, and that an amine, at least at high concentrations can permeate the membrane in the uncharged form are similar to previous studies of another amine containing subcellular organelle, the chromaffin granule (Johnson and Scarpa J. Gen. Physiol. 68, 601 (1976), Johnson et. al. J. Biol. Chem. 252, 1512 (1978), Casey et. al. Biochem.16, 972 (1977)). The combined study of these two embryologically distinct organelles may provide important insight into the role of the ΔpH in amine homeostasis. (Supported by GM 20246 and AHA 77-675)

1412 IMMUNOHISTOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE IN THE CEREBELLUM OF NORMAL HUMAN AND HUNTINGTON'S CHOREA BRAINS. K.-S. K. Kan, L.-P. Chao, and L.S. Forno (SPON: A. Yuwiler). Dept. Neuro., Sch. Med., UCLA, Los Angeles, CA 90024 and Stanford U. Sch. Med., Stanford, CA 94305.

Choline acetyltransferase (ChAc) catalyzes the synthesis of the neurotransmitter acetylcholine (AcCh) in the cholinergic neurons. There is no satisfactory histochemical method for the localization of AcCh at the cellular level. The histochemical localization of acetylcholinesterase and the published histochemical methods for the localization of ChAc in terms of free coenzyme A are not very specific for cholinergic neurons. The only alternative is to visualize the highly specific synthesizing enzyme marker, ChAc, by immunohistochemical techniques. ChAc from bovine brain has been purified in our laboratory and antibody has also been produced from guinea pigs. We successfully localized this enzyme in the anterior horn cells of bovine spinal cord which are known to be cholinergic.

Recently, the localization of ChAc in formalin-fixed, paraffin-embedded rabbit brain sections has been accomplished in this laboratory using the peroxidase anti-peroxidase immunohistochemical method and with antibody specific to bovine ChAc. ChAc was found to be localized in the mossy fibers and glomeruli of the rabbit cerebellum (Brain Res. in press). Further study has revealed that the antiserum cross-reacts with human ChAc. The present study is of paraffin sections of normal human cerebellum as well as those from Huntington's Chorea (HC). In the cerebellar folia of both brains there was no peroxidase reaction in the molecular layer, the Purkinje cells, granular cells or Golgi cells. The staining was restricted to the mossy fibers in the medullary layer and extended to the granular layer. Variations in the number of stained mossy fibers in the various folia was observed. No major differences could be observed between the normal and HC cerebellum. These immunohistochemical findings are in agreement with the localization of ChAc in rabbit cerebellum. Additional studies on the localization of ChAc in other areas of normal, HC and other pathological human brains are in progress. Supported by USPH Service Grant No. MS-11087

1413 CONDITIONED EFFECTS OF CHRONICALLY-ADMINISTERED PSYCHOMOTOR STIMULANTS. M. Marlyne Kilbey and Everett H. Ellinwood, Jr. Dept. Psychiat., Behav. Neuropharm. Sect., Duke Univ. Med. Ctr., Durham, NC 27710.

Several sets of data that relate to the question of whether conditioned effects operate in the augmentation of behavioral effects during chronic administration of psychomotor stimulants, as well as when these drugs are re-administered after drug-free periods, will be examined. Behavioral effects measured include: hyperactivity, stereotypy, and amplitude of movement in specific frequency ranges in rats; stereotypies and dysjunctive or dyskinetic postures in cats; and oral buccolingual dyskinesias in monkeys. Data will be discussed in terms of findings consonant with: (1) classical- and operant-conditioning principles; (2) discriminative properties of drugs; and (3) physiological mechanisms acting through specific and non-specific activation. Data addressing the differential contributions of these mechanisms to behavioral augmentation in various species will also be presented.

1414 GLUTAMIC ACID DECARBOXYLASE WITHIN LAMINAE OF THE OLFACTORY TUBERCLE. Neil R. Krieger and John S. Heller (SPON: A.M. Laties). Yale Univ., New Haven, Conn.

We have been interested in the localization of neurotransmitter related enzymes within the rat olfactory tubercle. Here we report on the distribution of glutamic acid decarboxylase (GAD) and gamma amino butyric acid (GABA) in this region of the limbic cortex. Three well-defined histological laminae occupy the 1mm depth of the tubercle: the plexiform, the pyramidal and the polymorphic. Our procedure uses direct enzymatic and chemical assays of homogenates prepared from frozen sections cut parallel to these laminae. The hemisected rat brain was frozen in powdered dry ice, the tubercle trimmed to a pedestal approximately 2mm on each side, and mounted in a cryostat at -15° C. Consecutive tangential 16µ sections were cut. For assays of GAD, groups of six sections (approximately 40µg of protein) were homogenized in a 0.6ml volume. Ten lambda aliquots were assayed using a method similar to that of Albers and Brady (JBC(1959) 234:926) in which enzymatic activity was assessed by the amount of CO_2 released from the substrate glutamic acid in a fixed time interval. GABA and other amino acid levels were determined with the use of a Durrum high pressure D500 amino acid analyzer.

Steep variations in GAD activity were observed as a function of depth in the tubercle. These were accompanied by corresponding but less marked variations in GABA levels. In the posterior medial region of the tubercle, GAD activity varied by as mueh as six fold ranging from 60nmoles of GABA formed hr/mg/protein in the outermost plexiform layer to 400nmoles/hr/mg/protein in the deepest layer of polymorphic cells. Such low activity in the plexiform layer suggests that GAD may play a very limited role in this lamina. In contrast, its high activity in the polymorphic layer rivals the highest known levels in other brain regions and leads to the further question whether this activity is attributable to cells intrinsic to this layer or to entering processes.

1415 NEUROGLIAL DEPOLARIZATION BY INTRACELLULAR INJECTIONS OF MONOAMINES. Y. Lamour*, K. Krnjević, J.F. MacDonald, A. Nistri* . Dept. Anaesthesia Research, McGill Univ., 3655 Drummond St., Montreal, H3G 1Y6.

We have previously reported that intracellular injections of monoamines (catecholamines especially) can have a striking depolarizing action on spinal motoneurons (Nistri et al., 1978, Canada Physiology, 9, 52). During our microiontophoretic studies with multibarrelled electrodes in the spinal cord, we frequently recorded from cells which could be identified as glial cells (high resting membrane potential, and input resistance, and lack of response to dorsal and ventral root or intracellular stimulation). We thus had the opportunity of testing the effects of intracellular injections of catecholamines and 5HT in cells of this type. The amines were injected by push-pull iontophoresis using currents of 5-20 nA for about 1 min. Most cells that were injected in this fashion with noradrenaline, dopamine, isoprenaline or 5HT showed a large fall in input conductance, which usually reversed within a few minutes after the injection was stopped. It was most commonly associated with membrane depolarization; however, the potential changes were sometimes minimal or in a hyperpolarizing direction. The conductance changes appeared to be genuine effects of the monoamines since they could not be produced by similar iontophoretic currents flowing through a barrel containing K-citrate at comparable pH. Moreover, these cells showed no consistent voltage-dependent conductance changes. We therefore conclude that intracellular monoamines appear to cause a marked primary blockade of the ionic conductance of the neuroglial membrane, presumably mainly by occluding potassium channels. Hence, glial depolarization could be a consequence of a rise in neuroglial monamine content, possibly as a result of certain forms of drug administration. This could be of functional significance, for example by slowing down of neurotransmitter uptake.

Supported by the Canadian Medical Research Council.

1416 INHIBITION OF HIGH AFFINITY GLUTAMATE ACCUMULATION BY KAINIC ACID - A KINETIC AND PHARMACOLOGICAL STUDY. John Lehmann, E. G. McGeer and H. C. Fibiger. Div. Neurological Sci., Dept. Psychiatry, University of British Columbia, Vancouver, B.C. V6T 1W5, Canada

Kainic acid (KA) reversibly inhibited the high affinity, sodium-dependent accumulation of labeled glutamate into crude rat striatal synaptosomes. Dixon plots indicated that the K_i was 7.5 x 10^{-4}M and was apparently non-competitive with glutamate In contrast, both glutamate and aspartate were competitive inhibitors, with K_i of 3.7 and 2.3 x 10^{-6}M respectively. KA itself was not taken up into synaptosomes under identical conditions, suggesting that its inhibitory action is mediated at the level of the plasma membrane. The non-competitive nature of the inhibition indicates that kainic acid exerts its action at a site distinct from the glutamate uptake substrate site. Inhibition by KA was not affected by various glutamate receptor-antagonists, tetradotoxin, ouabain, or omission of calcium from the medium. In contrast, agents which disturb structural units of the synaptosome such as neuraminidase, colchicine, and cytochalasin B, reduced control uptake velocity and tended to enhance KA's inhibition. Since KA is not taken up by synaptosomes, it is probably restricted to extracellular space when injected intracerebrally. It is suggested that concentrations of KA equal to those required to inhibit glutamate accumulation are probably attained by intracerebral injections of cytotoxic doses of KA.

Supported by the Medical Research Council.

1417 OPIATE AND CHOLINERGIC INDUCED CHANGES IN MEMBRANE POTENTIAL MONITORED BIOCHEMICALLY IN NEUROBLASTOMA X GLIOMA HYBRID TISSUE CULTURE CELLS. D. Lichtshtein*, H.R. Kaback* and A.J. Blume, Dept. Phys. Chem. & Pharm. and Dept. Biochem., Roche Institute of Molecular Biology, Nutley, NJ 07110.

Cultured neuroblastoma x glioma hybrid cells NG108-15 have a transmembrane electrical potential ($\Delta\Psi$) of -40 to -60 mV as determined by direct electrophysiological measurements. In an attempt to develop a biochemical method for monitoring changes in $\Delta\Psi$ in populations of cultured neuronal cells, accumulation of the permeant lipophilic cation [^{3}H]tetraphenylphosphonium (TPP^+) [bromide salt] has been studied. TPP^+ is accumulated against a concentration gradient by NG108-15 cells suspended in physiological buffers (high external Na^+) and in buffer containing 135 mM K^+ (high external K^+); however, the concentration gradient is about 9-fold higher in high Na^+. Since high external K^+ is known to collapse $\Delta\Psi$ in NG108-15, the difference in TPP^+ accumulation in high Na^+ versus high K^+ has been taken as an index of $\Delta\Psi$, and using the Nernst equation ($\Delta\Psi = 61 \log [TPP^+]_{in}/[TPP^+]_{out}$), a resting potential of $\sim$ -60 mV has been calculated for NG108-15 cells in suspension. TPP^+ accumulation is time dependent, achieving a steady-state in about 20 min, and is a linear function of cell number and TPP^+ concentration (i.e. $\Delta\Psi$ is independent of TPP^+ concentration up to 26 μM). Moreover, TPP^+ accumulation is decreased or totally abolished by carbonylcyanide m-chlorophenylhydrazone, dinitrophenol or ouabain. In contrast, the ionophore monensin which catalyzes electroneutral transmembrane exchange of Na^+ and H^+ causes an increase in TPP^+ accumulation. Alterations in TPP^+ accumulation are also induced by addition of ligands for certain plasma membrane receptors. Veratridine, a compound which opens a specific Na^+ channel, decreases TPP^+ uptake in high Na^+ to the same level as observed in high K^+. This effect is dependent upon the presence of external Na^+ and is blocked by tetrodotoxin. The cholinergic agonist carbamylcholine and the opiate peptide agonist D-ala^2-met^5-amide (DAMA) induce an increase in TPP^+ accumulation. The carbamylcholine effect is blocked by the specific muscarinic cholinergic antagonist QNB and the DAMA effect is blocked by the specific opiate antagonist naloxone. The data demonstrate clearly that (i) membrane depolarization and hyperpolarization can be monitored biochemically in cultured cell populations; and (ii) in NG108-15 cells, hyperpolarization is induced by agonist occupany of either opiate or cholinergic receptors.

1418 INTERACTION OF GABA MIMETICS WITH CENTRAL DOPAMINE (DA) NEURONS. Kenneth G. Lloyd, Branimir Zivkovic*, Bernard Scatton*, Paul Worms* and Giuseppe Bartholini*. Synthélabo-L.E.R.S., Research Department, 31,Ave Paul-Vaillant Couturier, 92220 BAGNEUX, FRANCE.

The current literature indicates that GABA neurons are involved in the feed-back regulation of DA neurons.In the present study several indirect acting GABA mimetics (e.g. dipropylacetamide, DPA; gamma-acetylenic GABA, GAG; amino-oxyacetic acid, AOAA; pyrrolidinone; gamma-butyro-lactone, GBL) as well as two direct acting GABA mimetics, muscimol (M) and SL 76.002 (SL), were utilized for studying DA neuron function.

Increasing GABA-receptor activity by M or SL (i.p.) did not appear to greatly alter the basal activity of DA neurons as neither drug induced catalepsy, altered apomorphine-induced stereotypies or mesh-climbing, or altered the kinetic state of striatal or limbic tyrosine hydroxylase. M or SL slightly decreased DOPA and DA synthesis in both limbic system and striatum ; DA turnover after α-methyl-p-tyrosine was decreased only in the striatum. After activation of the feed-back circuit by neuroleptics, the DA neurons became much more susceptible to GABA-related drugs. Thus, haloperidol-induced catalepsy was potentiated by M, SL, DPA, GAB, AOAA or pyrrolidinone.Bicuculline blocked the potentiation of catalepsy by M or SL. M, SL and AOAA also potentiated the catalepsy due to thioridazine or chlorpromazine. In correlation with these behavioural findings, M, SL and GBL all blocked the activation of tyrosine hydroxylase induced by haloperidol. M and SL reduced the haloperidol-activated DA turnover to a greater extent in striatum than in the limbic system. These observations indicate that increased GABA receptor activity reduces the feed-back activation of the nigro-striatal DA pathway therefore enhancing the cataleptic action of the neuroleptics. These results also imply that DA neuron function is much more sensitive to modulation by GABAergic mechanisms after activation of the neuronal feed-back loop and enhancement of DA cell firing. The following are consistent with the above conclusions :
i) neither AOAA nor pyrrolidinone induce catalepsy following sulpiride, a neuroleptic with minimal activity in the nigro-striatal DA pathway ; and ii) behavioural effects of direct stimulation of DA receptors by apomorphine are not overcome by SL or M.

1419 POSTSYNAPTIC PHARMACOLOGY OF CEREBELLAR NEURONS IN CELL CULTURE. R. L. Macdonald, G. Moonan*, P. G. Nelson. Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda, Md. 20014.

Cerebellar neurons grown in cell culture have been examined for responses to amino acid putative neurotransmitters, norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (5-HT). Cultures were derived from 17-19 day old fetal rats and were maintained for at least 4 weeks prior to study. Although these cultures contain at least three different cell types, all intracellular recordings were obtained from the largest neurons (soma diameter >15μm). These neurons were polygonal in shape with 1-5 dendritic shafts and fine dentritic arborizations, and appeared, on morphological grounds to be the in vitro equivalent of Purkinje cells. All neurons studied with membrane potentials >25mV and with an action potential (evoked by intracellular stimulation), responded to iontophoretically applied glutamic acid (GLU) (n=20 cells) and γ-amino butyric acid (GABA) (n=30), but few responses were elicited by glycine (2/15 cells), β-alanine (0/10) or taurine (0/6). Aspartic acid (ASP) produced small responses only at high currents (n=15). Responses were also elicited by NE (n=15), DA (n=8) and 5-HT (n=8).

GLU reversibly increased membrane conductance and was depolarizing with reversal potentials between 0 and -10mV (n=4). There was non-uniform sensitivity to GLU with high sensitivity occurring only on the dendrites; little sensitivity to GLU was present on the soma. GABA also reversibly increased membrane conductance but was hyperpolarizing with reversal potentials (n=4) between -50 and -40mV (10 to 15 mV more negative than resting membrane potential). Log-log dose-response curves were linear in the low charge region with GLU having a slope of about 1.0 (n=6) and GABA of about 2.0 (n=7), suggesting that GABA, but not GLU, interacts with its receptor in a cooperative manner. Although having little direct action, ASP was a potent modulator of GLU responses (n=15). ASP at low currents augmented GLU responses and shifted the GLU DR curve to the left while higher currents diminished GLU responses. NE and DA were both inhibitory and decreased or abolished spontaneous spike activity without alteration of membrane potential; at higher currents, depolarization was frequently recorded. 5-HT was excitatory and increased spike activity in all cases.

The results indicate that large cerebellar neurons in cell culture develop considerable neuropharmacological specificity similar to that of Purkinje cells in vivo and suggests that this culture system may be useful in the study of cerebellar neurotransmitter mechanisms.

1420 LOW AFFINITY GABA TRANSPORT BY A HIGH-AFFINITY TAURINE TRANSPORT SYSTEM IN GLIOMA CELLS. D. Martin* and W. Shain Chemistry Dept., University of MD., College Park, MD 20742 and Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Transport of GABA, taurine, and β-alanine was studied in a rat glioma cell line cloned from a spinal tumor (LRM55 cells) incubated in HEPES buffered Hank's balanced salt solution. Kinetic analysis of ^{3}H-GABA influx (concentration range 2.5×10^{-8} -2×10^{-3}M) carried out in the presence of 1×10^{-5}M aminooxyacetic acid revealed only a low affinity GABA transport system (K_m=700 μM) whereas analysis of ^{35}S-taurine and ^{3}H-β-alanine influx (1×10^{-6}M to 1×10^{-3}M) showed only high affinity uptake systems with K_m values of 30 μM and 80μ M respectively. Maximum rates of influx were 5.4±1.0, 1.7±0.3, and 4.6±1.4 nmole/min/mg protein for GABA, taurine and β-alanine respectively. The rate of GABA influx at all concentrations was nearly constant for over two hours whereas influxes of β-alanine and taurine were nearly complete after 1 hour of incubation. This difference is attributable chiefly to the differences in kinetic parameters of the uptake systems as shown by numerical integration of the transport rate equation. Net transport of GABA and taurine was demonstrated by direct measurements of the amino acid content of the cells. Influxes of taurine and GABA were both strongly dependent on Na^+. Reduction of (Na^+) to 5 mM by replacement with choline Cl^- reduced the influx of 30 or 300 μM taurine and 70 or 700 μM GABA to less than 5% of control values. GABA, β-alanine and taurine were found to be mutually competitive inhibitors of each other's transport. The inhibition constants for GABA, taurine, or β-alanine when used as inhibitors of the transport of the other two amino acids were similar to their K_m values when used as substrates. Ten compounds including glycine, 2,4-diaminobutyric acid, α-alanine and nipecotic acid were tested as inhibitors and found to have similar effects on GABA, taurine and β-alanine influxes. The results of these experiments suggest that low affinity GABA uptake may be carried out, at least in part, by high affinity taurine or β-alanine transport systems.

1421 GLYCINE, GABA, AND INHIBITORY SYNAPTIC TRANSMISSION ONTO GIANT RETICULOSPINAL NEURONS OF THE LAMPREY. Gary Matthews and Warren O. Wickelgren. Dept. Physiol., Univ. of Colo. Med. Sch., Denver, CO 80262.

Intracellular recordings were made from giant reticulospinal neurons (Müller cells) in the isolated brain of lamprey, and the physiological-pharmacological properties of the inhibitory postsynaptic potential (ipsp) evoked by ipsilateral vestibular nerve stimulation were compared with those of the hyperpolarization resulting from the iontophoretic application of glycine or γ-aminobutyric acid (GABA). The reversal potentials of the ipsp, the glycine response, and the GABA response were identical, averaging -83 mV or about 13 mV negative to the usual -70 mV resting potential of the cells. To determine if a change in Cl conductance was involved in the response, the Cl equilibrium potential was changed either by injecting Cl intracellularly or by lowering extracellular Cl. Both manipulations shifted the reversal potentials for glycine, GABA and the ipsp in a positive direction by identical amounts. Alterations in extracellular K also produced identical changes in the reversal potentials for the drugs and the ipsp, but, although the cell resting potential changed rapidly after a shift in extracellular K, the reversal potentials changed more slowly. This suggests that the effects of K on the reversal potentials were secondary to changes in internal Cl concentration. Picrotoxin or bicuculline at a concentration of 20 μM abolished the response to GABA but had negligible effects on the ipsp or the response to glycine. In contrast, 20 μM strychnine abolished both the ipsp and the glycine response but had little or no effect on the GABA response. For all Müller cells 1 μM strychnine was sufficient to reduce the glycine response by 50% or more within 5 min and, in those Müller cells located in the aqueductal region (the I cells), this was accompanied by a parallel reduction in the amplitude of the ipsp. However, in those Müller cells in the midbrain (the M cells), 1 μM strychnine, although rapidly abolishing the glycine response, had no effect on the ipsp, which required approximately 5 μM strychnine before it was substantially reduced. We conclude that for lamprey Müller cells glycine is a better candidate for the inhibitory transmitter than is GABA, particularly so for those cells (the I cells) in which the sensitivity of the ipsp and the glycine response to strychnine was identical. (Supported by NIH grants NS 09661 and 09660.)

1422 SEROTONERGIC FACILITATION OF FACIAL MOTONEURON EXCITATION: A MODULATORY EFFECT. Robert B. McCall* and George K. Aghajanian (SPON: Michael Davis). Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Pharmacological treatments which enhance stimulation of postsynaptic serotonin (5-HT) receptors produce a behavioral syndrome in the rat which includes resting tremor, reciprocal forepaw treading, and lateral head weaving. Although the precise mechanisms underlying this 5-HT motor syndrome have not been elucidated, a direct action of 5-HT on motoneurons is possible since many brain stem and spinal motor nuclei receive a dense 5-HT input. Therefore, the effect of 5-HT on motoneurons located in the facial nucleus of the rat was investigated by single-cell recording. Fluorescence histochemistry revealed the facial nucleus receives a dense 5-HT input as well as a lesser input from norepinephrine (NE)-containing fibers. Microiontophoretic application of 10-200 nA pulses of 5-HT lasting from 1-10 minutes failed to excite facial motoneurons. However, small amounts of 5-HT facilitated the excitation of these cells produced by iontophoretically applied glutamate. The current of glutamate required to produce an activation of facial motoneurons was reduced at least 50% by 5-HT. In addition, 5-HT markedly shifted to the left the cumulative dose-response curve of glutamate-induced excitation of motoneurons. Thus, 5-HT facilitated the excitatory effect of iontophoretically applied glutamate without directly exciting facial motoneurons. The 5-HT releasing agent p-chloroamphetamine (PCA) facilitated the excitatory effect of glutamate on motoneurons in control animals, but not in those pretreated with the 5-HT synthesis inhibitor p-chlorophenylalanine. This indicates the ability of PCA to facilitate motoneuron excitation is secondary to release of endogenous 5-HT.

Microiontophoretic application of large amounts of NE also failed to directly excite motoneurons. However, NE facilitated the excitatory effects of iontophoretically applied glutamate on facial motoneurons. In addition, both 5-HT and NE facilitated the excitation of motoneurons produced by stimulation of the motor cortex and the red nucleus. Thus, 5-HT and NE facilitated synaptically-mediated excitatory inputs as well as the excitation produced by glutamate. The facilitating effect of 5-HT, but not NE, was blocked by methysergide (1 mg/kg, i.v.). On the other hand, piperoxane (2 mg/kg, i.v.) blocked the effect of NE but not 5-HT. Thus the actions of 5-HT and NE appear to be mediated by distinct receptors in the facial nucleus. It is concluded that 5-HT and NE do not produce a direct excitation of facial motoneurons, but rather act as modulating transmitters that enhance the effects of excitatory inputs.

Supported by USPHS Grants MH-17871, MH-14459, MH-14276, and the State of Connecticut.

1423 ADENOSINE ACTIVITY IN RAT STRIATUM. Mary L. Michaelis and Elias K. Michaelis. Dept. Human Development, U. of Kansas, Lawrence, Ks. 66044.

Adenosine (Ado) and its non-metabolizable analog 2-Chloro-adenosine (2-Cl-Ado) have been shown to cause large increases in the cAMP content of brain slices and hyperpolarization of cortical and subcortical neurons. We have previously shown that 2-Cl-Ado can stimulate the striatal adenylate cyclases (AC) in broken cell preparations and that it can modulate the dopa-(DA) stimulation of AC activity (Neuroscience Abst. III, 410, 1977). Further pharmacological characterization of the putative Ado receptor which is coupled to a striatal adenylate cyclase has been obtained. The stimulation of the striatal enzyme activity shows excellent correspondence with the physiological activity of adenosine and adenine nucleotides on cortical neurons. Adenosine, AMP-PNP, β, γ-methylene ATP, and 5'-AMP are approximately equipotent in stimulating AC activity but less effective than 2-Cl-Ado. The potent methylxanthine, isobutylmethylxanthine, is a very strong inhibitor of the 2-Cl-Ado stimulation of AC while 2'-deoxyadenosine is essentially inactive. The dependence of 2-Cl-Ado stimulation of striatal AC on various divalent cations and its effects on the enzyme kinetics are currently under investigation.

In addition, we have previously shown that 2-Cl-Ado partially inhibits the release of ^{3}H-DA from preloaded striatal synaptosomes. The magnitude of the inhibition by 2-Cl-Ado of the depolarization (KCl)-induced release of DA is dependent on the strength of the depolarizing stimulus, i.e., KCl concentration, with the greatest effect at low KCl concentrations. 2-Chloroadenosine modulation of DA release is also observed when the releasing stimulus is exposure to various amounts of d-amphetamine. Once again, 2-Cl-Ado decreases the amphetamine-stimulated release of ^{3}H-DA with the greatest effect at the lower amphetamine concentrations. Both the KCl and amphetamine-induced release of synaptosomal ^{3}H-DA is enhanced by pre-incubation of the synaptosomes with the specific Ado metabolizing enzyme adenosine deaminase. These results suggest the presence of endogenous Ado which is continuously controlling DA release from nerve endings. 2-Chloroadenosine has no effect on high affinity synaptosomal DA uptake, thereby ruling out the possibility that the 2-Cl-Ado effects are due to increased uptake activity. It appears, then, that adenosine can have both presynaptic effects on striatal DA nerve endings as well as possible postsynaptic effects on striatal Ac activity.

Supported by HD-07066 from NICHHD to Kansas Ctr. for Mental Retardation and by AA-01911 and GM-22357 to E.K.M.

1424 HIGH AFFINITY UPTAKE SYSTEM FOR CYSTEINE IN SYNAPTOSOMES OF RAT CENTRAL NERVOUS SYSTEM. Chandra H. Misra, Robert C. Smith. Dept. Psychiat., Washington Univ. School of Medicine, St. Louis, Mo. and Tex. Res. Inst. Mental Sciences, Houston, Texas 77030.

The in vitro uptake of [^{35}S]cysteine was studied in synaptosomal preparation of the cerebral cortex of rat brain. The accumulation of cysteine was found to be temperature dependent and very rapid. It was linear at least for 4 minutes at 37^{0} with characteristics of saturable kinetics. This uptake was Na^{+} and K^{+} dependent, but contrary to the Na^{+}, high extracellular concentration of K^{+} has inhibitory effect on cysteine uptake. Cysteine was accumulated against concentration gradients by energy dependent, saturable mechanisms, and the double-reciprocal plot of the cysteine uptake suggests dual affinity system with the Km values for the high affinity uptake of about 16.6 x 10^{-6}M and for the low affinity uptake of about 4.0 x 10^{-4}M. This transport was also found significantly inhibited by oubain, a potent inhibitor of the Na-K dependent ATPase and other metabolic inhibitors.

1425 A COMPARISON OF THE EXCITATORY EFFECTS OF L-ASPARTATE AND L-GLUTAMATE ON PURKINJE CELLS AND OTHER NEURONS IN THE CEREBELLAR CORTEX OF RAT. Sandra L. Morzorati, R.C.A. Frederickson and William J. McBride, Departments of Psychiatry and Biochemistry and Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46202.

L-aspartate (Asp) and L-glutamate (Glu) were administered iontophoretically onto Purkinje cells and other cerebellar neurons of urethane-anesthetized rats. Both Asp (0.5 M, pH 8.5) and Glu (0.5 M, pH 8.5) produced an increase of spike frequency in all cells tested. The excitatory effects of both agents were characterized by a rapid onset (<2 sec), a gradual increase to a maximal firing rate and fast recovery. Depolarization block was seen with large doses of Glu and, to a lesser extent, Asp. In order to compare potencies graded doses of Asp and Glu were ejected onto Purkinje cells and unidentified neurons and dose-response curves were plotted employing percent excitation of maximal firing rate vs. the product of ejection current and time. Potency ratios (at ED_{50}) showed both cell groups, but especially Purkinje cells, to be more sensitive to Glu than Asp. Dose-response curves for Asp and Glu on Purkinje cells were generally non-parallel, implying different mechanisms of action for the two amino acids. Tests for antagonism showed glutamic acid diethylester to be a more effective blocker of Asp than of Glu while DL-α-aminoadipate was equipotent in antagonizing the actions of both agents. These data are compatible with the possibility that both Asp and Glu may be excitatory transmitters in rat cerebellum. (Supported in part by PHS Grants MH10695 from NIMH and NS13925 from NINCDS).

1426 LAMINAR DISTRIBUTIONS OF AMINO COMPOUNDS AND [^{3}H]LIGAND BINDING SITES IN THE DOG OLFACTORY BULB. N.S. Nadi*, J.D. Hirsch* and F.L. Margolis (SPON: C. Zomzely-Neurath). Department of Physiological Chemistry and Pharmacology, Roche Institute of Molecular Biology, Nutley, NJ 07110.

The olfactory bulb was dissected into several discrete layers useful for studying the distribution of amino compounds and [^{3}H] ligand binding sites. Dogs under barbiturate anesthesia were exsanguinated and the olfactory bulbs removed into liquid N_2 within 15 min of the onset of anesthesia. The tissue was warmed to -20°C and 1 mm thick coronal sections were cut and then free-hand dissected into four layers: Fiber layer (F)-fibers arising from the olfactory nerve; glomerular layer (GL)-olfactory nerve endings, the mitral cell dendrites and the periglomerular cells; mitral-granule cell layer (M-G)-mitral cell perikarya, granule cells and tufted cells; and white matter (W)-efferent and afferent axons. Tissue was either deproteinized and analyzed for amino compounds, using Fluram$^{(R)}$, or else membrane fractions were prepared for [^{3}H]ligand binding studies. Levels of several amino compounds and the binding of nine [^{3}H]ligands were measured. Binding of [^{3}H]carnosine (carn) was highest in GL and lower in F, M-G and W. [^{3}H]Dihydromorphine binding was high in both F and M-G, but was much lower in GL and W. [^{3}H]Kainic acid and [^{3}H]spiroperidol (Spi) binding were primarily localized in M-G, while [^{3}H]diazepam (Dz) and [^{3}H]muscimol (Mu) binding were predominant in GL and M-G. [^{3}H]QNB binding was uniformly distributed. α- and β-adrenergic binding was distributed differently with α, lowest in M-G and W, while β was lowest in GL. Carn levels were high in F and GL, low in M-G and non-detectable in W. GABA and tyr levels were high in M-G and low in F, GL and W. The levels of tau, β-ala, glu, asp, ser, gly, ala and phe showed no significant differences among the four layers. The distribution of carn and its binding site support previous reports from this laboratory implicating this dipeptide as a neuroeffector at the glomerular level. While the localization of GABA and Mu binding are consistent with data from other laboratories associating it with granule cell function, the significance of elevated levels of both tyr and Spi binding in M-G remain to be investigated. The generally high level of binding sites in M-G imply that this layer is a major region of physiological and pharmacological interaction in the bulb.

	μmol compound/g tissue				fmol ligand bound/mg tissue			
Layer	Carn	GABA	Tyr	Leu	Carn	Mu	Spi	Dz
F	1.27	1.45	0.09	0.09	4	7	1.2	2
GL	0.95	1.87	0.08	0.08	10	32	0.8	24
M-G	0.34	7.0	0.20	0.10	3	52	11.6	50
W	0	4.51	0.08	0.08	3.5	8	6	10

1427 ACETYLCHOLINE INDUCED SLOW-WAVE ACTIVITY IN CAT ESOPHAGEAL SMOOTH MUSCLE. D. O. Nelson and A. W. Mangel*. Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801.

The electrical activity of cat esophageal smooth muscle was investigated with pressure electrodes. Most preparations were quiescent although some gave brief periods of spontaneous slow-waves and spikes. Application of 10^{-5}M acetylcholine induced slow-wave and spike activity to otherwise quiescent preparations. Lower concentrations of acetylcholine induced spike discharge without slow-waves. Continued exposure to acetylcholine for periods greater than 45 minutes caused desensitization which could be reversed by incubation in normal solution. Slow-waves were insensitive to ouabain or TTX application but were eliminated by exposure to low sodium containing solutions. Application of EGTA produced prolonged potentials as demonstrated in other smooth muscle preparations (1). Esophageal slow-waves may result from a sodium requiring, TTX insensitive, process as in the guinea pig ileum (2). Release of Ach from myenteric plexus neurons may be the in vivo correlate of acetylcholine application.

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This work was supported by NSF PCMS 07143 and USPH AM 12768.

1428 ACETYLCHOLINE SYNTHESIS AND RELEASE BY THE ABDOMINAL GANGLIA OF LIMULUS. Robert F. Newkirk*, M. A. Maleque* and James G. Townsel
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Acetylcholine (ACh) is a neurotransmitter candidate in Limulus (Stephens and Greenburg, 1973, Histochem. Cytochem. 21:923; Townsel et al., 1976, Neurosci. Abs. 2:618). The ventral nerve cord of Limulus has been shown to contain significant levels of choline acetyltransferase (Malthe-Sorenson and Emson, 1976, J. Neurochem. 27:341) and a true acetylcholine esterase (Townsel, Baker and Gray, 1977, Comp. Biochem. Physiol. 58:29). Recent studies have demonstrated the presence of a Na^+ dependent, high affinity choline (Ch) uptake system in Limulus (Maleque and Townsel, 1977, Neurosci. Abs. 3:318). The present study was conducted to evaluate the conversion of Ch to ACh and the mechanism for ACh release by the abdominal ganglia of Limulus.

Isolated ganglia from the ventral nerve cord of the horseshoe crab, Limulus polyphemus were incubated in Chao's solution containing 2μM ^{3}H-choline (0.55μCi). The rate of uptake was linear for 90 min. The uptake was inhibited 73% and 70% by 1mM ACh and 100μM hemicholinium-3 (HC-3), respectively, and stimulated 88% by previous exposure to 90mM K^+. Homogenates of ganglia incubated for 1 hr. in 2μM ^{3}H-Ch were analyzed by high voltage electrophoresis. Analysis showed that the ganglia contained 0.226 ± 0.1 pmole ^{3}H-Ch per mg tissue and 0.109 ± 0.09 pmole ^{3}H-ACh per mg. This represents a conversion to ACh of 25.8% of the Ch taken up. Ganglia labeled with ^{3}H-Ch and perfused with 90mM K^+ released five times the background efflux of radioactivity. The K^+ induced release of radioactivity was found to be Ca^{++} dependent and inhibited by Mg^{++} and Co^{++}. Similarly, 200μM veratridine effected a Ca^{++} dependent release of radioactivity. Analysis of the perfusate revealed that better than 85% of the released radioactivity co-electrophoresed with acetylcholine.

Thus, the abdominal ganglia of Limulus take up Ch via a high affinity Na^+ dependent uptake process, convert substantial amounts of it to ACh and possess a K^+ triggered, Ca^{++} requiring mechanism for the specific release of ACh. These results give further support to the contention that the abdominal ganglia of Limulus contain a population of cholinergic neurons.

(Supported by NIH Grant No. HL 17370 and MARC Faculty Fellowship No. 1 F34GM06132A)

1429 DEVELOPMENTAL CHANGES IN BRAIN HISTAMINE: A REASSESSMENT. Edward L. Orr* and Burr Eichelman (SPON: H. Kubinski). Waisman Ctr., Univ. of Wis., Madison, WI 53706; and Wm. S. Middleton VA Hospital, Madison, WI 53705.

We have recently shown that, due to incomplete extraction procedures, previous investigators have underestimated the levels of histamine (Hm) in the adult rat brain by as much as a factor of 10 (Orr & Eichelman, Trans. Am. Soc. Neurochem., Vol. 9, 1978). Since brain levels of Hm in the developing rat brain may have been underestimated as well, we have reinvestigated the developmental changes in rat brain Hm using our modified extraction procedure. Brains from male rats of 1, 10, 25, 40, 53 and 70 days of age (postnatal) were examined. In other reports, "high" concecntrations of whole brain Hm of about 300-350 ηg/g wet wt. were found in rats between 1 and 10 days of age. Brain Hm levels then declined to about 50 ηg/g by 30 days of age, and remained at this low "adult" level for the rest of development. We, too, find high concentrations of brain Hm early in development (466 and 493 ηg/g for 1- and 10-day old rats, respectively), with a subsequent decrease to 338 ηg/g by 25 days of age. However, rather than continuing to decline, brain Hm concentrations remain at this level until the onset of puberty, whereupon Hm concentrations begin to increase, reaching adult levels of about 500 ηg/g between 53 and 70 days of age. Total brain levels (ηg Hm/brain) increased from 1-day postnatal until adulthood. However, between 10 and 40 days of age the rate of increase was much slower than the rates of increase earlier or later in development. In preliminary experiments, the late (pubertal) increase of brain Hm levels in the male rat was prevented by prepubertal castration, indicating a relationship between the hormonal status of the rat and the levels of Hm in the brain.

This research was supported in part by a National Research Service Award (#1 F32 HD05173) and grants from NIMH (#MH 30210) and the Medical Research Service of the V.A. Hospital, Madison, WI.

1430 Voltage-Dependent Excitatory Response to Serotonin in Aplysia. T. C. Pellmar and D. O. Carpenter, AFRRI, NNMC, Bethesda, Maryland 20014.

A slow voltage-dependent excitatory response to iontophoretic application of serotonin has been observed in voltage clamped neurons of Aplysia californica. The response has a time-to-peak of 15 to 30 seconds and a duration of 1 to 3 minutes. At potentials more negative than approximately -40mV, the response is absent. As the cell is depolarized, the serotonin-evoked inward current becomes progressively larger. The potential dependence of the serotonin response is similar to that of delayed rectification. The response is accompanied by an apparent decrease in conductance which can be attributed either to a decrease in conductance to K^+ or to a regenerative inward current. Changes in extracellular potassium concentration up to 30mM have little effect, but higher concentrations reduce the amplitude of the response. The actions of zero sodium solutions depend on the sodium substitute: glucosamine prolongs the response to serotonin; sucrose and mannitol greatly reduce the amplitude. Exposure to lithium-substituted sea water causes a gradual attenuation and subsequent replacement of normal sea water potentiates the response. The serotonin response is minimally affected by alterations in extracellular calcium but is blocked by cobalt and manganese. Observed changes in response amplitude are usually accompanied by consistent changes in delayed rectification.

It is difficult to determine the ionic basis of the serotonin response from these data. A regenerative inward sodium current can be ruled out; but such a current carried by calcium remains a possibility. Alterations of extracellular calcium may not alter the calcium gradient sufficiently to modify such current. The above data seems most consistent with a decrease in a potassium conductance. Yet, it is disturbing that moderate changes in potassium concentration have minimal effects.

1431 EFFECT OF TREATMENT ON CATECHOLAMINE METABOLITES FOLLOWING HEAD TRAUMA. E.W. Pelton, II, Louis R. Nelson, and Robert S. Bourke Dept. Neurol., Albany Med. Coll., Albany, NY 12208.

Head trauma with hypoxia has been shown to alter normal metabolism and inactivation of catecholamines (CA) in cats 1 1/2 hours after insult (Pelton et al., 1977). Injurious trauma is induced by oscillations for one minute with subsequent hypoxia for 60 minutes. As previously reported, early post-injury regional assessment of norepinephrine (NE) and dopamine (DA) and their principal metabolites methoxyhydroxyphenylglycol (MHPG), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) indicate interference with normal oxidative and energy requiring methods of inactivation of vasoactive catecholamines with no significant changes in levels of CA per se. Recent work assesses the effects of anti-edema agents on morbidity, mortality and monoamines. At the time of insult cats received solumedrol, ethacrynic acid, DCPI, or nothing, and were scored neurologically for 24 hours. CA metabolism was assessed in animals that died during the 60 minute post-shake hypoxia or survived 24 hours. The longer cats survive during the hypoxic period, the lower the concentrations of CA metabolites in certain discrete areas of brain, supporting hypoxia's interference with oxidative degradation. Twenty-four hour survivors showed an inverse correlation between neurologic score and levels of regional DOPAC and HVA, with normal levels in fully recovered animals, and elevations in others. We suggest inability to inactivate vasoactive compounds following hypoxia and/or trauma may affect neurologic recovery, and should be an area of further pharmacologic study of therapy.

(Supported by Grant #NS13042, National Institutes of Health)

1432 SPECIFIC CONDITIONING OF STRIATAL DOPAMINE METABOLISM IN THE RAT WITH METHADONE AS AN UNCONDITIONED STIMULUS. Jorge Perez-Cruet. Dept. Psychiatry at Missouri Institute of Psychiatry, Psychoneuropharmacology Unit, 5400 Arsenal, St. Louis, Missouri 63139.

Methadone is known to produce marked increases in dopamine metabolism and to increase homovanillic acid (HVA) in rat striatum as an unconditioned reflex (Experientia 28: 926, 1972. Previously it has been reported that a conditioned stimulus reinforced with methadone produced increments in HVA and in the synthesis of dopamine as a conditioned reflex with methadone, morphine or bulbocapnine as an unconditioned stimulus (Pavlovian J. Biol. Sc. 11: 237, 1976). The present study was designed to determine if brainstem serotonin metabolism is also conditioned during conditioning of striatal dopamine metabolism with methadone as an unconditioned stimulus. The conditioning experiments included four groups of rats. Group I, was never exposed to a conditioned stimulus or drug treatments with methadone. Group II, was exposed to the conditioned stimulus plus injections of normal saline and never exposed to methadone. Group III, was the conditioned group that was exposed to the conditioned stimulus reinforced with methadone as the unconditioned stimulus for at least 10 trials after which time saline was given instead of methadone. Group IV, was a drug control group receiving methadone injections at random without no association to the conditioned stimulus. The metabolism of serotonin was estimated by measuring the concentration of 5-hydroxyindoleacetic acid (HIAA), the main metabolite of serotonin, in the brainstem. The results showed a conditioned reflex increment in HVA in the striatum tissues to a conditioned stimulus previously reinforced with methadone in trained animals whereas no conditioned reflex changes in the concentration of HIAA were observed in the brainstem of these trained rats. These results suggest that methadone conditioning of striatal HVA was not accompanied by a conditioned reflex change in brainstem HIAA. It is postulated that the conditioning of dopamine metabolism to methadone as an unconditioned stimulus seems to be specific to the dopaminergic system and that brainstem serotonin metabolism was not conditioned.

1433 EFFECTS OF DELETION OF CEREBELLAR GRANULE CELLS ON THE REGIONAL CONTENT, SYNAPTOSOMAL LEVELS, AND HIGH AFFINITY UPTAKE OF GLUTAMATE, ASPARTATE, AND GABA. Michael A. Rea*, Brooks H. Rohde*, Jay R. Simon and William J. McBride. Departments of Psychiatry, Biochemistry and Pharmacology and Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46202.

Exposure of the cerebella of rats to focal low-level x-irradiation treatment at different days following birth can prevent the acquisition of late-forming granule cells (Altman, J., J. Comp. Neurol. 165: 49, 1975). The levels of glutamate (Glu), aspartate (Asp) and GABA were determined in the cortex (where the granule cell bodies and terminals are located), white matter and deep nuclei of the cerebella of control rats and of rats exposed to x-irradiation treatment from day 8 to 15 following birth. Rats were killed at 60 days of age. In the control group, the level of Glu in the cortex was almost twice the values found in the white matter and deep nuclei. Treatment with x-irradiation caused a significant ($P<0.001$) 28% decrease in the level of Glu in the cortex without altering its levels in the other two regions. The levels of Asp did not change in any of the three regions with x-irradiation. Treatment with x-irradiation did not alter the content of GABA in the deep nuclei but it did cause a significant ($P<0.05$) increase in the levels of GABA in the cortex and white matter of approximately 60%. The levels of Glu, Asp and GABA were also determined in a crude synaptosomal fraction (P_2) isolated from the cerebella of adult control animals and rats which had been exposed to x-irradiation treatment on days 12 to 15 following birth. The level of glutamate was significantly ($P<0.005$) reduced by 25% and the level of Asp was decreased ($P<0.02$) by 15% with x-irradiation. The level of GABA in the P_2 fraction was the same for both groups. The uptake of [^{3}H] Glu and [^{3}H]Asp at a concentration of 1 μM was approximately 20% lower in the P_2 fraction from the x-irradiated group than from the control animals whereas the uptake of [^{3}H]GABA was nearly the same in both groups. More detailed kinetic analysis of [^{3}H]Glu uptake revealed that the K_m value was not significantly changed but that the V_{max} value was significantly ($P<0.01$) lower (by 20%) for the x-irradiated animals than for the control group. The data are consistent with the idea that Glu levels are higher in granule cells than in other cells in the cerebellum and that Glu may serve as the excitatory neurotransmitter released from granule cells. Supported in part by Research Grant NS 13925 from the NINCDS .

1434 LOCALIZATION OF TWO GABANERGIC SYSTEMS IN MAMMALIAN RETINA. D. A. Redburn, C. Kyles*, T. Chantenez* and J. Ferkany*. Dept. Neurobiol. & Anat., U. Tex. Med. Sch., Houston, TX 70025.

GABAnergic systems have been identified within two subcellular fractions of rabbit retina. The first is a P_2 synaptosomal fraction containing conventional sized synaptosomes presumably derived from elements of the inner plexiform layer. This fraction demonstrated high affinity uptake and Ca^{++} dependent, K^+ stimulated release of GABA; and the presence of GABA receptors assayed by ^{3}H-GABA binding. The second fraction is a P_1 synaptosomal fraction which contains synaptosomes from photoreceptor terminals. It also demonstrated a high affinity uptake system with a K_m equal to that observed in the P_2 fraction but with a much lower V_{max}. In addition, the P_1 fraction released much less GABA when stimulated by K^+ and Ca^{++}. However, the GABA system in P_1 was stimulated by ACh. Both uptake and release of GABA was stimulated by ACh in P_1; no stimulation by ACh was observed in the P_2 fraction.

In bovine retina, a different distribution was observed, with a much more pronounced GABA system present in bovine P_1 as compared to rabbit P_1. These data are consistant with the suggestion that GABA is a neurotransmitter within both plexiform layers of the retina. Based on the subcellular distribution and species differences reported here and on previously published autoradiography analyses, the GABAnergic system in the inner plexiform layer may be associated with amacrine cells; in the outer plexiform layer it may be associated with horizontal cells which contact cones. In addition these GABAnergic horizontal cells may receive cholinergic input.

1435 SEROTONINERGIC INNERVATION OF RAT AND BOVINE PARENCHYMAL BRAIN BLOOD VESSELS: BIOCHEMICAL AND PHARMACOLOGICAL STUDIES. John F. Reinhard, Jr., Michael A. Moskowitz, Sherman R. Elspas*, and James E. Liebmann.* Laboratory of Neural and Endocrine Regulation, Dept of Nutrition & Food Science, M.I.T., Cambridge, Mass. 02139.

The neurotransmitter serotonin (5-HT) is a potent vasopressor substance whose presence within nerve terminals of brain intraparenchymal blood vessels has been recently suggested by autoradiographic and histofluorescent studies. Serotoninergic nerve terminals, in conjunction with noradrenergic and perhaps other neurons, may participate in the regulation of cerebral blood flow. Present studies provide biochemical evidence that 5-HT is present in nerve endings and not simply within blood of the vessel lumen. Microvessels were isolated from the brains of 450 gm male Sprague Dawley rats by sucrose density centrifugation and microsieving, after meninges and choroid plexi were removed. Blood vessels were examined histologically with H & E and stains for elastin and found not to contain glia, neurons, and myelin. 5-HT was measured by a radioenzymatic assay which converts 5-HT to tritiated melatonin. Tritiated compounds were then resolved by multiply developed unidimensional thin-layer chromatography. Uptake into nerve terminals was measured by a method previously described (J.P.E.T. 181:36, 1972.)

Microvessels were found to contain 15 pmoles of 5-HT per mg protein. The administration of p-chlorophenylalanine (300 mg/kg, I.P.), or p-chloroamphetamine (10 mg/kg, I.P.) 24 hours prior to decapitation reduced the levels of 5-HT in microvessels by 52% and 47% respectively. Levels of 5-HT in cerebral cortex were reduced by 87% in these animals, whereas trunk blood showed no change. Administration of the monoamine oxidase inhibitor, pargyline (75 mg/kg, I.P.) elevated microvessel 5-HT levels to 168% of controls. Saline perfusions of rat brain vessels did not alter microvessel 5-HT levels. Picomole quantities of 5-HT were also measured in microvessels isolated from bovine cerebral cortex: these vessels appear to possess an uptake mechanism for 3H-5HT which can be blocked by the specific inhibitor, fluoxetine, in vitro.

We believe these data provide the first biochemical evidence for the innervation of brain microvessels by serotoninergic neurons.

1436 PROBENECID ENHANCES HALOPERIDOL-INDUCED HVA ACCUMULATION IN CSF AND PLASMA OF MACACCA MULATTA. R. H. Roth, N. G. Bacopoulos*, and G. Heninger (SPON: Jacqueline N. Crawley). Departments of Pharmacology and Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06510

The metabolites of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured in the plasma and CSF of adult male rhesus monkeys by a gas-chromatographic mass fragmentographic method. A 24 hour fast decreased the initially very high levels of DOPAC and HVA in the plasma to stable baseline values (DOPAC = 8.8 ± 3.8 ng/ml, $\bar{x}$ ± S.E.M., N = 5, and HVA = 8.9 ± 1.1 ng/ml) without affecting the concentration of HVA (15.6 ± 7.8 ng/ml) in lumbar CSF. DOPAC was usually not measurable in the CSF of control animals. All subsequent experiments were conducted on animals fasted for 24 hours. Haloperidol, 1.0 mg/kg, i.m., induced variable effects on plasma HVA, doubling the level of this metabolite in 2 out of 4 animals. It was noted that the animals showing large increases in plasma HVA after haloperidol displayed the largest increases in CSF HVA as well.

Probenecid, 25 mg/kg, i.p., produced rapid and prolonged increases in the plasma and CSF concentration of HVA in all animals tested. Haloperidol 1.0 mg/kg, i.m., significantly enhanced the probenecid-induced accumulation of HVA both in plasma and CSF. A significant correlation was observed between plasma and CSF concentrations of HVA within animals, and across the entire group when data from all animals were pooled. The above drug effects and the correlation between plasma and CSF level of HVA did not apply to DOPAC. These results are consistent with the possibility that probenecid, 25 mg/kg, blocked the renal transport of HVA, as evidenced by the accumulation of HVA in the plasma, and only partially blocked HVA egress from the CNS as evidenced by the further increases in plasma HVA in probenecid treated animals induced by haloperidol administration. Application of the above procedure to human patients might expand the usefulness of currently used methods to assess the metabolic state of central dopamine neurons. (Supported in part by USPHS Grant MH-14092 and by postdoctoral fellowship USPHS 1 F32-MH-17146-02 to N.G.B.)

1437 CLASSICAL CONDITIONING USING DRUGS AFFECTING DOPAMINE TURNOVER--BIOCHEMICAL EVIDENCE OF TURNOVER CHANGES ASSOCIATED WITH CONDITIONED STEREOTYPY AND HYPERACTIVITY. Stanley R. Schiff*, Wagner H. Bridger, and Nansie S. Sharpless. Albert Einstein College of Medicine, Departments of Neuroscience and Psychiatry, Bronx, New York, 10461.

We have previously demonstrated that d-amphetamine (amp) induced stereotyped behavior and hyperactivity can be classically conditioned while the similar behaviors induced by apomorphine may not. The conditioned response (CR) to signals (CS) previously associated with amp administration was blocked by a low dose of haloperidol (hal, 0.2 mg/kg)[Fed. Proc. 34(3):860, 1978]. Furthermore, preliminary studies using hal itself as the unconditioned stimulus (UCS) in our conditioning paradigm have demonstrated a CR opposite in direction to the unconditioned behavior (UCR) observed following hal administration. The CR to the CS previously associated with hal injection includes increased sniffing and increased activity measures. Together, these observations suggested that some mechanism of dopamine (DA) release from terminals could be more easily conditioned than direct receptor stimulation or blockade. To determine whether an increased DA turnover was associated with the CR, we measured homovanillic acid (HVA) concentrations in the striatal (caudate) and mesolimbic (nucleus accumbens plus olfactory tubercle) regions of conditioned and control rats. Male hooded rats (N=16) were submitted to 10 daily conditioning trials with amp (2.6 mg free base /kg, i.p.) along with a CS (tone-1 min): then a placebo (saline, i.p.) was paired with the CS. Significant behavioral conditioning was seen in these rats as compared to controls throughout the 30 min interval following the CS - placebo pairing. Control rats (N=16) were pseudo-conditioned by injecting saline with the CS and amp (2.6 mg/kg) after return to the home cage following behavioral testing. Both conditioned and pseudo-conditioned rats were killed by decapitation 30 min after CS-placebo pairing. Caudates from 2-3 rats and mesolimbic areas from 4 rats were pooled and homogenized in 0.1N $HClO_4$. HVA was isolated on small columns of Sephadex G-10 and measured fluorometrically.

In the mesolimbic area, mean (± SE) HVA concentration was significantly higher ($p<0.01$) in the conditioned rats (495 ± 17 ng/gm) than in the pseudo-conditioned rats (421 ± 14) while, in the caudate, the difference was not significant (conditioned: 896 ± 68; pseudo-conditioned: 810 ± 38). The results support the hypothesis that the CR induced by the CS previously paired with amp administration is mediated by increased DA release in the mesolimbic region, the area believed to be most important in the mediation of those behaviors that we have been able to condition.

1438 EFFECT OF OPERANT BEHAVIOR ON THE REGIONAL SYNTHESIS OF ^{3}H-CATECHOLAMINES FROM ^{3}H-TYROSINE IN THE RAT BRAIN. L.S. Seiden and T.G. Heffner*, U. of Chicago, Chicago, IL 60637.

The relative rates of synthesis of norepinephrine (NE) and dopamine (DA) from tyrosine (TYR) in brain regions were compared in rats lever pressing for water and in non-performing control rats. A 10 ul solution of artificial cerebrospinal fluid containing 50 uCi of l-2,6-^{3}H-TYR(42.3 Ci/mmol) was injected into a lateral cerebral ventricle of unanesthetized rats through a chronically-implanted cannula. Rats were killed 5-20 min later, brains were dissected and brain regions analyzed for ^{3}H-NE, ^{3}H-DA and ^{3}H-TYR by alumina and dowex chromatography. Endogenous levels of catecholamines (CA) were determined by radioenzymatic assay while endogenous levels of TYR were determined fluorometrically. The ratio: (nCi ^{3}H-CA)/(nCi ^{3}H-TYR/nmol TYR) was used as an index of conversion of ^{3}H-TYR to ^{3}H-CA and was assumed to reflect the relative rate of CA synthesis from TYR. The conversion index for NE and DA increased linearly in all brain regions between 5 and 15 min after the injection of ^{3}H-TYR. Rats previously trained to perform on a fixed ratio of 5 operant schedule were injected with ^{3}H-TYR and placed in operant test chambers. Control rats not previously exposed to the operant schedule were returned to the home cages after ^{3}H-TYR. After a 1-5 min delay, rats exposed to the operant schedule began lever pressing and continued to do so for the rest of the session. In an initial experiment, all rats were killed 15 min after ^{3}H-TYR. The conversion index for DA in operant rats was 62% higher than in controls in the corpus striatum but unchanged in the mesolimbic area. The conversion index for NE in operant rats was 43% higher than in controls in the hypothalamus, but was unchanged in telencephalon, mesolimbic area and brainstem-mesencephalon. In a second experiment, groups of operant and control rats were killed 5 or 15 min after ^{3}H-TYR in order to better estimate the change in the conversion index with time. In this experiment, performing rats again displayed an increase in the apparent synthesis rate of ^{3}H-DA in the corpus striatum and ^{3}H-NE in the hypothalamus while no changes in the synthesis of either CA were found in other brain regions. These findings suggest that the performance of operant behavior is associated with an increased synthesis of CA from TYR in specific central CA projections. (Supported by USPHS Grants MH-11191; MH-14274 Trng., and 5-K02-10562.

1439 PURIFICATION OF L-GLUTAMATE DECARBOXYLASE FROM CATFISH BRAIN. Y. Y. Thomas Su*, J.-Y. Wu and Dominic M. K. Lam* (Spon: E. Peck) Cullen Eye Institute and Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

L-Glutamate decarboxylase (GAD) has been purified from mouse brains and its properties have been extensively studied (J.-Y. Wu, in "GABA in Nervous System Function", E. Roberts et al. , eds., Raven Press, N.Y., 1976). Unfortunately the antibody against this enzyme does not cross-react with the GAD from neural tissues of fishes. We have therefore begun to purify GAD from catfish brains in an attempt to obtain specific antibodies with a view to localizing GAD in GABAnergic neurons in teleost retinas. GAD was extracted from catfish brains by homogenizing the tissue in water containing 0.1 mM pyridoxal phosphate, 1 mM EDTA, 1 mM AET and 1 mM reduced glutathione, pH 7.2. About 70% of GAD activity was recovered in the supernatent of 100,000 xg. The enzyme was purified to electrophoretic homogeneity by a combination of ammonium sulfate fractionation, gel filtration, calcium phosphate gel and preparative acrylamide gel electrophoresis. The purified protein migrated as a single band on several different polyacrylamide gel systems. Furthermore, the protein band contained all the GAD activity. The enzyme was therefore used as an antigen to produce a specific antibody which will be used for immunochemical and -histochemical studies of the GABA system in teleost retinas.

Supported in part by the Retina Research Foundation (Houston), NIH grants EY 02423 and NS 13224 and a grant from Huntington's Chorea Foundation in memory of Mrs. Ruth Berman.

1440 THE RELATIONSHIP BETWEEN CALCIUM-DEPENDENT AND INDEPENDENT RELEASE OF [^{3}H]GABA FROM CORTICAL SLICES EVOKED BY POTASSIUM, VERATRIDINE AND ELECTRICAL STIMULATION. J.C. Szerb, Dept. Physiology & Biophysics, Dalhousie Univ., Halifax, N.S., Canada B3H 4H7.

Although the transmitter role of GABA in mammalian CNS is supported by extensive evidence, its release from brain slices is not consistently dependent on extracellular Ca^{2+}, raising the possibility that Ca^{2+}-dependent and independent release may occur from two distinct pools. To study this possibility the time course of the evoked release of [^{3}H]GABA was followed during 48 min superfusion with either 50 mM K^+ or 50 µM veratridine in slices which had been incubated for 15 min with 1 µM [^{3}H]GABA and 10 µM aminooxyacetic acid. The latter was also present in the superfusion medium. Both 50 mM K^+ and veratridine caused an initial fast efflux which declined in spite of continuous depolarization and both released about 1.6 nmol.g^{-1} in 48 min. However, while release by K^+ was decreased by 66% when Ca^{2+} was omitted, the release by veratridine was unchanged in the absence of Ca^{2+}. The release caused by both K^+ and veratridine was reduced by about 80% following a 48 min superfusion with 50 mM K^+ but release by both was preserved better if, during the first superfusion with 50 mM K^+, Ca^{2+} was omitted. Omission of Ca^{2+} following the initial superfusion with 50 mM K^+ and Ca^{2+}, reduced further the second release by K^+ but caused a 2.5 fold increase in release induced by veratridine as compared to release in the presence of Ca^{2+}. Release induced by 24 min electrical stimulation (64 Hz, alternating polarity) was delayed by the omission of Ca^{2+}. It was decreased by 80% after 48 minutes superfusion with 50 mM K^+ but this decrease was less if Ca^{2+} was omitted after the superfusion with 50 mM K^+. Results suggest that omission of Ca^{2+} affects little net [^{3}H]GABA release caused by depolarization due to an influx of Na^+ (veratridine or electrical stimulation) because it has two effects: it depresses release from the Ca^{2+}-dependent pool and enhances release from the Ca^{2+}-independent pool, probably by increasing the influx of Na^+. Depolarization by K^+ which causes only a limited influx of Na^+, activates less release from Ca^{2+}-independent pool even in the absence of Ca^{2+} hence the release appears to be largely Ca^{2+}-dependent.

(Supported by the MRC of Canada)

1441 PITFALLS IN THE USE OF THE AUTOMATED AMINO ACID ANALYZER FOR THE QUANTITATION OF SOME ACIDIC AMINO ACIDS IN BRAIN TISSUES. Ken H. Tachiki*, Roger A. Baldwin* and Claude F. Baxter. Neurochemistry Laboratories, V.A. Hospital, Sepulveda, CA 91343, and Dept. of Psychiatry, UCLA School of Medicine, Los Angeles, CA 90024.

The quantitation of amino acids in biological extracts by the use of an automated amino acid analyzer is predicated upon a constancy and uniqueness in retention time of the individual amino acids. Using a Durrum microbore column packed with Durrum DC-4A resin and the Durrum Pico Buffer IV elution system, we found that in amphibian brain tissue, the taurine content appeared to be 1.7 µmoles/gram of tissue. However, as with other automatic amino acid analyzers, the elution peak for taurine was not totally symmetrical. Upon acid hydrolysis of the amphibian brain extract, close to 90% of the "taurine" peak disappeared whereas an authentic sample of taurine was not affected by such treatment. This indicated that extracts of amphibian brain contain two or more compounds with retention times on the column similar to authentic taurine. The unknown compound in the taurine peak was isolated from protein-free extracts of amphibian brain using short columns of Bio-Rad AG50W-X8 resin, AG1-X8 resin and paper chromatographic techniques. In the last system, a butanol/acetic acid/water (12:3:5,v/v/v) solvent separated the unknown compound from taurine. Upon hydrolysis, large amounts of ethanolamine and phosphate were detected. However, the unknown was not phosphoethanolamine. Additional tests have identified the unknown as glycerophosphorylethanolamine. Several investigators have reported a loss of "taurine" upon hydrolysis of mammalian brain preparations (Mussini,E. and Marcucci,F., in Amino Acid Pools, ed. by J.T. Holden, Elsevier Publ. Co., New York, p.486-492, 1962; Lähdesmäki,P., Karppinen,A., Saarni,H. and Winter,R., Brain Res. 138:295-308, 1977). All of these findings reemphasize the tenuous nature of conclusions reached about taurine levels in brain tissues when such conclusions are based exclusively upon the identification of the amino acid by the retention times from an ion-exchange column. An acid labile compound in brain tissues which co-chromatographs with aspartate has also been detected.

Taurine levels in brain tissues have been linked to the containment of convulsive disorders. The function of glycerophosphorylethanolamine in the central nervous system is not well established. Experimental and clinical data for taurine levels often have been determined with the help of an automated amino acid analyzer. Some of this data in the literature deserves to be re-evaluated.

1442 CAGE CONVULSANT DRUGS INHIBIT PICROTOXININ BINDING TO MAMMALIAN BRAIN MEMBRANES. Maharaj K. Ticku and Richard W. Olsen, Depart. of Biochemistry, Univ. of California, Riverside, CA 92521

Bicyclophosphates [P-$(OCH_2)_3$-C-R], bicyclic orthocarboxylic acid esters [R'$(OCH_2)_3$-C-R], silatranes, and tetramine all have a symmetrical caged structure and produce tonic-clonic convulsions and death in mice in a manner similar to picrotoxin (Casida et al., Tox. App. Pharm. 36, 261, 1976). Bowery et al. (Nature 261, 601, 1976) have reported that bicyclophosphates antagonized GABA-induced depolarization (reversed by barbiturates) in a superior cervical ganglion preparation. We have examined the interaction of these 'cage' compounds with [^{3}H]-α dihydropicrotoxinin (DHP) binding sites in rat brain, sites previously shown (Ticku et al., Mol. Pharmacol. in press, 1978) to be related to GABA receptor-ionophores but distinct from the GABA recognition site. Bicyclophosphate esters (up to 100 µM) did not inhibit GABA binding (Na^+-independent) to its receptor sites; however, they did inhibit DHP binding with a potency profile: R = t-C_4H_9 > i-C_3H_7 = C_2H_5 >> CH_3. This agrees with their order of biological activity. t-Butyl bicyclophosphate, the most potent compound of the series, inhibited DHP binding with IC_{50} = 2 ± 1 µM. Orthocarboxylic acid esters [R'$(OCH_2)_3$-C-t-C_4H_9] also inhibited DHP binding in the micromolar concentration range. Compounds with a smaller R' substituent (e.g. -H) were more potent than those with a larger R' substituent (e.g. -C_6H_5). DHP binding was also inhibited by the convulsants tetramine (IC_{50} = 4 ± 2 µM) and p-chlorophenyl silatrane (IC_{50} = 9 ± 3 µM). Our results suggest that 'cage' compounds may produce convulsions by acting via the picrotoxin-sensitive sites at the GABA receptor-ionophores. DHP binding sites are also inhibited by convulsant and depressant barbiturates (Ticku and Olsen, Fed. Proc. 37, 907, 1978), consistent with a common site of action in the CNS for these drugs in modulating inhibitory synaptic transmission.

Supported by NSF Grant BNS 77-24414 and NIH Grant NS 12422.

1443 THE RELATIONSHIP BETWEEN CHOLINE TRANSPORT AND ACETYLCHOLINE RELEASE. Ken Vaca* (SPON: E. Shaskan). Physiology Section, Biological Sciences Group, Univ. of Connecticut, Storrs, CT 06268

The chick ciliary nerve-iris preparation was used to investigate the relationship between sodium-dependent, high affinity choline (Ch) transport and acetylcholine (ACh) release. Depolarization with high potassium was approximately an order of magnitude more effective than electrical stimulation (30 Hz) in releasing newly synthesized ^{3}H-ACh. A 10 min period of depolarization reduced endogenous ACh levels more than 40%. Upon repolarization in the presence of physiological concentrations of Ch (5 mM), the endogenous levels of ACh largely recover to control levels within 10 min. This recovery is dependent on Ch transport and is prevented by hemicholinium-3 or replacement of Na^{+} by Li^{+}. Depolarization in Ca^{++}-free, 10 mM Mg^{++} medium fails to significantly reduce endogenous ACh levels. The conditioning depolarization (in the presence of Ca^{++}) increased the V_{max} for Ch transport approximately 2-fold and ACh synthesis 5-fold after return to normal medium. A conditioning depolarization prior to preloading with ^{3}H-Ch increased the integral of the initial velocity of ^{3}H-ACh release, evaluated over a 2 min interval, several-fold relative to control. After a conditioning depolarization, extended preloading with ^{3}H-Ch resulted in an increase in the initial release of ^{3}H-ACh which maximized at 5-10 min preloading time. Although longer periods of preloading increased the amount of radiolabelled Ch taken up by the tissue, it did not increase the initial rate of release. However, more prolonged preloading with ^{3}H-Ch decreased the rate of decline of ^{3}H-ACh release with successive depolarizations. A comparison of the rate of release of ^{3}H-ACh with the rate of decline of endogenous ACh suggests that newly synthesized (radiolabelled) ACh is preferentially released relative to the older endogenous stores. It is hypothesized that the readily releasable pool of ACh consists of a saturable compartment, which is primarily dependent on Ch transport for its supply. Supported by NIH-NS10338 and the Univ. of Conn. Research Foundation.

1444 DESYNCHRONIZED SLEEP ENHANCEMENT BY BOTH CHOLINERGIC AND ANTIAMINERGIC AGENTS AT THE SAME PONTINE BRAIN STEM SITES. Ennio Vivaldi*, Robert W. McCarley, and J. Allan Hobson. Lab. of Neurophysiology, Harvard Med. School, Boston, Ma. 02115.

We have proposed that desynchronized sleep (D) periodicity results from the interaction between executive cells located in the pontine reticular formation (FTG) and a group of cells in the locus coeruleus (LC). FTG cells are postulated to be cholinergic, self-excitatory and excitatory to LC cells; LC cells are postulated to be noradrenergic, self-inhibitory and inhibitory to FTG cells. From this model one would expect that the administration to a group of FTG cells of either a cholinomimetic agent or an adrenergic blocker would increase their activity, and an increase of D would be observed. Extensive microinjection studies have supported the cholinergic hypothesis but direct injection of adrenergic blockers has not previously been attempted.

Following up on previously reported observations that lowering the volume of vehicle enhanced the cholinergic evocation of D, we have studied the effects of simple diffusion. The mere placement of a 30 ga. cannula stereotaxically aimed at the FTG and containing a 4 g/l solution of carbachol increased D to 63.3% in two hours as compared to 14.3% with a saline containing cannula. Effects were still observable 6 hours later.

Two cats were used to test the effect of propranolol diffusion: at the same sites where carbachol had increased D to 3.53 times control values the placement of a cannula containing 1 g/l of propranolol increased D sleep 2.12 times. The dose-response curve from a 3 dose counter-balanced propranolol microinjection study in two cats showed a graded increase in D from low (0.2µg) through medium (0.6µg) to high (2.0µg) dose levels. The respective D percents were 0.73, 3.04, and 4.05 times saline control values.

The results suggest that D sleep phenomena may be enhanced by either cholinergic stimulation or adrenergic blockade of the pontine reticular formation.

Supported by grants from NIMH (13923) and NSF (BNS 76-18336)

1445 REGIONAL DISTRIBUTION OF ALPHABUNGAROTOXIN RECEPTORS IN NORMAL AND PATHOLOGICAL HUMAN BRAIN. Bruce T. Volpe‡ Andrew Francis‡ Michael S. Gazzaniga, and Nisson Schechter‡(SPON: Fred Plum). Dept. of Neurology, Cornell Medical College, N.Y., N.Y. 10021, Dept. of Psychiatry, S.U.N.Y., Stony Brook, N.Y. 11794

Clinical and pharmacological studies have demonstrated a relation between central cholinergic function and memory processes. Other reports have shown loss of CAT activity in dementia,but no loss of muscarinic receptor activity. Conservation of nicotinic cholinergic receptor also would give further impetus to clinical trials of centrally acting cholinergic drugs. However,the distribution of nicotinic receptors in normal or pathological human brain tissue has not been studied. We have used regional differential binding of radio labelled alphabungarotoxin in human post-mortem brain tissue to identify nicotinic cholinergic receptors. Post-mortem tissue pieces were homogenized (5%w/v) in buffer (10mM sodium phosphate, 0.4mM phenylmethy sulfonyl fluoride, 0.013 mM dimethyl formamide,1mM sodium EDTA,0.02% sodium azide) at pH 7.2 using a teflon glass homogenizer. Aliquots of homogenate were incubated in the presence of excess ^{125}I-alphabungarotoxin (specific activity 10^{6} ci/mole) and counted by liquid scintillation after a centrifuge assay. All counts were corrected for non-specific binding by subtraction of controls obtained in the presence of a several hundred fold excess of native toxin. The demonstrated non-uniform binding of alphabungarotoxin seems unaffected by agonal state. To date, results in the normal human post-mortem brain are similar to other mammals, and reveal the highest activity in the mammillary bodies,hippocampus,amygdala, colliculi, and certain cortical regions; and the lowest levels in the cerebellum,pons,and caudate. Currently, parallel analyses of CAT activity and morphologic studies are in progress.

Aided by USPHS grant 25643 and the McKnight Foundation.

1446 GLUTAMATE AND EXCITATORY SYNAPTIC TRANSMISSION ONTO GIANT RETICULOSPINAL NEURONS OF THE LAMPREY. Warren O. Wickelgren and Gary Matthews. Dept. Physiol., Univ. of Colo. Med. School, Denver, CO 80262.

Intracellular recordings were made from the cell bodies and axons of giant reticulospinal neurons (Müller cells) in the isolated brain and spinal cord of adult lamprey. Glutamate added to the saline surrounding Müller axons produced a reversible, dose-dependent depolarization of up to 20 mV. However, there was no apparent drug-induced conductance change in the axon when potential-dependent conductance changes were blocked with tetrodotoxin and 4-aminopyridine. This, plus other evidence, including the lack of an axon response to iontophoretically-applied glutamate, suggests that the depolarization to bath application was an indirect effect of the spread of current from other spinal cells depolarized by the glutamate and electrically coupled to Müller axons. On the other hand, glutamate did produce a conductance change when iontophoretically-applied to the Müller cell bodies in the brain. The reversal potential of the glutamate depolarization was compared to that of the excitatory postsynaptic potential (epsp) evoked by electrical stimulation of the contralateral vestibular nerve. In undamaged cells (resting potentials about -70 mV) it was not possible to pass enough current to reverse the epsp or glutamate response, and their reversal potentials were estimated by extrapolation. The values obtained in one such experiment were -28 mV (epsp) & -33 mV (glutamate response). The errors involved in extrapolation were avoided by actually reversing the epsp and glutamate response in cells which had been damaged (resting potentials between -12 & -55 mV) by impalement with a low-resistance micropipette. In these experiments the reversal potentials for the epsp and the glutamate response were essentially identical, the average difference in 12 determinations being 0.54 mV. The absolute value of the epsp-glutamate reversal potential varied from -16 to -33 mV, the more negative values occurring in cells with higher resting potentials. Lowering the extracellular Na concentration to 1/10 normal shifted the glutamate reversal potential by about -14 mV, indicating that an increased Na conductance was involved in the depolarization. Injection of Cl intracellularly had no effect on either the glutamate or epsp reversal potentials. Since the epsp-drug reversal potential was negative to the Na equilibrium potential and Cl was not involved in the response, it appears that there is also an increase in K conductance, although this was not investigated directly. We conclude that glutamate is a candidate for the excitatory transmitter onto Müller cells. (Supported by NIH grants NS 09661 and 09660.)

1447 MODIFICATION OF ACETYLCHOLINE TURNOVER RATE IN SELECTED BRAIN REGIONS: EFFECT OF PARASYMPATHOLYTICS. P.L. Wood* and D.L.Cheney Lab. Preclin. Pharmacol., NIMH, Saint Elizabeths Hosp.,Washington, D.C. 20032

Previous work from this laboratory has examined the actions of benztropine and trihexyphenidyl on cholinergic function in the striatum (Racagni et al., JPET 196: 323, 1976). We now report the effects of these drugs on the turnover rate of acetylcholine in brain areas lacking a dopaminergic/cholinergic interaction. To estimate the turnover rate of acetylcholine, rats were infused with deuterated phosphorylcholine and sacrificed by microwave irradiation. The endogenous and deuterated acetylcholine and choline of specific brain areas were subsequently determined by gas chromatography-mass spectrometry.

Systemic administration of benztropine (20 mg/kg i.p.; 30 min) and trihexyphenidyl (20 mg/kg i.p.; 30 min) produce two types of effects on cholinergic mechanisms in rat brain areas. In hippocampus and thalamus there is an increase in the fractional rate constant for the efflux of acetylcholine, no change in acetylcholine concentration and consequently an increase of the turnover rate of acetylcholine. In frontal cortex, parietal cortex and striatum a decrease in the concentration of acetylcholine compensates for the increase in fractional rate constant for acetylcholine efflux. Thus, the turnover rate of acetylcholine remains unchanged. Disruption of the septal-hippocampal pathway by cutting the fimbria/fornix prevents the alterations of cholinergic parameters elicited by benztropine in either cortex or hippocampus. Moreover, intraseptal injection of phenoxybenzamine (15 nmol; 36 min) does not alter the effects of systemic benztropine. These results suggest that benztropine does not alter cholinergic mechanisms in cortex or hippocampus via an action on presynaptic cholinergic receptors but may produce its effects through a feedback control via the septum.

1448 PURIFICATION AND IMMUNOCHEMICAL STUDIES OF CYSTEIC ACID DECARBOXYLASE AND GLUTAMATE DECARBOXYLASE FROM BOVINE BRAIN. Jang-Yen Wu, M. S. Chen* and W. M. Huang* Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

Cysteic acid decarboxylase (CAD) and glutamate decarboxylase (GAD) which catalyze the decarboxylation of cysteic acid and glutamic acid to form taurine and GABA, respectively, have been purified to homogeneity from bovine brain. The purification procedures involved the initial extraction of CAD and GAD from gray matter of bovine brain, followed by ammonium sulfate fractionation, column chromatographies on gel filtration, calcium phosphate gel and DEAE cellulose and finally by preparative polyacrylamide gel electrophoresis. The purified enzyme preparations migrated as a single protein band on 7% polyacrylamide gel electrophoresis and was coincident with the enzyme activities. Previously, we have shown that in partially purified preparations, there were two forms of CAD, one of them, CAD I, contained only CAD activity and was believed to be the synthetic enzyme for taurine and the other one, CAD II, which possessed both CAD and GAD activities and was believed to be responsible for GABA synthesis (Trans. Amer. Soc. Neurochem. 9, 53 (1978)). The same results were also obtained with purified CAD I and CAD II preparations. Using rabbits, we have obtained an antibody specific to CAD II following five biweekly subscapular injections of 10 μg of the purified enzyme in complete Freund's adjuvant. The antisera thus obtained show a single precipitin band on Ouchterlony double diffusion tests against both crude CAD (1% pure) and the purified protein. Rabbits have also been immunized with purified CAD I preparation and the serum will be tested periodically for the production of antibody to CAD I. Using these antibodies, studies on the species and tissue specificity and immunohistochemical localization of CAD and GAD are in progress.

Supported in part by Grant NS 13224-02 from NIH and Grant from Huntington's Chorea Foundation in memory of Mrs. Ruth Berman.

PAIN

1449 Analgesic effect of glutamate injected into the periaqueductal gray (PAG) of the rat: Evidence for excitatory interaction between the PAG and nucleus raphe magnus (nrm).
Michael M. Behbehani and Howard L. Fields. Dept. of Physiology U. of cincinnati, cincinnati ohio and Dept. of Neurology U. of Cal. at San Francisco.

Both the electrical stimulation and injection of morphine into the PAG produces analgesia. It is believed that this analgesic effect is mediated by activating a descending system that involves the NRM. In studies reported here, rats were anesthetized with urethane and after tracheostomy and cannulation of the femoral vein, were stationed in a stereotaxic instrument. Using a microsyringe fitted with a 34 gauge needle, 0.5 µl of monosodium glutamate (50 mM) was injected into the PAG. The analgesic effect of glutamate was measured electromyographically by recording from the flexor muscle of the rigt hind leg and by application of noxious heat to the palm of that foot. Also the the effect of glutamate on the activity of the cells in the NRM was measured using single unit recording techniques. To test the specificity of the effect of glutamate, it was injected 2mm above, in the dorsal part, in the ventral part and 2mm below the PAG. It was shown that only when glutamate was injected into the PAG it caused analgesia and that this analgesic effect was correlated with an increase in the firing rate of the majority of the cells in the NRM. This analgesic effect could be abolished by either lesioning of the NRM and a small area in the reticular formation surrounding this nucleus or by naloxone 20 minutes following its i.v. injection. However, injection of naloxone directly into the NRM had no effect on the analgesia produced by glutamate. It is concluded that there is an excitatory connection between the PAG and the NRM and that activation of this system produces analgesia. Furtheremore it is concluded that this analgesic effect is mediated by a morphine like compound acting at sites other than the nucleus raphe magnus.

1450 ELECTRICAL STIMULATION OF THE MIDBRAIN ANALGESIC SYSTEM: EFFECT ON TRIGEMINAL NEURONS. M.A. Biedenbach. Dept. Physiol., U.T. Health Science Center, San Antonio, TX 78284.

The aim of this study is to identify trigeminal neurons that can be excited by electrical stimulation of the lingual nerve and/or the tooth pulp, and to establish the effect on these neurons of stimulating the midbrain analgesic system. In cats anesthetized with chloralose and paralyzed with Flaxedil, unit potentials were recorded in the part of the trigeminal nucleus that extends from 5 mm rostral to 3 mm caudal to the obex. Bipolar stimulating electrodes were placed on the exposed lingual nerve, into two canine tooth pulps and in the midbrain periaqueductal gray (PAG) at a site from which stimulation inhibited the jaw-opening reflex. PAG-effects were established by conditioning-testing stimulation where a stimulus train to PAG (200 Hz and 200 msec duration) preceded the test shock. Three types of test shocks were used, lingual nerve and tooth pulp stimulation, and, if the neuron was mechanosensitive, nonnoxious and noxious "pinches" were applied to its receptive field. The major PAG-effect was to inhibit responses to the three kinds of stimuli either completely or partially (decreased number of spikes per response). Inhibitory effects after a single PAG-stimulus train lasted in various neurons approximately from 0.1 to 1 second. Nearly all tooth pulp-evoked responses were completely or partially inhibited, only a few were not affected. PAG-effects on lingual nerve responses were less marked, most being only partially inhibited and a somewhat greater fraction was uninfluenced. Partial inhibition of tooth pulp or lingual nerve responses usually blocked the later spikes in a given response. Both lingual nerve and tooth pulp-evoked responses exhibited a considerable range of first-spike latencies. Neurons influenced by PAG spanned the whole latency range but those not influenced exhibited mostly shorter latencies. Among the tested responses to "pinches", a considerable portion was not affected by PAG, but in those which were, responses to both nonnoxious and noxious pinches were inhibited for similar periods. (Supported by Biomed. Res. Supp. Grant RR 05654.)

1451 DIFFERENTIAL ALTERATIONS FOLLOWING HYPOPHYSECTOMY IN ANALGESIA INDUCED BY MORPHINE AND DIFFERENT STRESSORS. Richard J. Bodnar, Murray Glusman, Alfred Mansour* and Dennis D. Kelly. New York State Psychiatric Institute and Columbia University, N.Y.,N.Y. 10032.

Pain threshold elevations in rats occur following acute exposure to such stressors as cold-water swims, inescapable foot shock, food deprivation, rotation and intraperitoneal injections of either hypertonic saline or 2-deoxy-D-glucose, an antimetabolic glucose analogue. The analgesia induced by cold-water swims or by inescapable foot shock is markedly attenuated in hypophysectomized animals, and is only minimally affected by opiate antagonists, which suggests that the analgesic effects of these particular stressors may be mediated by the pituitary and not by opiate-like neural systems. The present study examined the dose-dependent and time-dependent analgesic effects of insulin, 2-deoxy-D-glucose (2-DG) and morphine in three groups (n=6) of normal and three groups (n=6) of hypophysectomized rats. Tail-pinch thresholds were defined by the lowest pressure delivered at a linear increasing rate to a rat's tail which elicited a tail withdrawal response. Threshold determinations were made prior to, and then 30, 60, 120 and 180 min following all injections. Insulin (0, 1, 8, 16, 32, 64 U/kg, sc), 2-DG (0, 100, 200, 400, 600 mg/kg, ip) and morphine (0, 1, 2.5, 5, 10 mg/kg, sc) were administered to separate groups of normal and hypophysectomized animals; a minimum of 96 hrs elapsed between injections. Whereas insulin induced clear dose-dependent and time-dependent tail-pinch elevations in normal rats, the analgesia induced by insulin in hypophysectomized rats was greatly attenuated in terms of both analgesic magnitude and effective dose. By contrast, 2-DG induced greater analgesia in hypophysectomized rats than in normal rats. In addition, morphine-induced analgesia was not affected by hypophysectomy. Thus, insulin-induced analgesia, like that induced by cold-water swims and inescapable foot shock, seems to be dependent upon the integrity of the pituitary, while both 2-DG-induced and morphine-induced analgesia would seem to be mediated by different mechanisms. (Supported by NIH Grant #NS14449 and N.Y.S. Health Research Council Grants #365 and #922.)

1452 DISSOCIATION OF PAIN THRESHOLD AND FOOD INTAKE ALTERATIONS FOLLOWING CHRONIC 2-DEOXY-D-GLUCOSE ADMINISTRATION. Martin Brutus, Richard J. Bodnar and Dennis D. Kelly. New York State Psychiatric Institute and Columbia University, N.Y., N.Y. 10032.

Rats acutely exposed to 2-deoxy-D-glucose (2-DG), an antimetabolic glucose analogue, display dose-dependent and time-dependent elevations in both food intake and pain thresholds. The former seems likely to be mediated by acute cellular glucoprivation and the latter may represent another example of stress-induced analgesia. Since stress-induced analgesia shows adaptation with repeated exposure to the stressors, the present study examined whether chronic 2-DG administration would result in a diminution in either its analgesic or hyperphagic effects. Seven rats were trained on an operant psychophysical procedure in which pulsed foot shocks were presented on discrete trials for 10 sec unless the rat pressed a lever three times to abbreviate the shock train. Each liminal escape session consisted of 100 trials equally distributed over a range of five shock intensities in a randomized-blocks design. On alternate days, 60 minutes prior to liminal escape testing, each rat received in order: seven baseline placebo injections, eight 2-DG (600 mg/kg) injections and seven recovery placebo injections. Food intake was measured for six hours after each injection. The first three 2-DG injections induced profound elevations in liminal escape thresholds and in food intake. However, by the final three injections, 2-DG failed to alter liminal escape thresholds above baseline values, but it continued to induce significant increases in food intake. During recovery pain thresholds remained normal but food intake showed some increases, post-2-DG. Hence, these data demonstrate that the analgesic and hyperphagic properties of 2-DG are experimentally dissociable, and offer further evidence that 2-DG may increase pain thresholds via a stress-induced activation of pain-inhibitory systems. (Supported by NIH Grant #NS 14449 and N.Y.S. Health Research Council Grants #365 and #922.)

1453 LATERAL HYPOTHALAMIC MODULATION OF ESCAPE FROM STIMULATION OF NUCLEUS GIGANTOCELLULARIS IN THE RAT. Kenneth D. Carr* and Edgar E. Coons* (SPON: Samuel M. Feldman). Dept. of Psychology, New York University, New York, N.Y. 10003.

A double-pulse electrical stimulation technique was used to infer neurophysiological interaction between lateral hypothalamus (LH) and pain-associated nucleus gigantocellularis (NGC) of the medullary reticular formation.

Rats were trained to barpress for 3-second escape periods from stimulation of NGC. Trains of .1 msec pulses were delivered through monopolar electrodes. The interval between successive pulses to NGC was fixed at 40 msecs. Once stable escape-responding was established, a concurrent train of pulses was delivered to the ipsilateral LH so that each pulse to NGC was preceded by a pulse to LH. Although all trains were identical with regard to number and frequency of pulses to both sites, the interval by which the LH-pulse preceded the NGC-pulse was varied over trials and ranged from .1 msec to 20 msecs. The purpose of this parametric manipulation was to probe the excitability of the NGC-pain system as a function of time since stimulation of LH. The measure of NGC-pain system excitability was the rate of escape-responding.

When the intensity of LH-pulse trains was above stimulation-bound feeding threshold, escape-responding to concurrent stimulation of NGC was inhibited. Inhibition was greatest (rate of escape was least) at one short (.1 msec) and one long (10-15 msec) LH-NGC inter-pulse interval with less inhibition (high rates) being evidenced at intermediate intervals. Work done previously in our laboratory (Porrino, doctoral dissertation, N.Y.U., 1977) found there to be two separate peaks in the medial hypothalamic inhibition of LH reward. The similarity of the data profile in the present study suggests that here too there operates a biphasic inhibitory mechanism. As in the previous study, it may prove possible to demonstrate neurochemical and/or neuroanatomical dissociation of the two inhibitory peaks.

The modulation of NGC-escape behavior by LH may reflect an inhibition of the ascending aversion-message or competition for use of the final common behavioral pathway. (Supported by NIMH predoctoral fellowship #1 F31 MH07302-01)

1454 MIDBRAIN PROJECTIONS TO THE MEDULLARY RETICULAR FORMATION. J. M. Chung, L. H. Haber, R. F. Martin, and W. D. Willis. Marine Biomedical Inst. and Depts. of Anatomy and Physiology & Biophysics, U. Texas Med. Br., Galveston, TX 77550

Stimulation in the periaqueductal and periventricular gray inhibits reflex reactions to noxious stimulation in rats and cats and pain in humans. This "stimulus produced analgesia" (SPA) is thought to be mediated by descending pathways which relay in the lower brainstem. Part of the relay is probably in the nucleus raphe magnus (NRM). Since stimulation in both the NRM and in the nucleus reticularis gigantocellularis (NGc) can inhibit the activity of spinothalamic tract neurons, it is possible that the NGc may also contribute to SPA. To evaluate this further, we have mapped the cells in the midbrain which project to the NGc and adjacent reticular formation (RF).

Horseradish peroxidase (HRP) was injected into the medullary RF in 3 cats and 4 monkeys (Macaca fascicularis) anesthetized with sodium pentobarbital. A single 0.1-0.2 µl injection of 50% HRP was made in each animal through a glass micropipette. After 3 days, the animals were perfused with Ringers followed by 2.5% glutaraldehyde and 0.5% paraformaldehyde. After soaking in 30% sucrose, frozen sections of the brainstem were cut at 50 µm and reacted with o-dianisidine or tetramethylbenzidine.

The injection was centered over the NGc in 3 cats and 1 monkey. These animals had labelled cells bilaterally in the midbrain RF, periaqueductal gray, and the deeper layers of the superior colliculus. The RF and periaqueductal gray label was heaviest ipsilaterally and the superior colliculus label contralaterally. An injection ventromedial to the NGc in 1 monkey, with spread into the medial inferior olivary nucleus, labelled cells in the same regions and in the ipsilateral red nucleus. Two monkeys had injections centered laterally to the NGc. In an animal with a restricted injection, the labelled areas of the midbrain included the RF, periaqueductal gray, and mesencephalic nucleus of V, but not the superior colliculus. In a monkey with a diffuse injection, with spread to the auditory relay nuclei in the caudal pons, there was label in the inferior colliculus, the nuclei usually labelled from the NGc, and also the red nucleus and mesencephalic nucleus of V.

It is concluded that there are substantial projections to the medullary reticular formation from the midbrain RF and periaqueductal gray. These data are consistent with the hypothesis that stimulation within the periaqueductal and periventricular gray results in an "analgesia" due to activation of a number of pathways descending to the spinal cord from the lower brainstem.

This work was supported by NIH grant NS 09743 and by NIH postdoctoral fellowship NS 05087 (to L.H.H.).

1455 NARCOTIC ANALGESIA: CHANGES IN NEURAL ACTIVITY RECORDED FROM PERIAQUEDUCTAL GRAY MATTER OF RAT BRAIN. Hugh E. Criswell and Frederick B. Rogers*. Dept. Psychol., Williams College, Williamstown, MA 01267.

Multiple unit activity was recorded from the periaqueductal gray matter (PAG) and striatum (CPU) following systemic injections of morphine (20 mg/Kg) and following microinjection of 15 µg of morphine directly into the recording site via hollow electrodes. Animals which received microinjections of morphine were tested for analgesia 1 hour after the injection and were called analgesic if they did not vocalize or struggle following tissue distructive tail pinch. Animals which received systemic morphine were tested for analgesia using the same methodology following electrical stimulation through the recording electrodes. This testing was accomplished at least 48 hours after the last morphine injection. Analgesically active sites in the brain were defined as those where either electrical brain stimulation or microinjection of morphine produced analgesia. Systemic injections or morphine but not saline produced increased multiple unit activity at analgesically active sites within the PAG. No effect was observed at analgesically inactive sites within the PAG or the CPU. Morphine, but not saline microinjected into analgesically active sites within the PAG resulted in increased neural activity at the site of injection. There was no effect when morphine was microinjected into analgesically inactive sites within the PAG or CPU. The increased neural activity seen at analgesically active sites following morphine administration was partly but not completely antagonized by systemic Naloxone (2 mg/Kg). The similarity between systemic and central administration of morphine suggests that systemic morphine may produce analgesia by an action at analgesically active sites within the PAG and that the overall effect of morphine upon neural activity at these sites is an increased rate of neural firing. The overall increase in multiple unit activity in analgesically active sites may result from either a direct excitatory effect of morphine or an indirect effect mediated by small intraneurons.

1456 STIMULATION-PRODUCED ANALGESIA: ASSESSMENT BY DIFFERENT PAIN TESTS AND CORRELATION WITH SELF-STIMULATION. S. G. Dennis and M. Choinière*. Dept. of Psychology, McGill Univ., Montreal, Quebec, Canada H3A 1B1.

Stimulation of certain central grey and raphe regions of the rodent brainstem produces analgesia. The mechanism(s) of stimulation-produced analgesia are apparently related to the mechanisms of morphine analgesia (Mayer & Price, Pain, 2, 1976). Since morphine is known to be more effective on continuous pain of pathological origin than on transient thermal pain (Beecher, in: Soulairac et al., Pain, Academic Press, 1968), we compared, in rats, the analgesic efficacy of central grey stimulation using the formalin test (Dubuisson & Dennis, Pain, 4, 1977), which produces moderate continuous pain, and the tail-flick test, which relies on brief, radiant heat stimulation.

Electrodes were implanted in a region extending from 0-1 mm caudal to the intra-aural line, 0-1.25 mm lateral to the midline, and 0-1.6 mm ventral to the center of the aqueduct. Current thresholds--the amount of current needed to reach a criterion level of analgesia--were determined for each rat on both pain tests. Other stimulus parameters were held constant. 32 rats were used. The data show: 1) that analgesia to the tail-flick stimulus requires significantly more current (~200 µA) than analgesia to the formalin stimulus; and 2) that current thresholds on both tests rise sharply (i.e., less effective analgesia) as the midline dorsal raphe nucleus is approached. These data are not readily explained by known somatotopic organizations, motor deficits, or pain intensity factors. The results are consistent with the hypothesis that the ventrolateral central grey contains analgesia substrates which operate differently on different kinds of pain. It is not as yet clear whether there is a single system common to both kinds of analgesia, or whether separate but spatially mixed systems mediate the effects.

A separate group of rats (n=10) were taught to self-stimulate (bar-pressing) through electrodes implanted in the same vicinity. The current thresholds for self-stimulation were then compared to those for analgesia in the formalin test. A significant correlation was found ($r=0.94$; $p<.005$); the regression line has a slope of 1.2 and passes through the origin. This suggests either a common neural system for analgesia and self-stimulation, or two systems inextricably mixed in this region. Preliminary pharmacological studies suggest a noradrenergic link for both phenomena; no evidence has been found for a serotonergic link. This contrasts with pharmacological studies employing the tail-flick test for measuring analgesia (see Mayer & Price, 1976). Supported by NRC Grant A-7891 to Prof. R. Melzack.

1457 NALOXONE REVERSIBLE ANALGESIA PRODUCED BY D-PHENYLALANINE IN MICE. S. Ehrenpreis, J.E. Comaty* and S.B. Myles*. Dept. of Pharmacology, The Chicago Med. Sch., Chicago, IL 60612.

Two important limitations in using enkephalins or derivatives as analgesics are rapid breakdown by brain enzymes (Hughes, Brain Res. 88: 295, 1975) and development of tolerance to and dependence upon repeated administration (Wei and Loh, Science 193: 1262, 1976). In an attempt to obviate these difficulties, we have utilized the approach of increasing endogenous levels of the peptides by inhibiting the enzyme(s) responsible for their degradation. For this purpose, we have administered D-phenylalanine, an inhibitor of carboxypeptidase A (Delange and Smith, "The Enzymes," 3: 81, 1971) to mice and tested analgesia by the hot plate method. At a dose of 250 mg/kg, i.p., D-phenylalanine produced an increase in jump latency equivalent to 5-10 mg/kg morphine. Naloxone, 20 mg/kg, completely reversed analgesia as did the injection of carboxypeptidase A into the mice. Acutely injected L-phenylalanine was completely devoid of analgesic activity. Non-narcotic analgesics such as indomethacin and other prostaglandin synthetase inhibitors potentiated the effect of D-phenylalanine. Chronic administration of D-phenylalanine for nine days at a dose of 500 mg/kg/day did not result in tolerance to the analgesic effect. In fact, on day 9 the initial baseline analgesic threshold was higher than it had been on day 1. On the 9th day, naloxone reversed analgesia without precipitating any observable signs of withdrawal. These results are further evidence that enkephalins and/or endorphins may be involved in the modulation of pain and demonstrate that it is feasible to develop agents which produce analgesia without tolerance and dependence by preventing breakdown of the peptides at their sites of action in the body. (Supported in part by a grant from Hoffman-La Roche and by NIH - GRS funds.)

1458 STIMULATION OF THE PERIAQUEDUCTAL GRAY (PAG) ALTERS THE M PEAKS OF THE THALAMIC PAIN CODE. R. Emmers. Department of Physiology, College of P & S, Columbia University, New York, N. Y. 10032.

As reported in Brain Research 103:425, 1976, thalamic neurons which relay noxious stimulation are excited in a temporal pattern characterized by several activity peaks on spike density histograms. A short-latency I peak codes the intensity of stimulation; late spike potentials form M peaks that are related to the modality of the afferent activity: pain. The M peaks are produced by excitation of a positive feedback loop between the VB complex and the thalamic nuclei CM-Pf. These M peaks can be modified by electrical stimulation of the PAG. In rats given urethane and chloralose, when the first pulse of a pair of electrical stimuli is applied to the sciatic nerve and the second to a site in the PAG, the latter can either fill in the trough between two adjacent M peaks or augment any M peak. Either of these effects can be obtained simply by changing the interstimulus interval. Conversely, when the PAG site is stimulated with a train of pulses immediately preceding a single pulse applied to the sciatic nerve, all M peaks can either be augmented or deranged by changing the parameters of the PAG train (from 0.5 msec pulses at 50/sec for 200 msecs to 0.5 msec pulses at 70/sec for 400 msecs). This suggests that different modes of PAG stimulation could make an animal either more or less susceptible to pain. Moreover, electroanalgesia resulting from prolonged stimulation of the PAG is most likely produced by excitation of axons recently identified as ascending from the PAG to the parafascicular and the VB nuclei (Neurosci. Abstr. 3 # 1215, 1977). These axons, by altering the excitability of the positive feedback loop, could either augment or change the temporal pattern of the M peaks. And since stimulation of the sciatic nerve excites the site of the PAG which influences the feedback loop, electrical stimulation of the PAG simply interferes with the natural regulation of the excitability of this loop and thus modulates the perception of pain (Aided by grant NS-03266 from NINCDS).

1459 SUPERIORITY OF INTERMITTENT VERSUS DIRECT CURRENT ELECTROANALGESIA AT HIGH CURRENT LEVELS. R. Wayne Fields, Robert P. O'Donnell*, Richard B. Tacke* and Patrick J. Reynolds. School of Dentistry, University of Oregon Health Sciences Center, Portland, Oregon, 97201.

Using the monopolar stimulation configuration with a remote cathode and the anode applied to exposed dentin, we have previously demonstrated that direct currents of 70-100 μA block afferent activity from the tooth pulp (Fields et al., Exp. Neurol. 47:229-239, 1975) and that trains of rectangular pulses at 1000 pps having similar peak intensities but with duty cycles as low as 10% are equally efficacious (Fields et al., Exp. Neurol. 53:386-398, 1976). The present experiments were conducted to characterize the absolute and relative effects of monophasic and biphasic intermittent and of constant direct current electroanalgesia (EA) waveforms using intensities of 0-1000 μA, spanning much of the range of electroanalgesia and desensitization procedures reported in the clinical literature. This problem was examined using our previously described methods for recording thresholds of pulp-driven primary afferents in the ipsilateral Gasserian ganglion of cats to electrical stimulation of the test tooth (applied during brief 10 ms "windows" in the EA stimulation). Three EA waveforms were examined a) continuous direct current (dc), b) pulsating direct current of 1000 pps 10% duty cycle (pdc), and c) the pdc waveform capacitively coupled to the animal to effect an alternating current with zero net power transfer (ac). In each experiment, all three waveforms were studied in the order ac-pdc-dc to minimize accumulative effects (Fields et al., Exp. Neurol. 50:293-303, 1976). Each waveform was applied in an ascending series of intensity steps of 10 μA from 0-100 μA and of 200 μA from 200-1000 μA. For the dc and ac waveforms, the threshold of pulp-driven units progressively rose along essentially identical curves with increasing EA intensity, reaching 1000% of control thresholds at 400 μA. Pulpal thresholds also progressivlely rose to 1000% of control using pdc EA but the hypoexcitability was less pronounced than that of the other waveforms for EA intensities above 40 μA. Upon EA cessation, dc recovery was minimal, being 850% of control even after 120 min. The pdc and ac series exhibited recoveries after 30 min. to 350% and 200%, respectively, and the ac record further recovered to reach 150% of control after 120 min. post-EA. These results demonstrate that the ac waveform has equal efficacy to dc over the majority of the clinically significant range and exhibits far superior post-EA recovery properties under conditions of zero electrical power transfer.
(Supported by NIH Grant DE 04281)

1460 ASCENDING PATHWAYS INVOLVED WITH CARDIAC NOCICEPTION GENERATED BY MYOCARDIAL ISCHEMIA. Robert D. Foreman and Carl A. Ohata*. Department of Physiology and Biophysics, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190.

Studies from this laboratory have shown that myocardial ischemia following coronary artery occlusion (CAO) is an effective stimulus for increasing the discharge rate of spinal neurons responding to viscerosomatic convergence. This study was conducted to determine if the activity from cardiac nociceptors is transmitted by neurons which have axons ascending to higher levels of the central nervous system. The activity of cells in the T_2 to T_3 segments of the spinal cord was recorded extracellularly with a glass microelectrode. These cells were antidromically activated by electrically stimulating their axons in the contralateral, anterolateral quadrant of the C_1 segment of the spinal cord. All of the cells exhibited viscerosomatic convergence which was tested by electrically stimulating the T_2 to T_3 sympathetic chain and by manipulating the somatic fields, respectively. The heart was exposed with a thoracotomy made in the fourth intercostal space. Short segments of the anterior descending branch and the circumflex branch of the left main coronary artery were carefully dissected and ligatures were placed around them for occlusion. Cells increased their discharge rate several seconds following the onset of CAO. The increased activity was usually associated with configurational changes in the ECG suggesting that the heart was becoming ischemic. Cells remained active for a short period following the release of the CAO and then returned to their preocclusion discharge rate. Some cells increased their discharge rate immediately following the onset of CAO and then increased to a higher level of activity several seconds later, usually when changes occurred in the ECG. Not all cells were excited during CAO, even though they discharged following electrical stimulation of the sympathetic chain. Some of the cells had somatic receptive fields similar to the size described for the spinothalamic tract (Hancock et al. Exptl. Neurol. 47, 1975). Another population of cells had large, bilateral somatic fields similar to those observed for the cells of origin of the spinoreticular tract (Fields et al. Brain Res. 120, 1977). All cells responded to a noxious somatic stimulus, however, some cells also responded to touch and increased their discharge rate when a noxious pinch stimulus was applied. The results suggest that the information from cardiac receptors and nociceptors ascends in the anterolateral quadrant of the spinal cord toward suprasegmental levels of the central nervous system. Since these cells exhibit viscerosomatic convergence, they may demonstrate a possible mechanism underlying referred pain associated with coronary artery disease. (Supported by Grant #18728).

1461 THE EFFECT OF AN ACTIVE PLACEBO DRUG AND A NARCOTIC ANALGESIC ON VERBAL DESCRIPTOR SCALES OF THE SENSORY INTENSITY, UNPLEASANTNESS AND PAINFULNESS OF ELECTRICAL TOOTH PULP STIMULI. Richard H. Gracely*, Patricia A. McGrath* and Ronald Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD. 20014

Eighty subjects used verbal descriptor scales to rate electrical tooth pulp stimuli before and after the IV administration of either the narcotic analgesic fentanyl, the minor tranquilizer diazepam, saline, or fentanyl and diazepam in combination.

Exp I (n=40) used verbal descriptor scales of sensory intensity (e.g., weak, moderate, strong) and unpleasantness (e.g., unpleasant, annoying, distressing) to assess the effects of 0.11 mg/kg diazepam administered to mask the different subjective effects produced by subsequent administration of either 0.66μg/kg fentanyl or saline placebo. The diazepam and fentanyl combination significantly reduced sensory intensity ($F=8.05$, $p<.05$) but not the unpleasantness of the sensations evoked by these stimuli. Diazepam with saline significantly reduced unpleasantness ($F=6.21$, $p<.05$) but not sensory intensity. We have shown previously that fentanyl reduces only sensory intensity ratings and that saline reduces only unpleasantness ratings. Thus diazepam did not influence verbal judgements of the sensory intensity or unpleasantness of painful tooth pulp stimuli and therefore can be treated as an active placebo useful for masking subjective effects of putative pain control agents.

Exp II (n=40) used verbal descriptor scales of painfulness (e.g., slightly painful, moderately painful, very painful) to assess the effects of a diazepam pre-injection on the subsequent administration of either fentanyl or saline. Without a diazepam pre-injection, pain ratings were reduced significantly after the administration of fentanyl ($F=5.56$, $p<.05$), but not after saline. With the addition of a diazepam pre-injection, painfulness was significantly reduced ($F=9.46$, $p<.05$) after the administration of fentanyl, a result consistent with both the effects of fentanyl alone on pain ratings in Exp II and the effects of diazepam with fentanyl on the ratings of sensory intensity in Exp I. Diazepam with saline, however, also significantly reduced painfulness ($F=20.75$, $p<.01$). This result is not consistent with the effects of saline alone on the pain ratings in Exp II but does parallel the effects of diazepam with saline on ratings of unpleasantness in Exp I. This experiment shows that, unlike verbal scales of sensory intensity and unpleasantness, scales of painfulness may not separate the effects of an active placebo and a narcotic analgesic drug.

1462 TOOTH PULP PRIMARY AFFERENT DEPOLARIZATION (PAD) AND NALOXONE: ARE PRESYNAPTIC OPIATE RECEPTORS INVOLVED? J.W. Hu, J.O. Dostrovsky and B.J. Sessle. Fac. of Dentistry and Dept. of Physiology (J.O.D.), Univ. of Toronto, Canada.

Recent studies suggest the existence of opiate receptors on primary afferent terminals. These receptors may be involved in the production of presynaptic inhibition which is generally assumed to result from depolarization of presynaptic terminals (PAD). Because stimuli to the periaqueductal gray (PAG), which has been implicated in opiate-induced analgesia, depress trigeminal (V) brainstem responses to tooth pulp (TP) and noxious facial stimuli (Sessle et al., Can. J. Physiol. Pharmacol. 54: 66, 1976), we tested for a PAD contribution to the depression. The effect of naloxone, 0.4 mg/kg, i.v., was also tested since, if the PAD is mediated by the release of endogenous morphine-like compounds interacting with presynaptic opiate receptors, reversal of the PAD by this opiate antagonist might be expected.

Single unit TP potentials were recorded in the canine TP of chloralose-anesthetized adult cats. Antidromic activity could be evoked by microstimulation in the V nucleus caudalis and/or oralis. Single units displayed clear all-or-none, constant latency responses at threshold intensity and followed high frequency (' 100 Hz) stimulation. Conditioning stimuli were delivered to the PAG, and also to nucleus raphe magnus (NRM), ventroposterior medial nucleus of thalamus (VPM), TP (of other canine teeth), infraorbital nerve, and facial skin. PAD of TP afferents was determined by the increased probability of exciting an antidromic spike following a conditioning stimulus train. We found that all these stimuli could produce PAD which had a peak at 50 ms and lasted for 150-500 ms. Naloxone has been tested on 12 units (7 units antidromically activated from N. caudalis and 5 units from N. oralis), but had no effect on resting excitability and did not reverse PAD produced by PAG, NRM or facial stimuli. Thus this study has shown that the TP endings in N. caudalis and N. oralis are subject to primary afferent depolarization from PAG, NRM, VPM, and oral-facial sites; the ineffectiveness of naloxone to reverse this effect however implies that presynaptic opiate receptors might not be involved in the PAD.
(Supported by the Canadian MRC and NIH grant #1-ROL-DE04786-01).

1463 ANTIBODIES TO G_{M1} GANGLIOSIDE BLOCK MORPHINE ANALGESIA. S. E. Karpiak, R. J. Bodnar, S. Hanson*, M. Glusman, and M. M. Rapport, N. Y. State Psychiatric Inst. and Columbia Univ., Coll. Phys. & Surg., New York, N. Y. 10032.

The region of the periaqueductal gray matter (PAG) in the rat has a high concentration of morphine receptors[1], and it is well established that injection of small amounts of morphine into this region induces analgesia[2]. We have been studying the effects of antibodies to synaptic constituents, in particular G_{M1} ganglioside, in altering CNS functions[3,4], and it was of interest to determine whether these antibodies affect morphine analgesia. An excellent control for evaluating the effects of these antibodies was available by absorbing the specific antibodies from the antiserum with pure G_{M1} ganglioside. Pain threshold was determined by the flinch-jump technique in male Sprague-Dawley rats (250-300 g). The baseline response to an injection of morphine sulfate (10 mg/Kg,I.P.) was determined for each animal. One week later, the animals were injected in the PAG, under stereotaxic control, with 5 μl either of antiserum to total brain ganglioside or absorbed antiserum or saline. No alteration of pain threshold lasting longer than 24 hours was detected by baseline response on the flinch-jump test. Ten to 14 days after intracerebral (PAG) injection of antibodies, the rats were tested again for analgesic response to morphine sulfate (10 mg/Kg,I.P.). Animals injected with anti-ganglioside serum showed a decrease in analgesic response (84% inhibition; $p<.01$). Animals injected with saline or absorbed antiserum did not show differences in analgesic response (from those obtained with the morphine injection 17 to 21 days earlier). These results suggest that G_{M1} gangliosides are involved in the mechanism of action of morphine in the PAG and may be a structural part of the morphine receptor.

Supported by NIH Grant NS-14449 and N.Y. State HRC Grant 922.

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1464 STIMULATION-PRODUCED ANALGESIA AND LIMINAL ESCAPE BEHAVIOR: SYNERGY WITH MORPHINE AND REDUCTION BY NALOXONE. Dennis D. Kelly, Shannon K. Such*, Martin Brutus, Murray Glusman, Richard J. Bodnar. New York State Psychiatric Institute and Department of Psychiatry, Columbia University, New York, N.Y. 10032.

Liminal escape (LE) is an operant psychophysical threshold procedure that reflects both an animal's evaluation of the relative aversiveness of a range of shock stimuli and its motivation to terminate their presence. The aim of these experiments was to determine 1) whether intracranial stimulation (ICS) of midline mesencephalic and diencephalic sites previously reported to yield an analgesic depression of pain reflexes in rats would also elevate LE functions in an intensity-dependent manner, 2) whether subanalgesic doses of morphine and low-intensity ICS might interact to produce major LE shifts, and 3) whether opiate receptor blockade by naloxone would alter ICS-induced LE shifts. On each liminal escape trial, pulsed foot shocks (300-msec ON/300-msec OFF) were presented for 10 secs unless the subject pressed a lever three times to abbreviate the shock train. Sessions consisted of 100 trials distributed over five shock intensities (.2, .4, .6, .8, 1.0 ma) in a randomized-blocks design. Biphasic square-wave ICS trains (250-msec ON/400-msec OFF) were superimposed upon 40 preselected trials. Foot shock and ICS were interdigitated and never occurred simultaneously. ICS intensity was varied across sessions in 10-25 μa steps. Of 32 rats implanted with bipolar electrodes, 19 were fully tested in the liminal escape procedure, and only 5 displayed a gradient or family of threshold functions that was dependent upon ICS intensity. There was no evidence in any of these subjects of a post-stimulation time course of analgesia. Both their subsequent intertrial behaviors and their escape thresholds on post-ICS trials were normal. Moreover, these ICS-induced elevations in LE thresholds were significantly reduced by naloxone (1 & 10 mg/kg s.c.), which by itself had no effect upon normal thresholds nor upon the behavior of rats with non-analgesic ICS placements. At all effective analgesic placements, low-level subthreshold electrical stimulation was found to interact synergistically with subanalgesic doses of morphine (2.5 mg/kg, s.c.) so as to produce a profound analgesia. Naloxone at the same doses as above also eliminated the analgesic synergy of morphine and ICS. (Supported by New York State Health Research Council Grants #365 and 922 and by NIH grant #NS 14449.)

1465 SENSITIZATION AND DESENSITIZATION OF THE RESPONSES OF PRIMATE SPINOTHALAMIC NEURONS TO NOXIOUS HEAT STIMULI. D.R. Kenshalo, Jr., R.B. Leonard, J.M. Chung, and W.D. Willis. Marine Biomedical Inst. and Depts. of Physiology & Biophys. and Anatomy, U. Texas Med. Br., Galveston, TX 77550

The spinothalamic tract (STT) is thought to be the chief spinal pathway carrying nociceptive information in primates. Support for this comes from experiments in which the responses of STT cells are studied following stimuli known to activate nociceptors. An easily controlled noxious stimulus is heat.

STT cells were identified by antidromic activation from the contralateral diencephalon in anesthetized monkeys (Macaca fascicularis). A thermal stimulator having a surface area of 14 cm^2 was placed against the glaborous skin in the receptive field on the hindlimb. The stimuli were a series of temperature changes from an adapting level of 35° to 43, 45, 47 and 50°C (rate 2°/s). Stimulus duration was either 30 or 120 s with a 5 min interstimulus interval. The series of heat stimuli was then repeated to determine the effect of prior heating.

There were responses to heating in 40 of 41 STT cells examined. During the first series of stimuli of 30 s duration, there was a monotonic increase in the peak frequency of discharge as the temperature was elevated to progressively higher levels. The baseline discharge during the 30 s preceding each stimulus was also progressively increased. When a second series of identical stimuli was applied, sensitization was evidenced by an increase in the peak frequency and in the baseline discharge preceding the stimulus at each intensity. When 120 s duration changes in temperature were employed, peak frequency was enhanced except when the response to the second 50° heat stimulus was compared to the response to the first 50° stimulus. In this case, there was a desensitization, since the peak frequency was less. Furthermore, the baseline discharge was lowered after the second 45° stimulus.

In addition, to sensitization to repeated heat stimuli, we found evidence for cross-modality sensitization to mechanical and to intense cold stimuli.

It is concluded that the responses of STT neurons in the primate show sensitization and desensitization with characteristics very similar to those of C polymodal nociceptors. It is hypothesized that the responses of STT cells are due largely to the activity of these nociceptive afferents.

This work is supported by NIH grant NS 09743 and by NIH postdoctoral fellowships NS 05698 (to D.R.K.) and NS 05434 (to R.B.L.).

1466 EFFECT OF ACUPUNCTURE ON THE EXCRETION OF ADRENALIN, NON-ADRENALIN, 17-HYDROXYCORHCOSTEROID AND VANILLYL MAUDELIC ACID IN URINE. K. C. Kim. Dept. Anat. R. Heimburger. Dept. Neurosur., Sch. Med., IN U, Indpls., IN 46202.

Analgesic effect of acupuncture may explain neurogenic theory or increasing endorphine in brain by acupuncture. However, it is difficult to explain the prolonged improvement of antiinflammatory effect in various pain syndromes.

It has been shown that surgical stimulation increased the excretion of adrenalin (A), non-adrenalin (NA) and 17-hydroxycorticosteroid (17-OHCS) in urine. Many insertions of acupuncture needles may be considered a type of minor surgical stimulation and it may influence A, NA and 17-OHCS level in urine in chronic pain patients.

Chronic intractable low back pain patients, 3 females and 5 males, were admitted to the hospital and A, NA, VMA and 17-OHCS in the urine was measured for 2 days as control before treatment and for 8 days after the treatment.

15 acupuncture needles, made of stainless steel, were inserted on various loci in their back and left for 30 min. Special diet was given for consideration of catecholamine level. Pain was evaluated by patient themself.

Results: A: The mean control value of A was 6.4 ug/L, it was decreased by 21% and 35% on the first and second day respectively. The third day it returned to the control level and after the fourth day A was fluctuating. NA: The mean control value of 27.6 ug/L was decreased by 48% on the first day, the second day it returned to the control value and after the third day it fluctuated. 17-OHCS: The mean control value of 6 ug/L was increased by 44% and 80% on the first and second respectively. It then increased rapidly by 229% by the fourth day, then it decreased to 50% above the control level for the rest of the hospital days. VMA: The first day VMA was decreased by 66% of control, after that it fluctuated, but was significantly lower than the control. PAIN: Pain was relieved significantly in all patients on the first day, thereafter the pain score fluctuated, but was relieved over all.

1467 ANALGESIA INDUCED BY MICROINJECTION OF BACLOFEN AND MORPHINE AT SITES IN THE RAT BRAIN STEM. R. A. Levy and H. K. Proudfit. Dept. of Pharmacol., Univ. of Illinois Med. Center, Chicago, Illinois 60612.

Analgesia induced by both morphine and by the non-narcotic baclofen (β-4-chlorophenyl-GABA) is blocked following transection of the caudal medulla, but not by midcollicular transection (Proudfit and Levy, Eur. J. Pharmacol. 47(1978), 159. This suggests that the analgesia induced by these agents may reflect activation of the same neuronal substrates, albeit by different cellular mechanisms. To test this hypothesis we determined the analgesic capacity of both drugs when microinjected at sites in the periaqueductal gray (PAG) and raphe magnus (RM), brain stem nuclei shown previously to be active areas for morphine analgesia. On separate occasions, equimolar doses of both agents (1.5 µg baclofen, 2.5 µg morphine sulfate) were microinjected, in 0.5 µl saline, into the same site in rats chronically implanted with guide tubes. Sensitivity to pain was assessed with the tail flick assay and expressed as the analgesia index (AI). The AI expresses the drug-induced change in latency as a function of the greatest possible increase (14 sec.); AI = 0, no analgesia; AI = 1, maximum analgesia. AI $\geq$ 0.2 was defined as indicative of analgesic action. Baclofen and morphine were both more effective in producing analgesia when applied at caudal than at rostral PAG sites. Thus, baclofen caused analgesia when microinjected at 10 of 15 PAG sites located caudal to the interaural line, but only at 2 of 16 PAG sites located rostral to this plane. Similiarly, morphine caused naloxone-reversible analgesia when applied at 9 of 9 caudal sites but only at 8 of 12 rostral sites. Analgesia produced by morphine applied at the caudal loci (AI = 0.52 ±0.09) was significantly greater than that caused by morphine applied at active rostral sites (AI = 0.29 ± 0.05). Baclofen and morphine caused analgesia of equal magnitude when injected at their respective active sites in the caudal PAG; however, the relative potency of morphine among caudal PAG sites was poorly correlated with that of baclofen (r^2 = 0.16).

Morphine was more active than baclofen in the RM. Morphine caused a substantial naloxone-reversible analgesia when injected at 7 of 13 RM sites (AI = 0.46 ± 0.08, n = 7); baclofen caused a slight analgesia when applied at only two (AI = 0.2) of 11 RM sites.

Despite the greater potency of both agents at caudal as opposed to rostral PAG sites, the poor correlation in potency of these drugs in caudal PAG and the capacity of morphine but not baclofen to produce analgesia when injected into the RM suggest that different substrates are involved in analgesia induced by systemic administration of these agents. Supported by PHS Grant NS 12649.

1468 ANALGESIA ELICITED BY INTRACEREBRAL MICROINJECTIONS OF BACLOFEN AND MUSCIMOL. Jeffrey M. Liebman and Gary Pastor*. Research Dept., Pharmaceutical Div., CIBA-GEIGY Corp., Summit, NJ 07901.

Baclofen (Lioresal(R)), a muscle relaxant, elicits non-narcotic analgesia when administered systemically. Because analgesia can be elicited by morphine microinjections into caudal ventrolateral mesencephalic central gray, morphine analgesia appears to be at least partly mediated by this region (Yaksh et al., Brain Res. 114:83. 1976). The effects of baclofen (BF) microinjections into this region were, therefore, of interest, particularly as Proudfit and Levy (Eur. J. Pharmacol. 47:159, 1978) have implicated brainstem sites in the analgesic effects of both morphine and BF. Muscimol (MUS), a GABA-mimetic, was also compared to BF, which is a structural analog of GABA but differs from GABA-mimetics in some respects (Naik et al., Neuropharmacol. 15:479, 1976).

Male Wistar rats were stereotaxically implanted in dorsal tegmentum with guide cannulae (0.029" diam.), allowed to recover for at least one week, then injected intracerebrally through a needle protruding 1 mm below the cannula tip. Morphine (5 µg), BF (0.01-2 µg) and MUS (0.001-1 µg) were microinjected in a volume of 0.5 µl. In most cases, the three drugs were injected into a given placement in separate trials at intervals of one week. A pinch test of analgesia was employed, supplemented in some cases by a tail-flick test. Morphine-elicited analgesia was restricted to microinjections in or near ventrolateral caudal mesencephalic central gray, while BF and MUS produced analgesia when microinjected into various tegmental placements including mesencephalic central gray but also extending farther anterior and ventral. Naloxone (1 mg/kg i.p.) reversed the analgesic effects of intracerebral morphine, but not those of BF or MUS. Although ataxia was also noted after some intracerebral microinjections of BF or MUS, it was not invariably present in analgesic rats. MUS was more potent intracerebrally than BF, but further investigations which employed the intraperitoneal route in rats showed, at best, weak analgesic effects of MUS at physically debilitating doses, while BF showed a relatively greater separation between analgesia and obvious physical impairment.

These results indicate that the analgesic effects of BF may be mediated at least partly by central mechanisms. Further, selective activation of central GABA-ergic receptors, as by intracerebral MUS, may suppress nociception directly or indirectly. Because BF is not considered a directly-acting GABA-mimetic, the reason why its effects approximate those of MUS when microinjected intracerebrally remains to be fully elucidated.

1469 EVOKED POTENTIAL CORRELATES OF PAIN RATINGS IN RHESUS MONKEYS. Charles G. Lineberry and Albert T. Kulics*. Dept.Pharmacol., Sch. Med., Univ. of Pittsburgh, Pgh., PA 15261

In previous work, we have used a signal detection theory (SDT) model to assess pain sensitivity and response bias in monkeys responding to terminate trains of noxious electrical cutaneous stimuli. In the present study, we have recorded transcortical field potentials while subjects were performing in the SDT escape task in order to determine whether these potentials were correlated with behavioral responses as predicted from the SDT model. Bipolar, concentric recording electrodes were implanted in the post-central gyrus as well as in more posterior regions of parietal cortex. Subjects received escape trials at each of 5 stimulus intensities in daily sessions. On each trial, monkeys were required to bar-press within 1.5 sec after the presentation of a 20 msec train of electrical stimuli in order to prevent the occurrence of successive trains of identical stimuli. Response probabilities and response latencies were used as intensity ratings of the stimuli. For each stimulus intensity, trials on which escape responses were elicited were then grouped into blocks of 25 on the basis of behavioral response latency, with the first group consisting of the 25 trials having the shortest behavioral response latencies and each succeeding group of 25 trials including those trials with successively longer latencies. The final group for each stimulus consisted of 25 trials randomly selected from the trials on which no escape response occurred. The field potentials associated with each group were then averaged, and determinations were made of the peak to peak amplitude of each wave component.

Certain relatively late components (70 to 150 msec) of the primary somatosensory cortex response correlated well with behavioral response latencies; short latency behavioral responses (sensation rated as intense) were associated with large amplitude potentials, and the no-response groups had the smallest amplitudes. Further, groups of trials with similar response latencies (assumed to represent equal sensation magnitudes) tended to have similar late component amplitudes, regardless of stimulus intensity. Thus, the behavioral intensity rating and not stimulus intensity was best correlated with the amplitude of these later components. For the initial surface positive wave (18 to 20 msec latency), the reverse was true. Potentials recorded from other cortical areas showed little correlation with behavior. These findings indicate that late components of the primary somatosensory evoked potential correlate with behavioral ratings of noxious stimulus intensities and therefore may play a role in the perception of pain.

1470 ACUPUNCTURE AND NALOXONE MODIFICATION OF HUMAN SOMATOSENSORY EVOKED POTENTIALS. P. Maple*, R. Borison, H. Havdala* and B. Diamond. (SPON: C.M. Combs) Mt Sinai Hosp./Rush Med Coll., Chicago, IL.

Since by physiological and/or psychological means, acupuncture as well as naloxone must alter the central nervous system pathways involved in the perception of pain, we have sought to document this by means of measuring somatosensory evoked potentials (SEP) in man. SEP were measured in male and female volunteers free from pain, as well as in patients with pain. The SEP were analyzed by averaging 1000 sweeps of the electroencephalogram by a Computer of Averaged Transients. Acupuncture was performed by placing needles in the Hoku point and other relevant sites for shoulder pain, and needles were twirled every ten minutes. In some subjects naloxone (2 mg, intravenously) was immediately administered after acupuncture was terminated, or in place of acupuncture treatment. In 75% of our subjects the amplitude of the positive peak occurring at 28 msec (P_{28}) decreased and the amplitudes of the later positive peaks, P_{46} and P_{65}, decreased in 83% and 80% of our subjects respectively. We observed a concomitant decrease in latency to the P_{65} wave after acupuncture treatment. Moreover, the early negative peak (N_{18}) amplitude decreased, with the latency to and the amplitude of the late negative wave (N_{120}) decreasing after acupuncture. Furthermore, in studies where naloxone was administered, there appeared to be a noticeable reduction of the P_{46} potential induced by acupuncture. Moreover, when naloxone alone was administered to subjects, the P_{46} potential was markedly increased. Furthermore, in all our subjects there was a noticeable reduction in threshold for stimulation of the evoked potential during and after acupuncture. The early components of the SEP represent conduction of nerve impulses through the spinal cord and medulla to relays in the ventroposteriolateral nucleus of the thalamus, whereas late events indicate the firing of neurons in cortical association areas. Our data, which show dramatic changes in the early events of the SEP induced by either naloxone or acupuncture, would argue in favor of attentuation of stimuli in their conduction to the thalamus, rather than an alteration of perception solely at a cortical level. The action of naloxone on the P_{46} potential in particular shows the presence in man of a naloxone-sensitive pathway, and lends further evidence to an enkephalinergic mechanism mediating the pain response in man. Furthermore, the fact that we observe changes in the late events of the SEP, consistent withour subject's reports of arousal would indicate that acupuncture also produces changes in the supratentorial response to stimuli.

(Supported by Anesthesiology Research Fund, Mt. Sinai Hospital)

1471 PRIMARY AFFERENT DEPOLARIZATION EVOKED IN IDENTIFIED CUTANEOUS AFFERENTS BY STIMULATION IN THE MEDIAL BRAINSTEM. R.F. Martin, L.H. Haber and W.D. Willis. Marine Biomedical Inst. and Depts. of Physiology & Biophysics and of Anatomy, U. Texas Med. Br., Galveston, TX 77550.

Stimulation in either the nucleus raphe magnus (NRM) or the nucleus reticularis gigantocellularis (NGc) in monkeys (Macaca fascicularis) results in the inhibition of spinothalamic tract neurons. Both background activity and the discharges evoked by stimulation of the skin are inhibited. The possibility that at least a part of the inhibition might be presynaptic was investigated in experiments in which excitability tests were used to determine if primary afferent depolarization (PAD) results from stimulation in these brainstem nuclei.

Cats and monkeys were used. Anesthesia was maintained with either α-chloralose or a combination of this agent and an infusion of sodium pentobarbital (2 mg/kg/h). The spinal cord was exposed by laminectomy and the brainstem by occipital craniectomy and partial cerebellectomy. A freed segment of sural nerve was elevated onto a plastic platform and an incision made through the perineurium. A pair of stimulating electrodes was placed under a second freed segment. Glass microelectrodes (initially about 35 MΩ) filled with 4 M NaCl were used to record from afferent axons. Conduction velocities were determined from the latencies of spikes following stimulation of the nerve. Receptive field properties were determined. The excitability of each afferent was tested by stimulation with a steel microelectrode inserted into the dorsal root entry zone at S1. Firing indices were set at an intermediate value by adjustment of the stimulus strength. Conditioning trains of stimuli were applied through other steel electrodes introduced into the medial brainstem. An increase in the firing index was taken to signify PAD.

Stimulation in the NRM produced PAD in 20 Aδ fibers (12 high threshold and 8 D hair afferents) and 47 Aβ fibers (10 slowly adapting, 9 field and 28 hair follicle afferents). Stimulation in the area of NGc also produced PAD in all of these types of cutaneous afferent fibers. Threshold effects were seen with stimulus strengths as low as 25 μA. The latency of the PAD evoked by stimulation in the NRM was longer than for stimulation in the NGc; bilateral interruption of the dorsolateral fasciculi resulted in a loss of PAD from stimulation of the NRM but not of the NGc.

The results indicate that there are at lease two pathways originating in the medial brainstem in cats and monkeys which produce PAD and presumably presynaptic inhibition of transmission to spinal neurons from afferent fibers carrying information from cutaneous mechanoreceptors and nociceptors.

This work was supported by NIH grant NS 09743 and NIH postdoctoral fellowship NS 05087 (to L.H.H.).

1472 ANALYSIS OF ENDORPHINS IN CEREBROSPINAL FLUID (CSF) FROM PERSONS WITH LOWER BACK OR LEG PAIN. B.E. Miller, E.E. Codd*, A.L. Ungar* K. Mays*, and W.L. Byrne. Depts. Biochem. & Anesthes., U.T.C.H.S. Memphis, Tennessee 38163.

Since 1975 there have appeared 3 papers each reporting evidence for a functional relationship between CSF-endorphins and pain. The level of endorphin is apparently lower in patients with trigeminal neuralgia (Life Sci. 16: 1759-1764, 1975). Endorphin is elevated in pain patients after stimulation by analgesic electroacupuncture (Acta Physiol Scand. 100: 382-384, 1977). The CSF of persons with headache is lower than those without and these differences are not due to the rate of destruction of met-enkephalin in the CSF (Adv. in Biochem. Psychopharmac. 18: 363-366, 1978).

In the present study, CSF was removed from persons with lower back and/or leg pain while they were undergoing spinal anethesia for pain relief at the U.T. Pain Clinic. The CSF was then frozen. It was thawed and filtered through an Amicon PM-10 filter at 4°C. The filtrate was concentrated to 1/5 the original volume and loaded atop a Sephadex G-10 column and eluted with 0.2 N acetic acid. The column fractions were tested for endorphin activity with an opiate receptor binding assay (described in Prog. Neuro-Psychopharmac. 1: 259-264, 1977). In order to obtain enough material for other tests, several CSF samples were pooled and treated as a single sample. The pooled CSF sample provided enough material to also test the column fractions for endorphin activity with the mouse vas deferens bioassay (similar to: Brain Res. 88: 295-308, 1975). This assay confirmed most of the endorphin peaks indicated by the binding assay.

There is considerable variability in the number and amount of endorphins in the individual CSF samples. Peaks of endorphin activity consistently elute (though not in each sample) in the following positions from the Sephadex G-10 column: Volume(elution)/Volume(void) (Ve/Vo) ± 0.1 = 1.3, 1.7, 2.3, 2.7, 3.0, 3.5, and 4.5. The first four peaks usually account for more than 80% of the endorphin activity. The behaviour of some of these CSF endorphin components in reverse phase high pressure liquid chromatography systems suggests that most of these materials are not identical with as yet structurally identified endorphins.

1473 MEDIATION OF HYPALGESIA AND THALAMOCORTICAL MODULATION BY OPIATE RECEPTORS IN THE PERIAQUEDUCTAL GRAY. William J. Nowack,* Richard N. Johnson, Norman H. Bass and George R. Hanna, Department of Neurology and Clinical Neuroscience Research Center, University of Virginia School of Medicine, Charlottesville, Va. 22901

A major site for the modulation of responses to pain has been demonstrated in the periaqueductal gray (PAG); opiate receptors are found in the PAG. Both electrical stimulation in the PAG and intravenous morphine produce naloxone reversible behavioral hypalgesia. Similar changes in thalamocortical excitability are caused by intravenous morphine and PAG stimulation. We studied the effect of microinjection of morphine in the PAG to determine whether the PAG opiate receptors participate in hypalgesic thalamocortical modulation.

Pairs of pulses were delivered to the ventrolateral thalamus of anesthetized cats and the cortical evoked response following the second stimulus was recorded from ipsilateral sensorimotor cortex; the amplitude of the second stimulus and the interval between the two pulses (PI) were systematically varied by on-line computer. Three dimensional plots of the evoked responses, or evoked response profiles (ERP), were generated and analyzed statistically. Intravenous morphine, electrical stimulation of PAG and microinjection of 5 µg of morphine into PAG all produced similar PI dependent alterations in ERP. These observations suggest that opiate receptors in PAG modulate the thalamocortical processing of information interpreted as pain and presumably the perceptual threshold for pain.

(Supported in part by NIH grants nos. NS 07013 and DA 1330).

1474 OPTIMUM DUTY CYCLE AND FREQUENCY OF INTERMITTENT ELECTROANALGESIA CURRENT. O'Donnell, Robert P.*, R. Wayne Fields, Richard B. Tacke* and Patrick J. Reynolds. School of Dentistry, University of Oregon Health Sciences Center, Portland, Oregon 97201.

We have been studying the effect of electric current on the excitability of identified primary afferents from tooth pulp. Using the monopolar stimulus configuration with remote cathode and the anode applied to the exposed dentin of the test tooth, we have previously demonstrated that direct currents (DC) in the range of 70 to 100 µA block afferent activity from tooth pulp (Fields et al., Exp. Neurol. 47:229-239, 1975) and that trains of rectangular pulses at 1000 pps having similar peak current but duty cycles as low as 10% are equally effective in blocking pulp afferent activity (Fields et al., Exp. Neurol. 53:386-398, 1976). The present experiments were performed to further examine the range of duty cycle and frequency of pulsed electroanalgesia (EA) current that might produce blockade as effectively as DC. We examined this using our previously described methods for recording thresholds of pulp-driven primary afferents in the ipsilateral Gasserian ganglion of cats to electrical stimulation of the test tooth, applied at intervals during the EA administration. Duty cycles of 1.0, 3.16, and 10.0% were examined at a frequency (based on previous results) of 1000 pps Subsequently, frequencies of 100, 1000, 10,000 and 100,000 pps were studied at the identified optimum duty cycle of 10.0%. In each experiment the monophasic pulsed current waveform was compared to DC, and was applied in increasing steps of peak current from 0 to 100 µA. At effective current levels (70 to 100 µA) there was no difference between DC and the 10% duty cycle waveform, while the 3.16 and 1.0% waveforms were progressively less effective in afferent blockade. Maximal threshold increases for the DC and 10% duty cycle pulsed waveform were in the range of 400 to 500%. Return to within 150% of control excitability (arbitrary index of recovery) occurred in less than ten minutes for all waveforms. For the frequency variable only the 1000 pps waveform compared in effectiveness to DC (maximum threshold elevations of 400%) at similar current levels. The 100 pps waveform was intermediate in effectiveness (maximum 200% threshold elevation) while the 10,000 and 100,000 pps waveforms were totally ineffective. Recovery profiles were similar to those observed in the duty cycle experiments. The results permit identification of 1000 pps, 10% duty cycle as the optimal intermittent current waveform for use in lowering excitability of primary afferents from tooth pulp.

(Supported by NIH Grant DE 04281)

1475 STRESS-INDUCED ANALGESIA: CROSS-TOLERANCE STUDIES OF STRESSORS AND MORPHINE. Angela Spiaggia*, Richard J. Bodnar, Murray Glusman and Dennis D. Kelly. New York State Psychiatric Institute and Columbia University, N.Y., N.Y. 10032.

Rats display elevations in pain thresholds when initially exposed to such stressors as a forced cold-water swim (CWS), inescapable electric shocks, or an injection of 2-deoxy-D-glucose (2-DG), an anti-metabolic glucose analogue. Repeated exposure to each of these stressful situations results in adaptation of the analgesic response in much the same way that repeated administration of morphine results in tolerance. We have previously found that cross-tolerance fails to develop between CWS-induced and morphine-induced analgesia suggesting that the analgesic mechanisms underlying each are not identical. The present study investigated whether cross-tolerance would develop between the analgesia induced by CWS and 2-DG, and between the latter and morphine-induced analgesia. Six separate groups of six rats each were tested over a 22-day paradigm in which flinch-jump thresholds were determined on three pretreatment baseline days, the first of 14 chronic treatment days, the last treatment day, a cross-treatment day and four subsequent posttreatment recovery days. All treatments occurred 30 min prior to flinch-jump testing. Following baseline, the first two groups received 14 daily 2-DG injections (350 mg/kg) followed on the fifteenth, or cross-treatment day by morphine (10 mg/kg) in one group and CWS (2°C for 3.5 min) in the other. The third and fourth groups underwent 14 daily CWS or morphine injections respectively followed by 2-DG injections on the fifteenth day. The fifth group underwent 14 daily warm-water (28°C) control swims followed by 2-DG administration, while the sixth group received 14 placebo injections followed by CWS. Acute exposure to 2-DG, CWS and morphine all produced analgesia; control swims and injections did not. Repeated exposure to the same conditions resulted in adaptation or tolerance. Full development of 2-way cross-tolerance occurred between CWS and 2-DG, in other words, rats chronically treated with either 2-DG or CWS failed to display analgesia when switched to the other. Full cross-tolerance also occurred in chronic morphine-treated rats subsequently exposed to 2-DG, but only partial (50%) cross-tolerance occurred in chronic 2-DG-treated rats subsequently exposed to morphine. Chronic exposure to the warm water control and placebo conditions did not alter the normal analgesic effects of acute exposure to 2-DG and CWS. (Supported by NIH Grant #NS 14449 and N.Y.S. Health Research Council Grants #365 and #922.)

1476 ELECTROPHYSIOLOGIC ANALYSIS OF SYSTEMS SUBSERVING BASAL GANGLIA INHIBITION OF INTRALAMINAR THALAMIC NEURONS. Howard K. Strahlendorf and Charles D. Barnes. Department of Physiology, Texas Tech University School of Medicine, Lubbock, TX 79409.

Intralaminar thalamic nuclei are throught to play an important role in the extralemniscal sensory system since responses in these structures can be evoked by peripheral noxious stimuli. Conditioning electrical stimulation of caudate nucleus (CN) or substantia nigra (SN) evokes long lasting inhibition of driven units in centrum medianum-parafasicular complex of thalamus (CM-Pf) suggesting a modulatory function of the basal ganglia on central nociceptive processing. Latency analyses have suggested CN effects on CM-Pf units are not relayed through SN since onset latencies for both CN and SN are nearly identical.

We have conducted a series of experiments in order to physiologically characterize the neural substrates of CN and SN inhibition on CM-Pf. Units in CM-Pf which responded in a characteristic manner to sural nerve stimulation were recorded with tungsten microelectrodes stereotaxically placed in chloralose anesthetized cats. Discrete conditioning stimuli to CN or SN (200 Hz, 0.2 msec, 2-5 pulses, < 500 µA) produced prolonged inhibition of CM-Pf units with an onset latency of 20-50 msec and a duration of approximately 300-400 msec. Similarly, conditioning stimulation of nucleus accumbens, Fields of Forel (FF), thalamic reticular nucleus (RN), globus pallidus (GP), endopeduncular nucleus (EN), locus coeruleus (LC), periaqueductal gray (PAG), and raphe (R) markedly inhibited firing of sural evoked CM-Pf units. Acute electrolyte lesions were placed in SN, GP, EN, FF, LC and RN. Lesions in EN, FF and LC effectively reduced inhibition from CN while only lesions in SN and FF altered SN induced inhibition.

To investigate the possibility that descending spinal inhibitory mechanisms may have suppressed afferent impulses at sites caudal to the recording electrode in CM-Pf, a stimulating electrode was placed into either PAG or the central tegmental field (FTC). Units in CM-Pf which responded convergently to sural stimulation and single shocks to PAG or FTC (0.1 msec, 0.1 mA to 0.5 mA) were tested by conditioning CN and SN. CN and SN conditioning affected units driven by sural and FTC/PAG indifferently. These data suggest CN and SN induced inhibition of intralaminar thalamic units is mediated via similar but distinct supraspinal neural pathways.

(Supported by a grant from the Tarbox Parkinson's Disease Institute of Texas Tech University School of Medicine).

1477 ELECTRICALLY-INDUCED HYPALGESIA OF THE HUMAN DENTAL PULP AND ORO-FACIAL STRUCTURES. Richard B. Tacke*, R. Wayne Fields and Bhim S. Savara*, (SPON: Richard E. Talbott) University of Oregon Health Sciences Center, School of Dentistry, Portland, Oregon 97201

Our laboratory has described the successful induction of hypalgesia in the human dental pulp by electrical stimulation of nearby oral mucosa. (Tacke, Fields, Sakellaris, and Savara, Neurosci. Abst. 1: 119, 1975), and a comparison of the effects of varied waveforms (Tacke, Fields, Sakellaris and Savara, Neurosci. Abst. 2: 921 1976).

The present experiment compares effectiveness of the administration of electro-analgesic (EA) current to the oral mucosa with EA current applied to selected extra-oral anatomic sites on the head and neck. A 'standard' EA waveform composed of a continuous train of bidirectional rectangular pulses @ 100 pps and @ 50% duty cycle, at an intensity 10-20% below perceptual threshold was utilized. The intra-oral sites selected included the buccal oral mucosa near the test tooth and the salivary foramina. The extra-oral sites included areas adjacent to the infraorbital and mental foramina.

Electrode configurations are also compared by evaluation of data recorded when placing electrodes ipsalateral or contralateral to the test tooth. Bilateral active electrode configurations are included. The data from all comparisons will be presented and indicate that EA currents applied to both the buccal oral mucosa and to the mental foramen result in significant EA effects, however, induction time required between the two locations differs significantly.

The application of the 'standard' EA waveform, bilaterally to the mental formina of thirty six patients suffering from chronic head and neck pain has resulted in the following observation: thirty-three percent of the patients treated with EA current show a significant relief from their pain after single sixty minute session, one hundred percent of these subjects have received significant relief after two sixty minute sessions.

The patients treated suffered from chronic pain of the head and neck including trigeminal neuralgia, temporal mandibular joint pain, migraine headache, maxillary and mandibular pain resulting from trauma or surgical intervention. Evaluation of the effectiveness of EA current adminstration is achieved utilizing the model described by Melzack (Pain 1: 277-99 1975).

Preliminary experiments evaluating the efficacy of the use of EA current in operative dentistry will also be discussed.

1478 A NEUROANATOMICAL STUDY OF ANALGESIA AND CATATONIA INDUCED BY ETORPHINE IN THE RAT. B.E. Thorn and R. A. Levitt, Dept Psychol. and Sch. Med., Southern Ill. Univ., Carbondale, IL 62901

Etorphine hydrochloride is a fast-acting narcotic analgesic, several thousand times more potent than morphine. We studied the analgesic and catatonic properties of etorphine when microinjected into one of 7 neuroanatomical sites: periaqueductal gray; cerebellum; caudate putamen; basolateral amygdala; cortico-medial amygdala; hippocampmus; globus pallidus.

The flinch-jump technique was used to assess pain sensitivity and the bar test to study catatonia. Etorphine was administered in either a lug or a 2 ug dose. Each animal was used only once in one experiment and was administered etorphine only one time. The animals received a baseline bar test followed by a flinch-jump test followed by a second bar test (taking 10 min). They were then injected with drug (either water or etorphine in a lul solution) into one neuroanatomical location. All animals received both water and etorphine, presented in a couterbalanced order and separated by a four-day interval.

Neuroanatomical location ranged from AP +3.0 to AP -9.0. The region showing maximum sensitivity to the intracerebral administration of etorphine in the elevation of the nociceptive threshold lay within the periaqueductal gray (N=16). A dose of lug etorphine elicited significant analgesia and catatonia, while 2ug elicited progressively stronger effects. The area showing the least responsivity to etorphine-elicited analgesia or catatonia was the cerebellum (N=8). Neither lug nor 2ug doses elicited analgesia or catatonia. Injections into other areas produced variable results. While lug etorphine had no effect when injected into either the basolat. amygdala (N=8) or cortico-medial amygdala (N=8), 2ug did elicit analgesia and catatonia in the BLA but not in the CMA. Likewise, analgesia and catatonia were elicited in the caudate-putamen (N=8) by a 2ug but not by a lug dose of etorphine. Etorphine injections of 1 or 2ug into the globus pallidus (N=8) or hippocampus (N=8) did not produce reliable analgesia or catatonia. All results were significant at the .01 level. Interesting results due to differences in needle placement will be discussed.

This study suggests that etorphine-elicited analgesia and catatonia seen after injection into the brain is site specific, is related to affinity for the site to bind with etorphine, and is not just a result of diffusion following microinjection of a lipophilic substance into the brain. Moreover, the high correlation between degree of analgesia and presence of catatonia may suggest a common mechanism and substrate for these two actions of the narcotic system.

1479 RECOVERY OF PAIN REACTIVITY FOLLOWING SEQUENTIAL LESIONS TO THE SPINOTHALAMIC TRACT AND OTHER CORD SECTORS OF MONKEYS. Charles J. Vierck, Jr. and Mary Margaret Luck. Dept. Neuroscience and Center Neurobiological Sciences, Univ. of Fla. Col. Med., Gainesville, Fla., 32610.

Cebus albifrons monkeys were trained to escape electrical stimulation of either leg at five intensities, spanning a range from mild tingle to intense but tolerable pain (as judged by human observers). The average duration of shock received by the animals at each intensity was plotted for each leg over the required recovery period following ventrolateral spinal chordotomy. Similarly, recovery of escape responding was observed following subsequent lesions to the spinal cord, in an attempt to define the pathways that subserve pain conduction after readjustment from chordotomies that produced substantial deficits of escape behavior. The most enduring elevations of shock duration by lesion I (left chordotomy) were produced by lesions that involved all of the ventrolateral column and most or all of one or both ventral columns. Secondary lesions of the dorsal columns, Lissauer's tract and the dorsolateral columns, in various combinations, did not produce long term effects on escape responding. In contrast, a complete ventral hemisection produced a pronounced bilateral deficit that did not recover fully over 305 postoperative days. The major conclusions are: (1) that the dorsal pathways do not play a major role in the rostral conduction of information critical for pain perception in monkeys, even though these pathways receive input from high threshold receptors; and (2) in order to produce a lasting decrease of pain sensitivity in primates with spinal surgery, the lesion must be bilateral and must involve both the ventrolateral and ventral columns.

1480 SOME NEURONS OF THE SUBSTANTIA GELATINOSA PROJECT IN THE SPINOTHALAMIC TRACT. W.D. Willis, D.R. Kenshalo, Jr., and R.B. Leonard. Marine Biomedical Inst. and Depts. of Anatomy and of Physiology & Biophysics, U. Texas Med. Br., Galveston, TX 77550.

The substantia gelatinosa (SG) of the spinal cord dorsal horn plays a key role in the processing of information from nociceptors, thermoreceptors and mechanoreceptors. The SG is believed by many investigators to be coincident with both laminae II and III of Rexed. The cell types within the SG include the limitroph and central cells of Cajal. The limitroph cells are in the dorsalmost part of lamina II and have dendrites which project ventrally. The central cells are distributed throughout laminae II and III and have radially oriented dendrites in transverse section and rostrocaudally oriented dendrites in longitudinal section. There are also cells in the ventral part of lamina II with dorsally projecting dendrites, as well as transitional cell types. All of the cells of the SG are thought to have relatively short axons. However, we have found that some neurons of the SG project to the diencephalon.

Horseradish peroxidase (HRP) was injected into the diencephalon in each of 8 monkeys (Macaca fascicularis). The monkeys were anesthetized with sodium pentobarbital. The amount of HRP injected was 0.2-1.0 µl (50% sol'n.) in 1-4 sites. The animals were perfused after 3 days with Ringer's followed by 2.5% glutaraldehyde and 0.5% paraformaldehyde. After further fixation the brain and spinal cord were soaked in 30% sucrose. Frozen sections were made at 50 µm and reacted with either O-dianisidine or tetramethylbenzidine. A total of 4439 cells (average of 555 cells per animal) were identified in the spinal cord at L5 and caudally as belonging to the spinothalamic tract (STT) by the presence of HRP reaction product following retrograde transport. Most of the cells were in laminae I and V when HRP was injected into the lateral thalamus and in laminae VI-VIII when the injection was in the medial thalamus. An unexpected observation was an occasional labelled cell within the SG. To date, 50 such cells (1.1% of the total population of STT cells) have been found, excluding large cells near the boundary between lamina III and IV. Most of the labelled SG cells were contralateral to the side of the thalamic injection, and most could be classified either as limitroph (25) or central cells (17). The labelled cells were found in lamina II (34) and III (16). STT cells were observed in the SG in 6 of the 8 monkeys. In one negative experiment, the injection site was too rostral to label many STT cells in any cord region.

We conclude that the SG contains neurons which contribute to long ascending tracts, and thus the SG is not a completely closed system as is usually assumed.

This work was supported by NIH grant NS 09743 and NIH postdoctoral fellowships NS 05698 (DRK) and NS 05434 (RBL).

PLASTICITY

1481 COMPENSATORY CHANGES IN CEREBRAL TYROSINE HYDROXYLASE ACTIVITY FOLLOWING INTRAVENTRICULAR 6-HYDROXYDOPAMINE. Ann L. Acheson* and Michael J. Zigmond. Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

Previous studies have shown that the destruction of sympathetic nerve terminals by the intravenous administration of 6-hydroxydopamine (6-HDA) results in an increase in tyrosine hydroxylase (TH) activity in the adrenal medulla. The present studies were designed to investigate whether a similar increase in TH activity might occur in residual norepinephrine (NE)-containing nerve terminals in brain following intraventricular injection of 6-HDA. Rats were given a single intraventricular injection of 6-HDA (250 µg in 20 µl) or vehicle (0.9% NaCl, 0.1% ascorbic acid) and sacrificed by decapitation 3-21 days later. Cerebellum, hippocampus and the area containing the locus coeruleus were dissected out and frozen. Tissue was homogenized in a Tris-HCl buffer and spun at 49,000 x g for 60 min. Soluble TH activity was then determined in the supernatant by measuring the evolution of $^{14}CO_2$ from carboxyl-labeled ^{14}C-L-tyrosine (75 µM) in the presence of an excess of DOPA decarboxylase and saturating 6MPH$_4$. 6-HDA treatment was found to irreversibly deplete NE in hippocampus and cerebellum by >80% within 3 days, suggesting that no more than 20% of the NE terminals remained. In cerebellum, values for TH activity rose sharply above that predicted by NE levels. By 3 days, TH was 65% of control, where it remained for the duration of the study. Hippocampal TH activity increased from 20% on day 3 to 32% of control on day 12, and reached 66% of control by day 21. In locus coeruleus, an area containing the cell bodies of noradrenergic neurons which terminate in hippocampus and cerebellum, TH activity was 188% of control on day 5, was back down to control values by day 12, and was 75% of control on day 21. These results are consistent with the hypothesis that sub-total destruction of NE terminals induces an increased synthesis of TH in locus coeruleus. TH is then transported to the nerve terminals via a slow axonal transport mechanism, reaching cerebellar terminals, which are in close proximity to the cell bodies, within 3 days, and reaching the more distant hippocampus by 21 days. Similar time courses for the appearance of increased TH activity have been reported following reserpine. Further experiments will determine whether the increased TH activity is actually a result of an increase in the number of TH molecules and whether it is accompanied by an increase in the functional capacity of the remaining terminals for NE release. If so, the observed compensatory increase in TH activity may help explain the lack of behavioral deficits in these lesioned animals.

(Supported, in part, by USPHS grants MH00058 and MH20620.)

1482 EFFECTS OF NEONATAL LATERAL HYPOTHALAMIC DAMAGE UPON SENSORIMOTOR, SUCKLING, AND REGULATORY BEHAVIORS. C. Robert Almli, David L. Hill*, Nathaniel T. McMullen, and Robin S. Fisher*. Dept. Psychol., Ohio Univ., Athens, Ohio 45701.

Newborn albino rats (males and females) sustained bilateral destruction of the lateral hypothalamic area (LHA) at one day of age (24 hours ± 12 hours of age, Day-1). Following brain damage, the pups were tested daily for sensorimotor behavioral capacity (e.g. olfactory orientation, vibrisseal placing, activity level), and at 2, 3, and 14 days of age, the pups were tested for suckling-attachment behavior using an anesthetized dam. Body weight was measured daily, and responses to dipsogenic challenges were measured at 50-60 days of age.

Pups sustaining bilateral LHA damage typically lost body weight for no more than one day postlesion. Thereafter, they maintained an attenuated growth curve such that the pups were 20-25% below control body weight at 60 days of age. The suckling-attachment tests revealed deficits in the pups' attachment to the nipples, and these suckling deficits may underlie the attenuated growth displayed by the LHA rats.

The sensorimotor testing and evaluation of clinical signs revealed "no deficits" in the ontogeny of behavioral capacity of the brain-damaged pups. The pups did not display any alteration in orientation to multimodal sensory stimulation, and they failed to display any alteration of motor reflexes or locomotor behaviors.

Regulatory testing revealed that the pups respond to feeding and drinking challenges with intakes that range within the "low normal" level.

Bilateral LHA destruction sustained by neonatal rats failed to produce many of the symptoms of the classical "Lateral Hypothalamic Syndrome". The pups did not cease suckling or become adipsic or aphagic, they did not display significant residual feeding and drinking deficits, and they did not display sensory neglect, motor, postural or activity level deficits. The effects of LHA destruction were dependent upon the age of the rat at the time of brain damage, and early brain damage does not result in deficits as the animal ages.

1483 PROTEIN PHOSPHORYLATION IN REGULATION OF NEUROTRANSMITTER RELEASE; THE BLOOD PLATELET AS A MODEL. W.F. Bennett*, G. Lynch, M.D. Browning*, Dept. of Psychobiol., Univ. Calif., Irvine, CA 92717.

Serotonin release by blood platelets is a process similar in many respects to neurosecretion by synaptic terminals in the brain. Protein phosphorylation has been correlated with aggregation and release in blood platelets just as it has been suggested to control neurosecretion. We have undertaken to characterize the proteins affected during stimulation for 5-HT release, and have found that a basic protein (pI=9.0) of 40K daltons becomes phosphorylated in a manner which correlates with the time course and extent of release. Additionally a 20K dalton protein (pI=4.5-5.0) shows similar effects, but to a lesser degree. We have observed phosphorylation in the absence of release, but not release without phosphorylation, and have concluded that 40K phosphorylation is necessary, although not sufficient in itself, for release. The 40K basic protein has tentatively been identified as troponin-T, and in view of the known role of troponin in regulation of calcium sensitivity in muscle contraction, we propose that the phosphorylation of troponin-T confers calcium sensitivity upon the process of serotonin secretion.

1484 VISUAL CAPACITIES OF ADULT CATS WHICH WERE REARED WITH A LESION IN THE RETINA OF ONE EYE AND THE OTHER OCCLUDED. M. Berkley, S.M. Sherman, D.S. Warmath*, J. Tunkl. Dept. Psychology, Florida State U., Tallahassee, FL 32306; Dept. Physiology, U. Virginia, Charlottesville, VA 22903.

Cats monocularly deprived of form vision during the first few months of life suffer severe visual defects in the deprived eye when tested as adults. Perimetry testing in such animals indicates that they respond only to targets presented in the monocular segment of the visual field of the deprived eye, a region shown by electrophysiological and histological studies of the lateral geniculate nucleus (LGN) and visual cortex to be less affected by monocular deprivation.

These findings led to the suggestion that during development there is competition between the two eyes for control of neurons in those portions of the visual system in which binocular vision is represented. This hypothesis was strengthened by histological and perimetry studies of kittens raised with a lesion in the retina of one eye and the other occluded. In the LGN of these cats, a small segment of normal cells was found corresponding to the locus of the retinal lesion (critical segment), and perimetry tests demonstrated that the cats responded to visual stimuli presented in this region of the visual field.

To determine the visual capacity of the critical segment, in 2 kittens the eyelids of one eye were sutured closed and a lesion made close to the area centralis (AC) of the retina of the other (nondeprived) eye. When tested monocularly with the nondeprived (retinal lesion) eye as adults, the cats performed normally on light vs. dark and 0 vs. + problems. Grating acuity was somewhat low (2.0 c/d) in one cat and normal in the other (4.5 c/d). Reconstruction of the retinal lesions from fundus photographs and whole mounts of the retinae showed that in both cases the lesions were to one side of the AC. In the animal with reduced acuity, the lesion was smaller and medial to the AC, probably interrupting fibers of passage.

Informal perimetry tests of the deprived eye confirmed the presence of a small region of vision in the area of the visual field corresponding to the locus of the lesion in the nondeprived eye. Acquisition of the form and acuity tasks with the deprived eye was extremely poor and similar to that seen in ordinary monocularly deprived (MD) cats. Unlike ordinary MD cats, the critical segment cats reached a high level of performance on the visual tasks on some test days and after binocular training were able to reach criterion performance with the deprived eye. These latter findings suggest that the cats are capable of seeing with a small critical segment but may not learn to utilize it unless they have been given prior binocular training on the discrimination task.

Supported by EY 00953, 01565 and NSF BNS 7513837, 7706785.

1485 FORELIMB MOVEMENTS AFTER DORSAL RHIZOTOMY WITHOUT VISUAL FEEDBACK DURING TRAINING OR TESTING. D.Berman, A.D.Blau*, J.E.Herskovic* and A.J.Berman. Dept. Neurosurg., V.A.Hosp., Bronx,N.Y. 10468 and Dept. Psychol., Queens College, Flushing, N.Y. 11367.

Previous studies indicate that bilaterally dorsal rhizotomized monkeys can touch targets without visual guidance. These animals however, were permitted vision of their rhizotomized limb position in relation to the target during training. Subsequent performance could therefore be explained on the basis of mechanisms established when visual feedback was available. In the present study, three monkeys were trained to use unilaterally dorsal rhizotomized (C2-T3) forelimbs to touch targets which they were not permitted to see at any time, before or after surgery.

Training and testing were carried out in darkness, utilizing a 3x3 display of touch activated buttons mounted in front of the chaired animal. Initially, the monkey was shaped to touch a sequence of two lit buttons. The first (start) button remained lit until touched, then was turned off and the second (Rf) button lit. Touching the Rf button resulted in delivery of a food pellet. Once these visually guided responses had been trained, the monkey was required to touch intervening (bridge) targets without visual guidance to turn on the Rf light. Bridge positions were visually cued by brief light pulses at an intensity and duration such that the human observer could not see the target or its surround. Repetition rate was slow (0.67/sec) so that monkeys did not keep their hands over the target to obtain visual feedback from subsequent flashes. If the monkey failed to touch the bridge within 15 sec the trial was terminated.

Preoperatively, monkeys reached criterion (bridge touched 9/10 trials without error) with randomly presented single or dual bridges within 7-30 sessions. When tested 14-21 days after surgery, performance with the intact limb was unaffected. Movements of the rhizotomized limb, however, had to be shaped. This was accomplished by covering the response panel, except for the start and Rf buttons, and reinforcing successive approximations to the required movements with the room light on. This required 10-12 sessions, after which testing was continued in darkness and bridges were introduced. Monkeys were successful in touching visually cued targets with their rhizotomized limbs. There was a longer latency, however, more errors and more variability from session to session with the rhizotomized than the intact limb.

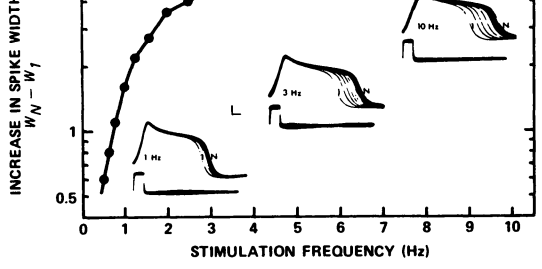
Several alternative explanations for these results are offered. Central representations of relevant movements may be established before surgery. Eye movements to the pulsed bridges may become paired with efferent commands after surgery. Transfer from the intact limb may have facilitated performance. Finally, ventral root afferents may mediate the behavior observed. (Supported by NIH Grant #2RO1-NS 12330-02A1 to AJB)

1486 INFLUENCE OF CATECHOLAMINES ON THE REGULATION BY PATTERNED PREGANGLIONIC NEURAL ACTIVITY OF TRANSMITTER STORES IN A SYMPATHETIC GANGLION. R.I. Birks (SPON: T.L. Sourkes). Dept. Physiol., McGill Univ., Montreal, Canada H3G 1Y6.

The presynaptic stores of acetylcholine in the cat superior cervical ganglion increase by 30-70% during prolonged (30-420 min) preganglionic stimulation with patterns of pulses whose mean frequencies are within physiological limits (0.2-3.0/s). The increases in stores occur when the pulses are grouped into brief (0.05-1.0s) trains at high frequency (10-60/s); but not when they are equally spaced in the patterns. When the mean frequency of the pattern is greater than 5/s the increase occurs only after termination of the stimulation (Birks, R.I., J. Physiol. In Press, 1978). It has now been found that these changes in transmitter stores can be reduced or prevented when patterned stimulation is carried out in the presence of circulating catecholamines at concentrations that are known (Buhler, H.U., Da Prada, M., Haefely, W. and Picotti, G.B. J. Physiol. 276, 311-320, 1978) to occur in animals under stress. The increase in stores was reduced or prevented by arterial infusion of nor-epinephrine or epinephrine at 70-250 ng/kg/min during patterned stimulation in animals that retained their ganglionic circulation intact. Similar depression of the response to patterned stimulation was obtained in ganglia perfused with choline-Locke when the solution contained nor-epinephrine or epinephrine at a concentration of 5 ng/ml. Since the increase in transmitter stores has also been shown to be reflected in a corresponding increase in transmitter output (Birks, R.I. J. Physiol. 271, 847-862, 1977), the present and previous results suggest that transmitter release at ganglionic synapses is subject to modulation both by the form of neural input from the CNS and by the level of hormonal output from the adrenal medulla, acting on the amount of transmitter stored at preganglionic nerve terminals. (Supported by the Muscular Dystrophy Association of Canada).

1487 LOW-FREQUENCY CALCIUM SPIKE BROADENING IN CULTURED MOUSE DORSAL ROOT GANGLION CELLS. Thomas H. Brown, A. Page Chiapella* & John H. Peacock. Department of Neurology, Stanford University Medical School, Stanford, CA 94305

Calcium action potentials recorded from the soma of cultured mouse dorsal root ganglion cells were found to increase in duration during a low-frequency stimulus train. The characteristics of this use-dependent plasticity were studied in 271 cells at 37°C in a defined low NaCl (4 mM) test medium (maintained at 330 mosmols and pH 7.2) which included $MgCl_2$ (2 mM) and either $CaCl_2$ (8 mM) or $BaCl_2$ (0.8 to 8 mM). Cells were impaled with two intracellular microelectrodes, one for passing current and the other for recording voltage. Ca spike broadening and its frequency dependence are illustrated below. Spike broadening in these cells is never accompanied by an increase in the maximum rate of spike rise (dV/dt) or in the spike amplitude; if anything, both decrease during a train. Tetraethylammonium (TEA, 5 to 50 mM), added to test solutions, causes Ca spikes to broaden but it eliminates use-dependent broadening. Barium spikes are also very broad and they sometimes show extreme (> 100-fold) use-dependent broadening. The amount of broadening during a train can be reduced or eliminated by interpolating hyperpolarizing current pulses between Ca spikes. A similar low-frequency Ca spike broadening has also been observed in certain identified molluscan neurons, where it has been hypothesized (Thompson & Getting, Neurosci. Abstr. 3, 189, 1977) to result from cumulative inactivation of a TEA sensitive outward K^+ current rather than augmentation of an inward Ca^{++} current. Our results are consistent with this potassium inactivation hypothesis. (Supported by NIH Grant NS12151).



1488 EVIDENCE FOR AN ORDERLY ARRANGEMENT OF OPTIC AXONS IN THE CENTRAL PATHWAYS OF VERTEBRATES AND ITS IMPLICATIONS FOR THE FORMATION AND REGENERATION OF OPTIC PROJECTIONS. S.M. Bunt*+ and T.J. Horder* (SPON: M. Jacobson) Dept. Anat., South Parks Rd., Oxford, G.B. (+Present address, Dept. Anat., Univ. of Utah, S.L.C., Utah 84132.)

The topographical arrangement of optic fibers within the optic nerve (ON), tracts and tectal bundles was investigated by following to the tectum, using light microscopy of 2μ toluidene blue stained plastic sections, or where necessary, electron microscopy, the degenerating fibers caused by a trans-scleral retinal lesion.

In the fish (Cichlasoma biocellatum), possessing a flattened, ribbon-like ON; the observations confirmed our previous work on the cylindrical ON of the goldfish. (J. Physiol. 171,10-12P (1977)). A dorsal retinal lesion resulted in a line of degeneration along the center of the ON which passed ventrally; a ventral lesion resulted in a strip down each side of the ON that passed dorsally as the degeneration was traced centrally. Thus,as the fibers reach the tectum they are arranged "tectotopically". In 5 day old chicks and the newts Triturus vulgaris, alpestris, and cristatus, the resultant degeneration could be followed as a discrete patch from the fundus to the tectal surface. In adult Xenopus and Rana a concise lesion produced degenerating myelin figures across the ON section. When HRP was applied to the retinal cut it was found that the majority of the tectal bound unmyelinated axons remained segregated in the ON while the few myelinated axons destined for diencephalic nuclei spread at random. When a retinal quadrant was removed from St. 42 Xenopus tadpoles and the ON, (with on av. 250 unmyelinated fibers), observed at St. 45; the resultant degeneration was still limited to 1/2 to 1/3 of the ON cross section. This suggests that the order of the adult ON is not the result of random growth followed by selective death.

These results, together with older reports on the rat, cat, rabbit, oppossum, monkeys and man, suggest that optic fibers reach their termination sites in a topographically ordered array with little intermingling of fibers from different retinal areas at any point along their route. Without evidence for active resorting of fibers, the observed order could be produced during development by new fibers growing alongside the older fibers using only "passive" mechanisms such as contact guidance and fasciculation. The ordered termination pattern on the central nuclei could result from, in development, fibers terminating on the first available tectal cell they encounter and, in regeneration, by the cells they encounter being limited by their position within the fiber array as it crosses the tectal surface. Where the tectum influences the formation of the optic projection, the contribution of the fiber organization to the formation of the nearest neighbor order of the terminations makes it unnecessary to postulate labelling of individual tectal cells. (M.R.C.,G.B., Wellcome trust and NSF #BNS 77-20219).

1489 THE EFFECT OF ALTERED LIMB MOBILITY ON GROUP Ia EPSPs IN DEFINED TYPES OF CAT MEDIAL GASTROCNEMIUS MOTOR UNITS. R. E. Burke, R. F. Mayer, K. Kanda*, B. Walmsley* and J. A. Hodgson*. Lab. of Neural Control, NINCDS, NIH and Dept. Neurol., Univ. of Maryland.

As part of a study of the effect of chronic immobilization on medial gastrocnemius (MG) motor units, we examined the peak amplitude of composite homonymous (MG) and heteronymous (lateral gastrocnemius - soleus, or LGS) group Ia EPSPs in motoneurons of defined motor unit type (Burke et al., J. Physiol. 234,723,1973). The left hindlimb was immobilized in 10 cats by inserting stainless steel pins through bone, spanning knee and ankle joints, under aseptic conditions. After short (3 and 5 weeks in 2 cats) and long (17 - 29 weeks in 8 cats) survival periods, Ia EPSPs and motor unit properties were examined under pentobarbital anesthesia using methods of muscle nerve stimulation and intracellular recording with hyperpolarization block of antidromic spikes as in a previous study of Ia EPSPs in normal cats (Burke et al., J. Neurophysiol. 39,447,1976). Table 1 shows the comparison between Ia EPSPs in normal and immobilized animals.

TABLE 1

Unit Type	EPSP Source	Normal (N=10)	Short Term (N=2)	Long Term (N=8)
FF	MG	4.1 ± 1.5 (58)	2.8 ± 1.3 (14)*	3.0 ± 1.2 (59)*
	LGS	1.2 ± 0.6 (58)	0.6 ± 0.1 (15)*	0.7 ± 0.3 (52)*
FR	MG	7.5 ± 1.9 (23)	4.2 ± 2.4 (7)*	4.4 ± 1.6 (12)*
	LGS	2.0 ± 0.9 (21)	1.1 ± 0.3 (7)	1.1 ± 0.3 (11)
S	MG	9.1 ± 1.8 (27)	6.2 ± 1.5 (3)	5.9 ± 1.5 (24)*
	LGS	2.8 ± 1.1 (26)	1.1 ± 0.5 (4)*	1.6 ± 0.6 (20)*

* = control and experimental means differ with $p < 0.001$

The data in Table 1 show that both homonymous and heteronymous group Ia EPSPs are, on the average, between 25% and 45% smaller in MG motoneurons after chronic hindlimb immobilization in all motor unit types when compared with EPSPs in normal cats. Group Ia EPSPs in MG motor units of cats with paraplegia (complete cord transection at Th12) or monoparesis (cord hemisection at L1) of comparable duration were not different in amplitude from normal. Immobilized ankle extensors were not "disused", since chronic EMG recordings showed appropriate activation patterns during stepping. The many factors that can influence EPSP amplitude were not systematically studied but we assume that immobilization affects (probably decreases) Ia spindle afferent firing and that this causes a net decrease in group Ia synaptic efficacy.

1490 INCREASED DENDRITIC BRANCHING IN HEMISPHERES OPPOSITE EYES EXPOSED TO MAZE TRAINING IN SPLIT-BRAIN RATS. Fen-Lei F. Chang and William T. Greenough. Dept. Psych. and Neural and Behavioral Biology Prog., Univ. IL at Urbana-Champaign, IL 61820

The amount of branching of dendrites is affected by the rearing environment. Preliminary studies also indicated small but consistent differences in dendritic branching between extensively maze-trained adult rats and handled rats. These differences appeared in the upper region of the apical dendrite of layer 5 pyramidal cells of occipital cortex.

To study general stimulation versus specific aspects of the maze training experience, we used a split-brain preparation and a contact eye occluder system to direct visual aspects of the training experience to a single hemisphere. Six groups of 60-70 day old male littermate triplet Long-Evans hooded rats were used (18 animals). The full extent of the corpus callosum was sectioned in a way which did not damage cerebral hemispheres, subcortical structures, or the midsaggital sinus. Two littermates of each set received 25 days training, 25 trials per day, on a series of Hebb-Williams maze problems for water reward. Each animal had an opaque contact lens inserted in one eye during the 2-3 hr. training period. For group T-T, the lens was alternated from one eye to the other on successive days, such that both hemispheres were opposite "trained" eyes. For group T-N, the lens was always placed in the same eye, directing visual training experience to one (T) hemisphere. Group N-N animals had either alternated or unilateral lens placement and were merely handled and given water several times each day.

Ten to 20 Golgi Cox stained layer 5 pyramidal neurons from each hemisphere of each rat were traced and analyzed with computer-aided microscope system by experimenters unaware of individual tratments. Intersections between concentric 20 μm spheres and the 3-dimensional dendritic field were counted. Neurons in hemispheres opposite eyes exposed to the training experience (both T-T group hemispheres and hemisphere T from the T-N group) had significantly more intersections, reflecting dendritic length, in regions of the apical dendrite beyond 200 μm from the cell body than those in hemispheres opposite the occluded eye in group T-N or opposite either eye in group N-N. Of 12 possible within-litter comparisons (T-T vs N-N; T-N (T) vs T-N (N)), 10 favored the T hemispheres or animals. The results indicate that visually-mediated aspects of maze training experiences may alter dendritic branching patterns in young adult rats. Supported by NSF BMS 75-08956 and BNS 77-23660 and PHS RR 07030.

1491 GLUCOCORTICOIDS DECREASE REACTIVE SYNAPTOGENESIS IN THE RAT DENTATE GYRUS. Carl W. Cotman, Stephen W. Scheff and Larry S. Benardo*. Dept. Psychobiology, Univ. Calif., Irvine, CA 92717.

We have investigated whether or not glucocorticoids play a role in modifying reactive synaptogenesis in the hippocampus following lesions of the entorhinal cortex. Corticosteroids are known to control and prevent cerebral edema and are sometimes used clinically following head injury. In previous studies we have shown that the hippocampus displays robust axonal growth and new synapse formation following partial denervation. The hippocampus has also been shown to have specific receptors for cortisol, one of the drugs utilized to control edema. Consequently this area of the brain is well-suited for testing the effects of glucocorticoids on the sprouting reaction. We have examined the response of various hippocampal afferents following a unilateral entorhinal lesion in control animals and those treated with cortisol.

Animals were given daily injections of cortisol six day preoperatively and six or fifteen days post-operatively. Control animals were given cortisol and no lesion and still others were injected with the vehicle only and lesioned. The brains were examined for changes in septal input by means of AChE staining and for changes in the commissural-associational (CA) fiber plexus by means of the Holmes fiber stain. Animals treated with cortisol and lesioned consistently showed a marked decrease in the spread of the CA fiber plexus and in the rate at which the intensification of AChE staining occurs. Additionally the brains were stained for astroglia. In both lesioned and non-lesioned animals treated with cortisol there was a marked hypertrophy of astrocytes, a response which was not present in animals treated with only the vehicle. Thus the administration of glucocorticoids significantly limits reactive synaptogenesis in the hippocampus possibly through an inhibitory action of hypertrophied glial cells.

These findings raise the question of whether corticosteroids administered to treat edema decrease axon sprouting. Moreover, these findings may account in part for the reduction in axon sprouting observed in aged animals. Previously it has been reported that aged animals exhibit both high corticosteroid levels and hypertrophy of astrocytes. Thus altered hormonal levels may be one of the factors responsible for reduced axon sprouting following cell loss in aged animals. (Supported by research grants AG 00538 and NS 08597)

1492 EXPANSION OF RAT CEREBROVASCULAR ANASTOMOSES. Peter Coyle and Pamela G. Brubaker*. Dept. Anat., Univ. Mich. Med. Sch., Ann Arbor, MI 48109.

Anastomoses exist between branches of the three major cerebral arteries in humans and rats. Objectives were to determine if the anastomotic branches could adequately nourish the vascular field of the middle cerebral artery following its interruption near the proximal end and to note chronic size and spatial pattern changes, if any, of the anastomoses.

Wistar rats of each sex ranging from 23-53 days of age were anesthetized with Ketalar 125-200 mg/Kg body weight. Skin over the right zygoma was shaved and a nearly 0.5 cm vertical incision was made extending from the zygomal vibrissa dorsalwards for removal of most of the temporalis muscle and to allow a 2-2.5 mm horizontal by 0.5-1.0 mm vertical trephine hole to be drilled in the squamosal bone extending from the zygomatic point rostralwards. Under stereozoom microscope observation the dura mater was reflected and the major ramus of the middle cerebral artery was transected superior to the rhinal fissure. Bleeding was not a problem. The removed muscle was replaced, the wound sutured with silk, 0.5 ml of 2% Evans Blue made with physiologic saline was injected subcutaneously, and the animal returned to its cage. Of the reported 16 animals, 8 underwent the above procedures, 4 were operated to the point of cutting the dura mater and 4 had minor branches of the middle cerebral vessel transected. All received Evans Blue and 15 were injected with vultex, a latex rubber to demonstrate the cerebral arterial tree at time of sacrifice 5-9,14,19,20 & 27 days later. Of the 12 brains having surgery all showed Evans Blue at the transection site and one brain had ipsilateral leakage concentrated at the occiput. The data suggest an adequate blood supply is usually provided through anastomotic vessels. Only rats with a transected vessel had expanded anastomoses between branches of the ipsilateral anterior, posterior and middle cerebral arteries. No anastomotic changes were observed in the contralateral hemisphere nor in controls. The dorsal expansion was present at 5 days and lateral anastomoses between branches of the supra- and infra-rhinal fissure portions of the middle vessel were evident. The expansion was characterized by greatly increased vessel diameters and a seemingly increased vessel tortuosity. Possibly the number of anastomoses increased. Project received support from the Michigan Heart Association and a Univ. of Mich. Biomedical Research Grant.

1493 CHANGES IN THE ORGANIZATION OF THE LATERAL POSTERIOR NUCLEUS OF THE GOLDEN HAMSTER AFTER NEONATAL SUPERIOR COLLICULUS LESIONS. Barbara J. Crain and William C. Hall, Department of Anatomy, Duke University Medical Center, Durham, N.C. 27710.

The rostrolateral subdivision of the lateral posterior nucleus (LP) in the golden hamster is normally characterized by synaptic clusters in which medium-sized axon terminals synapse around and along a central dendrite. The vast majority of these terminals are contributed by axons from the ipsilateral superior colliculus, although within restricted regions of the subdivision some are contributed by the contralateral superior colliculus and the contralateral retina. In contrast, axons from the ipsilateral posterior neocortex form very large terminals which make numerous synaptic contacts with complex dendritic appendages of a single dendrite. These terminals occasionally extend into the synaptic clusters but they are easily distinguished there from the medium-sized terminals. All four afferent pathways also contribute small terminals to the neuropil outside the synaptic clusters.

When a unilateral lesion of the superior colliculus is made on the day of birth, the ipsilateral LP develops in the absence of the major input to its synaptic clusters. Under these experimental conditions, the retina (Schneider, Brain Behav. Evol. 3:295, 1970) and the remaining colliculus contribute many more terminals to LP than usual. By removing the contralateral colliculus and injecting the contralateral eye with ^{3}H-leucine and then processing adjacent sections for anterograde degeneration and autoradiography, we showed that these two projections to LP expand to share a common border but do not overlap. Typical synaptic clusters are still present in the rostrolateral subdivision of LP under these conditions. Degeneration studies indicate that most of the medium-sized axon terminals in the clusters are now contributed by either the contralateral superior colliculus or by the retina.

These results suggest that the contralateral superior colliculus and retina normally compete with the ipsilateral superior colliculus for postsynaptic sites within the synaptic clusters of LP. In the absence of the ipsilateral tectal pathway, these two inputs form more synaptic terminals but continue to compete with each other for postsynaptic space. Supported by NINCDS Grant #NS-09623 to W. C. Hall.

1494 NORMAL AND ABNORMAL CALLOSAL CONNECTIONS IN THE OCCIPITAL CORTEX OF ALBINO RATS. C. G. Cusick* (SPON: R. D. Lund). Dept. Biol. Struct., Univ. Wash. Sch. Med., Seattle, WA 98195.

The total pattern of callosal projection was studied using tangentially cut sections of flattened hemispheres stained with a bleached Fink-Heimer method and viewed with dark field illumination. Area 17 is outlined by callosal degeneration except anteriorly. The 17/18a border splits anteriorly to form a ring-like pattern of degeneration, a prominent landmark in pigmented as well as albino rats. It is located posterodorsal to the PMBSF which is identified in this material by a callosal projection to the barrel septae. A band of degeneration extends laterally from the ring along the anterior border of 18a and probably marks the position of the region "D" vertical meridian representation of Montero. Area 18b, lying medial to area 17, receives a homotopic callosal input and a previously undescribed heterotopic projection. The region receiving the heterotopic projection is rostromedial to area 17 in the approximate position designated as region "E."

The stability of the pattern of callosal termination was tested in a size disparity experiment. Within 24 hours of birth, rats from several litters received large lateral cortical ablations or slit lesions to block callosal connections to and from the more lateral cortex of one hemisphere. The pattern of callosal connections was tested with Fink-Heimer and autoradiography 5-16 weeks later. In animals in which the 17/18a border remained intact, callosal fibers originating from regions lateral to this border tended not to invade area 17 or the 17/18a border. In animals in which the 17/18a border was removed at birth, callosal axons are found to end on the damaged side in patches situated rostromedially in areas 18b and 17. Double mapping experiments show that the projection to area 17 comes predominantly from around the 17/18a border on the intact side while 18b projections originate more laterally. Reorganization of the total callosal projection was studied in tangential sections after callosal section. In animals in which a large amount of cortex remains, the 17/18a border with its anterior "ring" can be seen and the acallosal area 17 is approximately its normal size. When a small amount of cortex remains, the acallosal region is reduced and the normal terminal pattern is obscured. The 17/18a callosal projection is broader after removal of the ipsilateral thalamus. These results suggest that the reorganization of the callosal projection in the visual area relates to both the boundaries of visual maps and the integrity of the geniculocortical system. (Supported by USPHS Grant GM-07108 from the National Institutes of Health.)

1495 INDEPENDENCE FROM ENVIRONMENTAL INFLUENCE OF THE DEVELOPMENT OF THE CIRCADIAN PACEMAKER OF MICE. Fred C. Davis* and Michael Menaker. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

The circadian rhythm of running-wheel locomotor activity was measured in *Mus musculus* (C57BL) raised on light cycles with periods (T) different from 24 hours. Parental mice were placed in L:D 11.67:8.33(T=20) or L:D 16.33:11.67(T=28). These cycles have the same ratio of light to dark as a standard cycle of L:D 14:10. The locomotor activity rhythms of all mothers were observed to be entrained prior to mating and were continuously monitored until 3 weeks post weaning. The offspring were weaned at 3 weeks and group housed (by litter) until 7 weeks of age. 15 males and 5 females from each group were then placed in individual running-wheel cages where they remained on T=20 and T=28 for 3 weeks. All of the animals but one were entrained, and at 10 weeks of age were placed into constant darkness (DD). An initial difference in the average free-running periods (τ) of the two groups was measured, but after 50 days (in DD) had disappeared. Similar temporary effects of L:D cycles of T≠24 hours have been shown with adult *Mus musculus* (Pittendrigh and Daan. J. Comp. Physiol. 106: 223, 1976). We have shown that such cycles have no greater effect on the circadian pacemaker of developing animals than they do on adults. Despite long-term entrainment, the period of the circadian pacemaker of mice develops uninfluenced by light/dark and maternal cycles with periods very different from 24 hours. Supported by NIH grant HD-03803.

1496 HETEROSYNAPTIC CONTROL OVER SYNAPTIC MODIFICATION IN THE DENTATE GYRUS. Robert M. Douglas (SPON: G. V. Goddard). Dalhousie Univ., Halifax, N. S. CANADA B3H 4J1

Very long-lasting increases in synaptic efficacy of the perforant path - granule cell synapses (enhancement) can be reliably produced by brief periods of high-frequency activation of the perforant path fibers. The perforant perforant path axons make excitatory synapses in the outer two thirds of the molecular layer. A commissural projection terminates in the inner third of the molecular layer. The predominate effect of stimulation of this input is inhibition of perforant-path-evoked granule cell discharge.

High-frequency trains (eg. 8 pulses at 400 Hz) applied to the commissure had little or no long-term effect on the size of either the commissural or perforant-path-evoked responses. However, when the commissure trains were combined with perforant path trains, enhancement of the perforant path response was greatly reduced. In some cases enhancement was completely blocked. Short-term changes in the perforant path response were largely unaffected. The timing of the onsets of the two trains was critical. The reduction in enhancement was obtained if the start of the commissure train preceded the start of the perforant path train by as little as 2-3 msec, whereas perforant path trains that preceded the commissure trains by the same amount produced almost as much enhancement as normal.

High-frequency activity on the commissure was essential. When the perforant path train was preceded by a single commissure pulse, enhancement was unaffected. Since the single pulse produced considerable inhibition of the perforant-path-evoked population spike, it suggests that the action of the commissure on perforant path plasticity was not mediated via manipulation of post-synaptic discharge.

1497 2-DEOXY-GLUCOSE STUDIES OF MOUSE AND RAT SmI BARREL CORTEX FOLLOWING EARLY LESIONS OF THE VIBRISSAE. D. Durham*, C. Welt and T.A. Woolsey. Dept. Anat. and Neurobiol., Washington U. Sch. Med., St. Louis, MO 63110 and Res. Dept. Central Wisconsin Center for the Developmentally Disabled, Madison, WI 53704.

Early lesions to the vibrissae have been shown to alter the normal arrangement of both barrels and the specific thalamocortical afferents in the mouse and the rat SmI cortex. It is of interest to know what functional changes, if any, accompany the morphological ones. These changes have been assessed in the rat by electrophysiological methods (Welt, 1977). Another way to determine the functional properties of the cortex in these animals is to use radioactive 2-deoxy-glucose (^{14}C-2-DG), which is a marker for functionally active brain regions. In this study, various combinations of vibrissae were lesioned in newborn rats and in mice of various postnatal ages. Thirty to sixty days after birth, the animals were examined with the 2-DG technique (Durham and Woolsey, 1978). To stimulate selectively particular groups of cortical cells, various combinations of vibrissae were left intact and the remainder clipped prior to injection of the 2-DG. For example, animals in which Row-C vibrissae were lesioned were divided into two groups: i) all whiskers clipped except those that had regrown in Row-C, and ii) all whiskers clipped except those in the adjacent Rows B and D. The former were used to test whether the regrown vibrissae were capable of driving cortical cells and the latter to determine whether the adjacent vibrissae could activate neurons in the cytoarchitectonically altered regions. All tissue was processed for film autoradiography and subsequently stained with thionin as described previously. In addition, similarly lesioned littermates were examined using the SDH histochemical method to demonstrate thalamocortical afferents.

We found that: 1) The patterns of thalamocortical terminals as seen with SDH match the cytoarchitectonics seen in the Nissl stained material; 2) In cases where the cortical anatomy was most severely disrupted, there was no evidence that regrown whiskers could drive cortical cells. If only the whiskers in adjoining rows were left intact, they activated only the appropriate barrels. There was no spread of activity into the altered zones; 3) In cases where the cortical alterations were less severe there was evidence that regrown whiskers activated the cells in the altered zone. The whiskers in the adjacent rows did not drive the barrels associated with the damaged whiskers, and 4) the results were similar for mouse and rat. The extent of cortical damage produced by vibrissal lesions in newborn rats varied considerably. This presumably is related to differences in gestational age of the animals at birth.

Supported by an NSF Graduate Fellowship (D.D.), NIH NS10244 and EY01255 (T.A.W.).

1498 ASYMMETRICAL DENDRITIC DEVELOPMENT OF NEURONS IN THE ACCESSORY SUPERIOR OLIVARY NUCLEI OF ALBINO RATS RAISED UNDER MONAURAL "DEPRIVATION".* Albert S. Feng. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

Neurons in the mammalian accessory superior olivary nucleus receive inputs from the ventral cochlear nuclei at the two sides of the brain. Their dendrites extend primarily laterally and medially. Those dendritic branches oriented medially receive input from the contralateral cochlear nucleus and those oriented laterally from the ipsilateral cochlear nucleus (Stotler, 1953; Liu and Liu, 1971). These neurons serve as an excellent model system in the present investigation for studying the plasticity of dendritic development. Monaural "deprivation" during the early stage of development of the albino rats resulted in asymmetrical dendritic development, i.e., the dendritic branches that normally receive input from the "deprived" ear were poorly developed in comparison to branches that receive input from the intact ear. This pattern is reflected in the accessory superior olivary neurons at both hemispheres, i.e., the lateral dendritic branches of neurons ipsilateral to the deprived ear and the medial dendritic branches of neurons contralateral to the deprived ear developed relatively poorly. Ontogenetical developmental patterns of these neurons were also examined and were found to follow a certain time course. These results demonstrate the environmental effect on the development of dendritic processes.

[Supported by N.I.H. grant no. NS 14488]

*This study was partially done in Dr. T.H. Bullock's laboratory at the University of California, San Diego and supported by N.I.H. and N.S.F. grants to T.H. Bullock.

1499 CEREBELLAR PLASTICITY: MODIFICATION OF DENDRITIC BRANCHING BY DIFFERENTIAL REARING IN MONKEYS. M. K. Floeter*, W. T. Greenough. Dept. Psych. and Neural and Behavioral Biology Prog., Univ. IL., Champaign, IL 61820 and G. P. Sackett* . Dept. Psych and Regional Primate Res. Ctr., Univ. Washington, Seattle, WA 98195.

Eight newborn monkeys, Macaca fascicularis, were reared in one of three environments for the first six months after birth. Isolation reared monkeys (I) were reared in small cages, providing limited environmental stimulation and no social contact. Socially reared monkeys (S) were kept individually in small, adjacent cages and allowed to play in pairs for four hours each day. Semi-naturalistically reared monkeys (N) were raised in large colony rooms with several other monkeys and with fixed structures and objects for play. Monkeys were killed at eight months and the uvulae were stained with a nissl stain. Measurements were made of the diameter of 100-200 Purkinje cell bodies for each animal. N monkeys averaged significantly larger soma diameters than S and I monkeys. The paraflocculi were stained by the Golgi-Cox method and computer tracings were made of portions of the Purkinje cell spiny branchlet dendritic tree, twenty for each animal. A Sholl analysis in which intersections between dendrites and a series of concentric spheres were recorded, showed that N monkeys had significantly more intersections, per spiny branchlet unit, indicating more dendritic material, than S monkeys. Computer tracings of granule cell dendrites from the paraflocculus, thirty per animal, showed no group differences with a Sholl analysis. The results indicate that experience can selectively modify the morphology of certain cerebellar cell populations in monkeys. Studies are presently underway to determine the effects of experience on other cerebellar areas.

Supported by Grants MH 28529, NSF BMS 08596, and PHS RR 07030.

1500 ALTERED PATTERN OF ASSOCIATIONAL HIPPOCAMPO-DENTATE CONNECTIVITY FOLLOWING LESIONS IN THE NEONATAL RAT. Russell A. Fricke, Dept. Biol. Sciences, Stanford University, Stanford, CA 94305.

Extrinsic afferents to the molecular layer of the rat dentate gyrus have the ability to expand into neighboring deafferented areas. For example, extensive lesions of the entorhinal cortex result in the spread of hippocampal afferents into the adjacent entorhinal terminal fields of the molecular layer (Lynch et al., Br. Res., 50:174, 1973). Similarly, when both the commissural and associational components of the hippocampo-dentate (H→D) projection are eliminated, neighboring entorhinal afferents appear to fill in the vacated hippocampal synaptic zone (Zimmer, Br. Res., 72:137, 1974). If, on the other hand, only the commissural afferents are disconnected, no such entorhinal expansion occurs. This is presumably due to the selective growth of associational afferents; however, this idea is difficult to test, since commissural and associational H→D afferents are co-extensive in the molecular layer of the dentate gyrus.

To address this exclusion hypothesis directly the hippocampal commissures and rostral hippocampus of one side were ablated in rat pups before the third postnatal day. Three to six months after surgery the same animals were stereotaxically injected with ^{3}H-proline (0.1-0.5μCi) at sites within the intact hippocampus. Injections into subfields CA_{3C} and CA_4 located approximately halfway between the septal and temporal poles of the hippocampus, always give rise to a band of silver grains in the proximal 3rd of the molecular layer of the dentate gyrus. In normal animals, the number of grains appearing over the external blade of the ipsilateral dentate exceeds the number found over the internal blade on the same side by a margin of at least 2-3:1 (Gottlieb & Cowan, Br. Res., 41:452, 1972). Early surgical elimination of commissural H→D afferents results in the disappearance of this particular quantitative asymmetry. Since the commissural projection has approximately the opposite quantitative pattern as the associational in normal animals, these results suggest that: (i) the total number of potential synaptic sites in both blades of the dentate gyrus is approximately equal; (ii) associational fibers occupy postsynaptic space vacated by the commissural afferents; and (iii) the amount of expansion is determined by the amount of degeneration.

It appears that following lesions which eliminate commissural hippocampal afferents, non-hippocampal classes of afferents are excluded from the hippocampal zone in the dentate gyrus by preferential growth of the intact associational hippocampal fibers. It remains uncertain, however, whether this is merely due to a spatial advantage or involves some residual, perhaps cytochemical, features which serve to distinguish the different extrinsic classes of afferents from each other during development.

1501 AGE-DEPENDENT REACTIONS OF OLFACTORY CORTEX TO DEAFFERENTATION. B. Friedman and J.L. Price, Dept. Anat. & Neurobiol., Wash. Univ. Sch. of Med., St. Louis, MO 63110.

The reaction of piriform cortex to deafferentation has been studied in rats using the Timm stain (Haug, 1973) and the autoradiographic method (Cowan et al., 1972) following ablation of the olfactory bulb in developing and mature rats. The Timm method stains heavy metals in axons, and in normal animals it reveals a striking bilaminar pattern in layer I of piriform cortex. The superficial lamina (Ia) is very pale while the deep lamina (Ib) shows a dark rust colored reaction. In addition, layer Ib and to a lesser extent Ia contain a granular reaction which is most prominent at the outer edge of Ib. The pale and colored laminae correspond precisely to the layers of termination of axons from the olfactory bulb (in Ia) and of the association fibers from the olfactory cortex (in Ib) (Price, 1973), but the granular reaction does not correspond to any known extrinsic fiber system. Some indication of a bilaminar Timm reaction in layer I is seen by the first day after birth (P-1) but the fully mature pattern does not develop until 17-18 days of age.

Following ablation of the olfactory bulb on P-1 (2-12 weeks survival) layer I develops to its normal thickness but the granular and rust colored reactions are found throughout this layer. This corresponds to an extension of the association fibers into the superficial part of layer I, demonstrated in parallel autoradiographic experiments.

In contrast, following ablation in adult rats (2-8 months survival) layer I is reduced in width by about 25%, probably due to dendritic degeneration. The normal color reaction in Ib is preserved but there is a profusion of the granular reaction in Ia which becomes much more intense with longer survivals. The association system, demonstrated autoradiographically, is largely confined to Ib, in correspondence to the color reaction.

Following ablations at ages 4-14 days (2 month survival) there is a reduction in the width of layer I (20%) which is almost comparable to that seen after ablations made in adult animals. However, the staining pattern is similar to that after P-1 ablations; the density of the stain is fairly homogeneous throughout layer I although the rust colored reaction tends to be concentrated deeply and the granular reaction superficially. Autoradiographic experiments show that the association fibers extend throughout layer I but are concentrated in its deeper part. Supported by NIH Grants NS09518 and NS07057.

1502 ROLE OF EARLY EXPERIENCES IN BEHAVIORAL ASYMMETRY IN THE RAT. J. Garbanati*, G. Sherman*, G. Rosen*, D. Yutzey*, and V. H. Denenberg. Depts. Biobehav. Sci. & Psychology, Univ. of Conn. Storrs, CT. 06268.

The human right hemisphere has been implicated as having a major role for affective processing. This phenomenon is not restricted to humans since rats which are handled in infancy and raised in complex environments exhibit differential involvement of the cerebral hemispheres in emotionality as measured by activity in the open-field test (Denenberg et al., Neurosci. Abs., 1977). A right cortical ablation produced a large decrement in activity in contrast to scores of left lesion and control animals.

Another major function of the right cerebrum is to monitor the spatial environment. Since the open-field test also involves locomotion through space, this test was used to assess the effects of early experience to induce a spatial bias. Spatial asymmetry was measured by observing the direction (left-right) of initial movements of rats in the open field. As in the previous study, we attempted to induce brain asymmetry by variation of early experience. This consisted of handling or not handling litters of pups during the first 20 days of life. At 105 days, 4 male littermates were given: a) a right neocortical ablation, b) a left ablation, c) a sham operation or d) no surgery. After a 30-day recovery period, all animals were tested in the open field for activity and wall-hugging behavior. One directionality score was computed for each animal using the responses which were recorded from 4 successive days of testing. The means of these scores for each group are presented in Table 1. Unilateral lesions resulted in the expected bias of movement along the wall ipsilateral to the side of lesion. Nonhandled sham and control rats showed no behavioral asymmetry. However, handled sham and control rats exhibited a behavioral bias in the left direction. These results provide evidence consistent with the hypothesis that early experience induces a spatial bias as well as the central asymmetry of affective processing found by Denenberg et al. (1977). These findings may reflect: a) the simultaneous inducement of two independent functions of affective and spatial processing, or b) the manifestation of a spatial bias which is due to a central asymmetry evoked during an emotional task.

Table 1. Group means for directionality scores[a]

	Right	Left	Sham	Control
Nonhandled	.509(34)[b]	-1.028(31)	.113(33)	-.047(30)
Handled	.329(31)	- .739(27)	-.530(24)	-.285(34)

[a]Directionality scores for individual animals were derived by the following formula (D.S. = R-L/ R+L); a negative score indicates a left bias, and a positive score indicates a right bias.

[b]( ) = n.

1503 ANOMALOUS CROSSED CORTICO-CAUDATE PROJECTIONS IN THE POSTNATAL MONKEY INDUCED BY PRENATAL RESECTION OF FRONTAL NEOCORTEX. Patricia S. Goldman, NIMH, Bethesda, Md. 20014

Primates, including man, exhibit remarkable sparing of behavioral performance following circumscribed brain injury that occurs early in life. It is not known, however, to what degree primates exhibit a capacity to respond to such injury by reorganization of axonal connections. To examine this issue, the prospective dorsolateral prefrontal cortex was resected in one hemisphere of a fetal rhesus monkey 6 weeks prior to birth. Following surgery, the fetus was returned to the uterus and subsequently delivered near term. On the 5th postnatal day, the homotopic region of the dorsolateral cortex contralateral to the cortex that was resected prenatally was injected with microquantities of ^{3}H-leucine and ^{3}H-proline. The monkey was sacrificed one week later and its brain processed for autoradiography. Autoradiograms from normal 5-day old monkeys that had been injected with the same quantities of tritated amino acids into the same cortical location were used to determine the normal projections from prefrontal cortex in neonates.

In the postnatal monkey whose prefrontal cortex was resected before birth, the projection to the ipsilateral caudate nucleus is similar in topography and configuration to that observed in normal monkeys of the same age. However, the projection to the contralateral caudate nucleus differs markedly from the controls. Whereas in normal monkeys, the contralateral nucleus contains grains which only barely exceed background level, in the prenatally operated case, dense concentrations of label can be traced in consecutive serial sections throughout the entire extent of the head of the nucleus. Significantly, the projection is greatly expanded in that portion of the nucleus that would normally receive ipsilateral projections from the prefrontal area that was removed. Further, the pattern of grain distribution is reminiscent of the intricate patches found normally on the ipsilateral side, indicating further that the anomalous projections have a predilection for deafferented areas.

The axonal rearrangement reported here is comparable to that described so far in other species. It is probably significant that a considerable degree of plasticity can be induced in the primate by a lesion performed prior to birth when major developmental events such as neuron genesis, cell migrations and elaboration of basic connections are occurring at a rapid rate. Mechanisms proposed for other systems may account for neuronal reorganization in the primate, among them, that cortico-striate projections expand their terminal fields to occupy vacated synaptic spaces made available by loss of input from the ipsilateral cortex.

1504 DIFFERENTIAL RESPONSE OF MOTOR AND SENSORY FIBERS TO LIGATION OF CAT HINDLIMB PERIPHERAL NERVES. T.Gordon, J.A.Hoffer and R.B.Stein. Physiol. Dept., Univ. of Alberta, Edmonton T6G 2H7, Canada.

Last year we reported that in cats with implanted neural recording electrodes, neural signals generated during locomotion declined after nerve section and resuture, but recovered following reinnervation (Hoffer et al, Neurosci.Abstr.3:863,1977). Motor signals recovered to near-control levels, while sensory signals did not. One reason could be that sensory fibers atrophied more severely than motor fibers following section. Such a differential effect could not be ascertained from our chronic recordings of evoked compound action potentials from whole nerves. We therefore have developed a method based on measurements of compound action potentials recorded at spinal roots when individual peripheral nerves are stimulated electrically, that has allowed us to measure changes separately in sensory and motor fiber populations.

Under sterile conditions we sectioned and ligated the lateral gastrocnemius-soleus and tibial nerve branches in one hindlimb and the medial gastrocnemius, common peroneal, and sural branches in the other hindlimb of cats. In acute experiments 45 or 150 days later we exposed the ligated nerves and contralateral controls, and also the spinal roots L6, L7 and S1 bilaterally. As each nerve was stimulated we measured amplitude and area of compound action potentials evoked at corresponding cut dorsal and ventral roots. We also measured the electrical impedance of each spinal root, to control for variations in compound action potential amplitude due to filament size. From these parameters we calculated the total electrical charge delivered by fibers mapping from each nerve onto each root, and added separately the contributions of the dorsal root and of the ventral root subpopulations. The ratio of these quantities, when compared with the ratio for each intact contralateral nerve, gave a measure of the relative shrinkage of each fiber subpopulation due to previous nerve ligation.

Root ratios were not significantly different from control values 45 days after ligation, but dorsal root fiber contributions were significantly more reduced than ventral root fiber contributions 150 days after ligation.

Our results indicate that sensory fibers are more affected by interruption of their peripheral processes than motor fibers. The reason for this differential effect is obscure. It could be due to the greater dependence of sensory fibers on trophic factors from the periphery, or to the electrical silencing of sensory, but not motor fibers, following ligation.
(Supported by MDA and MRC of Canada).

1505 SUB-SYNAPTIC PLATE PERFORATIONS: A NEW FORM OF SYNAPTIC PLASTICITY? William T. Greenough, Roger W. West, Timothy J. DeVoogd, and Janice M. Juraska. Dept. Psych. and Neural and Behavioral Biology Prog., Univ. IL at Urbana-Champaign, IL 61820 and Dept. Psych., Memorial Univ., St. Johns, Newfoundland, Canada.

Several forms of potential plasticity at the synaptic level have been described following exposure to differential rearing conditions. In studies of differential postnatal and postweaning experience upon synapses, we have examined interruptions in the darkly staining region underlying the post-synaptic membrane. Peters and Kaiserman-Abramof (Z. Zellforsch., 1969, 100: 487) demonstrated through serial sectioning that these interruptions represented perforations in a sub-synaptic plate. We have found that the relative frequency of these sub-synaptic plate perforations (SSPP's) varies with age and experience in the occipital cortex of rats.

In one experiment, one member of each of 11 male littermate triplet sets of Long-Evans hooded rats was reared from weaning in a toy-filled complex environment with other rats, a second littermate was reared with one other rat in a standard laboratory cage, and the third littermate was reared alone in a standard laboratory cage. At 55 days of age, occipital cortical tissue was studied electron microscopically at 41,800x. The percentage of asymmetric round vesicle synapses in which there was an interruption of .05 μm or greater in the post-synaptic opaque region was determined in layers 1, 3 and 4 (as determined from adjacent light microscopic sections). Across layers, complexity-reared rats had 25% more synapses with SSPP's ($p<.05$) than the isolates in the 5,537 synapses examined. Socially reared rats were intermediate. While percentages differed, the effect was seen in all 3 layers.

A second experiment compared male littermates housed either in pairs or in isolation for 130 days following weaning. The socially-housed rats' synapses had a 45% higher frequency of occurrence of SSPP's than those from isolates ($p<.05$).

Additional experiments have shown that extensive sensory stimulation shortly after birth can increase the relative frequency of SSPP's by more than 200% at 10 days of age and that the relative frequency of SSPP's increases by an equivalent amount from 10 to 60 days of age in isolation housed rats. Studies of the relationships of SSPP's to presynaptic anatomy are in progress. These studies suggest a change in post-synaptic anatomy in response to experience which could underlie functional changes in synaptic efficacy or permanence.

Supported by Grants HD 06862, NSF BMS 75-08596, and NSF BNS 77-23660.

1506 TWO-DIMENSIONAL MAPPING OF PROTEINS FROM RAT SYMPATHETIC GANGLIA: CHANGES IN SYNTHESIS RATES FOLLOWING DEAFFERENTATION AND AXOTOMY. Michael E. Hall and David L. Wilson. Dept. of Physiology and Biophysics. Univ. of Miami Sch. of Med., Miami, FL 33152

Superior cervical sympathetic ganglion (SCSG) cells respond to nerve section (axotomy) with a number of morphological, electrophysiological and biochemical changes. Section of the preganglionic axons (deafferentation) induces some of the same changes, such as separation of pre- and post-synaptic elements. Both treatments lead to changes in protein synthesis. By analyzing these changes in protein synthesis, using two-dimensional gel electrophoresis for protein mapping, we hope to gain insight into the regulatory mechanisms underlying gene expression in SCSG neurons.

Rat SCSG were deafferented by section of the pre-ganglionic sympathetic trunk. Three days later, SCSG were removed, desheathed, and incubated in the presence of ^{14}C-leucine for 1 hr. Labeled SCSG were then homogenized and subjected to two-dimensional gel electrophoresis as previously described (Anal. Bio., 83, 33, 1977).

A number of the more abundantly synthesized SCSG proteins exhibited significant changes in relative synthesis rates (RSR) following deafferentation. When these results were compared to the results of a previous analysis of changes in RSR after axotomy (SN abstr., 3, 426, 1977), it was observed that, of the 36 sample protein spots analyzed in detail following both deafferentation and axotomy, 16 protein spots exhibited changes in RSR of the same magnitude and direction after both treatments. Of the remaining sample proteins, one changed RSR only after deafferentation, five changed RSR after axotomy only and 14 did not change with either treatment. Furthermore, there were no protein spots that completely disappeared with either treatment, nor did any new protein spots appear. The RSRs of actin and tubulin were unchanged by deafferentation.

These results will be discussed in terms of what they indicate about the complexity of gene expression in SCSG neurons.

Supported by NIH grant NS12393. MEH is a postdoctoral trainee (NS 7044).

1507 SPROUTING OF CORTICORUBRAL TERMINALS IN THE CEREBELLAR DEAFFERENTED CAT RED NUCLEUS. Joseph Hanaway and Jeanne Smith. Dept. Neurol., Wash. U. Sch. Med., St. Louis, MO 63110.

We have investigated collateral sprouting or reactive synaptogenesis in the mammalian motor system using the cat red nucleus as a paradigm because of the distinct terminal fields of its two major afferent projections. Ultrastructural studies of normal corticorubral terminals have shown them to be exclusively on distal dendrites mostly in the rostral quadrants of the nucleus with a few found caudally. Cerebellorubral endings were found on somata, dendritic shafts and on distal dendrites mostly in the caudal quadrants of the nucleus but also rostrally. Cerebellar afferents were contralateral and cortical afferents ipsilateral.

To produce the environment for collateral sprouting from corticorubral terminals, lesions were placed in the nucleus interpositus and superior cerebellar peduncle in 4 cats and we waited one month for the degenerated endings to disappear. Ipsilateral corticorubral fibers were then transected in the hemisphere and after 3 days animals were sacrificed and perfused slowly(hours) with 3L of 1% paraformaldehyde and 1.5% glutaraldehyde. The red nucleus was identified on 250 um sections of the midbrain and was quartered into rostral(medial and lateral) and caudal(medial and lateral) blocks which were prepared for electron microscopy. One control cat with only cerebellar lesions was sacrificed at 37 days to see if any degenerated terminals persisted that could be misinterpreted in our double lesion series. 3.2% endings on the somata and 2.4% on dendritic shafts had not been phagocytosed, to our surprise, and were considered artifacts that were subtracted from our final counts. We found, after the double lesions, that corticorubral endings, normally found on distal dendrites, were now in greatly increased numbers on somata, dendritic shafts and distal dendrites and that the concentration was greater in the caudal "cerebellar quadrants" of the nucleus than in the rostral quadrants. We concluded that considerable collateral sprouting from corticorubral terminals had occurred into vacated postsynaptic sites and new ones.

1508 SEIZURES EVOKED BY FIBERS FROM THE CONTRALATERAL ENTORHINAL CORTEX WHICH REINNERVATE THE DENTATE GYRUS AFTER KINDLING AND ABLATION OF THE IPSILATERAL ENTORHINAL CORTEX. E.W.Harris*, J.Messenheimer and O.Steward,Depts. Physiology and Neurosurgery, Univ. of Va Sch Med,Charlottesville,VA 22901 (SPON:E.W RUBEL)

Unilateral stimulation of the entorhinal cortex (EC) leads to the gradual development of seizures in response to the stimulation (kindling), which may result from transynaptic changes in the targets of the kindled structure (Goddard et al.,Exp.Neurol. 25,1969). A major target of the EC is the ipsilateral dentate gyrus (DG). Because unilateral EC lesions result in the reinnervation of the DG by the contralateral EC, we tested whether these sprouting connections gain access to circuitry which when activated could evoke seizures. Chronic stimulating electrodes were implanted bilaterally in the EC of rats and daily stimulation was delivered to one EC until 5 seizures had been evoked. The kindled EC (EC1) was then ablated, and after 2 weeks (sufficient time to permit sprouting), kindling of the contralateral EC (EC2) was begun. An average of 24.3 trials preceded the first seizure for kindling of EC1 while an average of only 2.0 trials were required to evoke the first seizure from EC2. We have tested the hypothesis that the more rapid kindling of EC2 results from sprouting connections which gain access to structures transynaptically altered by the initial kindling, by using 3 treatments: 1)By omitting the lesion we examine the effects of primary kindling alone on the rate of EC2 kindling. In this group where the primary site of kindling was not destroyed, kindling of EC2 required an average of 6.1 trials, a rate significantly slower than that for kindling following destruction of EC1; 2)By omitting the initial kindling prior to ablation we examined whether post-lesion sprouting would increase the rate of kindling. This group required an average of 23.5 trials for kindling, which was not significantly faster than the initial kindling rate in normal animals; 3)By beginning EC2 kindling on day 1 rather than day 14 post-lesion, we examinedwhether the enhanced transfer can take place before sprouting has occurred. EC2 kindling required an average of 10.3 trials in this group, which was not significantly faster than the rate of secondary kindling observed in animals with no lesion. These results are consistent with the hypothesis that reinnervation of the DG by fibers from contralateral EC after kindling of the ipsilateral EC results in the sprouting fibers gaining access to circuitry which has been transynaptically altered by the kindling stimulation.

(Supported by NIH Grant 1 RO1 NS12333 to O.S.)

1509 MORPHOLOGY OF GOLGI-IMPREGNATED NEURONS IN MOUSE CORTICAL BARRELS FOLLOWING VIBRISSAE DAMAGE AT DIFFERENT POSTNATAL AGES. R.M. Harris* and T.A. Woolsey (SPON: R. Grubb). Dept. Anat. & Neurobiol., Washington U. Med. Sch., St. Louis, MO 63110.

The cytoarchitectonic appearance of and the pattern of thalamocortical afferents to the barrels in mouse SmI cortex can be altered by lesions to the contralateral vibrissae in the early postnatal period. Damage to the middle row of vibrissae (row C) prior to the sixth day of life (PND-6) prevents the formation of the middle row of barrels (row C) (Woolsey and Wann, '76). The earlier the vibrissae are damaged, the smaller the resultant row C zone becomes; the adjacent row B and D barrels enlarge as if to "compensate" for areal losses in row C.

To study the effects of vibrissae damage on the morphology of individual barrel neurons, the vibrissae in row C of mice were cauterized on PND's 1,2,3,4, or 5. The animals were sacrificed on PND-60. The brains were processed by the Golgi-Cox method, sectioned serially at 100 µm parallel to the plane of layer IV, and counterstained with thionin. The barrel fields were reconstructed, the position and extent of dendritic fields of impregnated neurons plotted, and their somata drawn using a camera lucida.

The findings are: 1) The altered cytoarchitectonic patterns of the barrels in these specimans are comparable to those previously described. Typically there were from 20-30 impregnated neurons in the altered C zones. 2) Impregnated neurons of both Class I and Class II (Woolsey et al., '75) were found in the C-zones and in the enlarged barrels in the adjacent rows B and D. Computer-based quantitative measures of somal area, three-dimensional distance to dendritic branches and to dendritic ends were similar for cells in the C-zones and in the expanded barrels in adjacent rows; they are comparable to like measures of barrel neurons in normal animals. 3) However, the spatial distribution of dendritic fields with respect to the barrel or zone boundaries showed that the earlier the vibrissal damage, the larger the fraction of C-zone cell dendritic fields extending out of the C-zone became. From PND-2 on, a majority of C-zone cells had their dendrites confined to that zone. The spatial distribution of processes of cells in the enlarged B and D barrels was the same as in normal animals.

These data suggest that a number of important structural properties of barrel neurons are determined intrinsically, but that the spatial orientation of the processes is largely determined by extrinsic influences, in particular the specific thalamocortical afferents.

Supported by NS10244 and EY01255.

1510 NOREPINEPHRINE LEVELS IN VARIOUS BRAIN AREAS, WITH FLUORESCENCE MICROSCOPIC CORRELATIONS, AFTER 6-HYDROXYDOPAMINE LESIONS OF THE LOCUS COERULEUS. Dennis P. Healy*, Jean Jew, and Asa C. Black, Jr. Dept. Anatomy, Univ. Iowa Coll. Med., Iowa City 52242.

Reis and Ross (1973) have shown that the closer a lesion is placed to the cell body of a noradrenergic neuron, the greater the neuronal degeneration, resulting in a diminished regenerative response. While electrolytic lesions have been shown to destroy central noradrenergic neurons (thus eliminating any regenerative response), the effect of 6-hydroxydopamine (6-OHDA) on central noradrenergic cell bodies is less well understood. We wished to determine what the effects of injection of 6-OHDA into the locus coeruleus (LC) itself would have on the morphology and catecholamine levels of this homogeneous population of noradrenergic cells. We have assessed the long-term effects of lesions of the LC on norepinephrine (NE) levels in selected areas of rat brain known to receive LC projections. We have also sought to determine whether the prolonged time course of anterograde degeneration in central noradrenergic systems produced by axonal injury is also seen following lesions of the cell body. A stereotaxic technique was used to inject 1 µl of a 2 µgm/µl solution of 6-OHDA into the LC of male Sprague-Dawley rats (150-160 gm body weight). Control animals received 1 µl of a physiological salt solution (McIlwain's solution). Animals were sacrificed at 1,2,4 and 8 weeks after lesion. Lesion placement was checked using a glyoxylic acid fluorescence microscopic technique. Portions of brain from the ipsilateral frontal cortex, parietal cortex, hippocampus, hypothalamus, and cerebellum were removed and assayed for NE using a radioenzymatic technique.

Brain Area	1 week†	2 weeks†	4 weeks†	8 weeks†
Frontal Cortex	85%(5)	40%(6)*	35%(6)*	35%(5)*
Parietal Cortex	76%(5)	52%(6)*	46%(4)*	67%(2)
Hippocampus	86%(5)	42%(6)*	36%(6)*	47%(6)*
Cerebellum	55%(4)*	30%(6)*	18%(3)*	22%(3)*
Hypothalamus	67%(4)	25%(6)*	71%(5)	47%(4)*

†Percentage control value. Number of samples in parenthesis.
*P<0.01, Student's "t" test.

Significant reductions of NE levels were found in all brain areas. These were followed by a levelling off within 2 weeks after lesioning. No increases were noted (with possible exception of the hypothalamus at 4 weeks). Morphological studies of terminal areas are under way at this time to determine the extent of damage produced by 6-OHDA. (Supported in part by NS-11650 to T.H. Williams from the N.I.H.).

1511 NEONATAL HAMSTER EYE ENUCLEATION AND TECTAL MIDLINE DAMAGE: EFFECTS OF PARTIAL COLLICULAR ABLATION ON RETINOTECTAL PROJECTIONS. K.K.-J. Hsiao and G.E. Schneider. (SPON: S. Corkin). Dept. Psychol., M.I.T., Cambridge, MA 02139.

The following three surgical procedures were performed on eight newborn hamster pups: a) right-eye enucleation; b) 1.5-mm deep slit made through the skull along the midline between the superior colliculi; and c) ablation of part of the lateral right superior colliculus (SC) made by heat applied to varying proportions of the overlying skull. When the pups were six-weeks old, the remaining left eye was enucleated, and the brains were processed two days later using the Fink-Heimer method for visualizing degenerating terminals and fibers. Each case was charted so that both the surface area and volume of the terminal region could be measured.

The inter-collicular slit impeded the formation of the normal midline tissue, and in five cases resulted in partial fusion of superficial gray on the left and right sides. In all cases, fibers from the left eye crossed the tectal midline and innervated the medial portions of the intact left SC, as well as the entire remaining right SC. This occurred even when the right SC was entirely undamaged, showing that retinotectal fibers can project to a supernormal tectal area. Preliminary electrophysiological work indicates that fibers which recross at the midline terminate in the left SC in a mirror image fashion (G. Sachs and K.K.-J. Hsiao)

Both the thickness of the brachium of the SC and the total retinotectal terminal volume decreased as the damage to the right SC increased, suggesting that direct axonal damage during neonatal collicular ablation, or some indirect post-ablative effect which leads to axonal degeneration, compromises the viability of retinotectal fibers.

When the entire right SC was ablated, the entire left collicular surface was innervated. On the other hand, when none of the right SC was ablated, only the medial third of the left SC was innervated. In either case, however, the recrossed terminal volume in the left SC was the same. Despite a constant recrossed terminal volume, the surface areal distribution of the fibers in the left SC increased and spread laterally in proportion to the damage done in the right SC. Current studies are focussed on an analysis of retinotopic organization in both the partially ablated right SC and in the intact left SC in similarly prepared cases.

Supported by NIH grant # EY00126, by Insurance Medical Scientist Scholarship Fund, Mass Mutual Life Insurance Co., and by Harvard-MIT Health Sciences and Technology Division.

1512 PROLONGED CHANGES IN BRAINSTEM TYROSINE HYDROXYLASE ACTIVITY FOLLOWING NEONATAL CEREBELLECTOMY, L. Iacovitti, T.H. Joh, D.J. Reis. Laboratory of Neurobiology, Cornell Univ. Medical College, 1300 York Ave., New York, NY 10021.

Treatment of neonatal rats by systemic administration of the catecholamine (CA) neurotoxin 6-hydroxydopamine produces in the pons-medulla (p-m) an apparent increase in: the number of CA containing fibers as visualized by histofluorescence, the content of norepinephrine (NE), and the activity of CA biosynthetic enzyme tyrosine hydroxylase (TH) (Jonsson and Sachs, Med Biol 54:286,1976). These results have been attributed to a "pruning effect" whereby widespread destruction of distal collaterals of immature NE neurons of the nucleus locus coeruleus (LC) stimulates compensatory growth of sprouts from uninjured branches. We sought to establish in developing rats whether: (a) a lesion restricted to a single projection of LC, either that projecting to cerebellum or that projecting through the median forebrain bundle (MFB) to innervate forebrain and hippocampus would also stimulate increased TH activity in p-m and LC; (b) such changes depend upon the postnatal age at the time of the lesion; (c) damage to one projection will elicit changes in TH in remote uninjured collaterals; and, (d) such lesions will alter developing cholinergic and gabergic neurons as reflected by changes in the activities of choline acetyltransferase (CAT) and glutamic acid decarboxylase (GAD), respectively. The cerebellum was totally ablated in rat pups on postnatal days (d) 3,6,9, or 18, and the rats killed 30-40 d thereafter. TH, CAT and GAD were assayed in LC, p-m and hippocampus. In other rats the MFB was transected on d 3, and 30 d later TH, CAT and GAD were measured in LC, p-m, and cerebellum. Controls were age matched, unoperated, or sham operated rats. Cerebellectomy performed on d 3 resulted in a 30-60% increase in TH in p-m and LC ($P<0.001$); a smaller but significant difference when performed on d 6 and 9; but no change when performed on d 18. These brainstem changes were unaccompanied by TH changes in the hippocampus. Cerebellectomy on d 3 did not alter the number of LC neurons (1466 ± 32 vs 1501 ± 109; $n=4$) 30 d later, nor did it increase the number nor distribution of TH containing fibers visualized by immunocytochemical staining with a specific antibody to TH. MFB lesions placed on d 3 resulted in no changes in TH activity in LC, p-m, or cerebellum 30 d later. Changes in CAT and GAD were not observed in any region following either lesion. We conclude that neonatal cerebellectomy results in an age-dependent and prolonged increase in TH activity in LC and adjacent brainstem, which is not reflected in uninjured collaterals, and without evident changes in the number of TH containing fibers. Cerebellectomy in early development may produce profound but restricted changes in CA synthesizing capacity in rat brain. (Supported by NIH grants HL 18974 and NS 03346.)

1513 VISUAL EXPERIENCE AND THE DEVELOPMENT OF THE EFFERENT SYSTEM TO THE CORPUS CALLOSUM; G.M. Innocenti and D.O. Frost. Institut d'Anatomie, Université de Lausanne, Lausanne, Switzerland.

In normal adult cats, neurons projecting through the corpus callosum (callosal neurons) are located within a limited region (callosal zone; CZ) spanning the area 17/18 boundary. At birth, however, the CZ is much wider. It extends throughout all of areas 17 and 18 and its medial limit reaches the fundus of the splenial sulcus. The CZ shrinks gradually and is almost adult-like by the end of the first postnatal month. The rules guiding this process and the fate of the callosal neurons lost are unknown. We have investigated whether visual experience is i) necessary for, or ii) can modify, the normal development of callosal connections. Three different experiments were performed: a) two kittens were deprived of pattern vision in both eyes and b) two in one eye, by eyelid suture maintained from postnatal day 6 or 7 (prior to spontaneous eye opening) on. Two days before sacrifice, (4th - 5th postnatal month) all these animals received unilateral multiple injections of horseradish peroxidase (HRP) alone or combined with radioactive leucine/proline, in their visual cortices (contralateral to the open eye for monocularly deprived cats). The postlateral and lateral gyri were completely filled by the injections. c) Convergent strabismus was provoked in two kittens and divergent strabismus in one, by bilateral section of either the lateral or medial recti muscles. At 7-8 months of age, these animals could perform standard visual reaching and placing tasks with either eye. At 8 or 9 months of age they received cortical injections as in a and b. They were sacrificed two to three days later at the end of an experiment in which microelectrode penetrations aimed at assessing the degree of binocularity of cortical neurons were performed in area 17, 18 or both. Thirty six to 44 single units were recorded in each animal. The overwhelming majority had strictly monocular receptive fields (groups 1, 2, 6 and 7 of Hubel and Wiesel). The degree of squint estimated in paralyzed animals from the projection of the retinal landmarks was 26-39 deg. Coronal sections from each brain were processed for HRP (with diaminobenzidine or o-dianisidine), autoradiography or both. Only the HRP results are reported here. The distribution of callosal neurons in each brain was determined from computer aided reconstructions. In all the animals, as in normal cats, the heaviest packing density of callosal neurons in areas 17 and 18 was found near the 17/18 boundary. The medial edge of the CZ appeared in the binocularly deprived animals in its normal adult position. From caudal to rostral it moved gradually from the crown of the postlateral gyrus onto the medial bank of the hemisphere. It reached the fundus of the suprasplenial sulcus only at its most rostral levels. In the three squinted and in one of the monocularly deprived cats, callosal neurons extended further medially. They reached the fundus of the suprasplenial sulcus or, posteriorly (where this sulcus is not yet apparent), comparable distances from the crown of the postlateral gyrus. A few were also found below the suprasplenial sulcus. Assuming unaltered cortical retinotopy, the CZ in these cats can be estimated to extend to regions in area 17 representing 7-12 degrees (depending on vertical excentricities from area centralis) from the vertical meridian, against 1-5 deg in the normal. Radial location and morphology of callosal neurons are not obviously affected. Thus, normal vision does not appear to be necessary to the development of the main morphological features of the CZ. However, the outcome of the developmental process can, to some extent, be modified by manipulations affecting the animal's visual experience.

1514 CATECHOLAMINERGIC TERMINALS IN KITTEN VISUAL CORTEX: THE NORMAL DISTRIBUTION AND ITS CHANGES FOLLOWING THE LOCAL MICROPERFUSION OF 6-HYDROXYDOPAMINE. Toru Itakura*, Takuji Kasamatsu and John D. Pettigrew*. Div. Biol., Calif. Inst. Tech.,Pasadena CA 91125

We have presented physiological evidence that the local availability of catecholamine (CA) is necessary for maintaining the high level of the synaptic plasticity in the developing visual cortex which can be demonstrated during the early postnatal life (Science, 194, 1976; Nature, 271, 1978). In the present study we present the morphological evidence that the cortical CA had been depleted locally in the cortical area from which our samples of single neurons were collected for the physiological assay of plasticity.

First, the normal distribution pattern of CA fibers was studied by a modified fluorescence histochemistry. The animals (4-8 weeks of age) were perfused with a mixture of glyoxylic acid and paraformaldehyde in a buffer solution which was rich in magnesium. Frozen sections were cut with a cryostat. The preterminal CA fibers are parallel to the cortical surface in the white matter and they give off perpendicularly collateral branches toward the deep layers in the gray matter. Some of these collaterals appear to be the stem CA fibers judged on their strength of fluorescence. In the course of their extension, perpendicularly or obliquely, toward the cortical surface, there are found many short and long collaterals in various directions. Giving off these fine branches, the stem fibers continue to stretch up to the molecular layer where they start to run horizontally as fine terminal fibers. The density of CA terminal fibers (with varicosities) seems to be highest in the superficial layers (layer II + III) but not in the molecular layer. The dense plexus formation in layer II + III) is the most characteristic pattern of the CA innervation in kitten visual cortex.

When the visual cortex was depleted of its CA by a week-long microperfusion of 6-hydroxydopamine (6-OHDA) (4.0 mM-40 μM) the yellowish-green fluorescence disappeared completely from the area near the perfusion site. The extent of lesion in which no CA fibers were found varied in the proportion of the concentration of 6-OHDA. Next to this primary lesion area, fragmented CA fibers appeared in the background of autofluorescence and those fibers were thicker in size and brighter in their intensity than the normal, suggesting the accumulation of CA at the sites proximal to the lesioned terminal fibers. When we moved further away from the perfusion site finally we started to see nearly normal patterns of fiber distribution. But the intensity seemed still lower than that at the corresponding site in the opposite hemisphere of the same animals. Work supported by the Spencer Foundation, and by MH25852 and NSF-BNS77-19433.

1515 APPARENT "CRITICAL PERIOD" AND DOMAIN CHARACTER OF NERVE SPROUTING IN MAMMALS. Patrick C. Jackson and Jack Diamond. Dept. of Neurosciences, McMaster University, Hamilton, Ontario, Canada, L8S 4J9.

We have examined the ability of cutaneous nerves in the mammal to enlarge their peripheral fields by collateral sprouting into adjacent denervated skin. Mechanosensory nerve fields were mapped by recording afferent impulses in whole nerve trunks during mechanical stimulation of the skin with a fine bristle. Surprisingly, we found no enlargement of a remaining mechanosensory skin field either in the adult rabbit or adult rat during periods up to 90 days after denervation of adjacent skin. In the neonatal rat however the situation is quite different.

In the rat the dorsal portion of each trunk dermatome is supplied by a dorsal cutaneous nerve (D.C.N.), the medial branch of which serves an area of skin bordering the midline, the lateral a comparable area within the dermatome, immediately lateral to the first. Consequently an "island" of innervated skin is left by cutting all cutaneous nerves supplying a region of the back except for the medial branch of one D.C.N. Nerve fields normally enlarge in proportion to the skin area as an animal grows in size. However, remaining "islands" produced in 10 day old rat pups, by 20 days had increased to an area almost double that of controls. This "extra" sprouting of intact nerves resulted only when adjacent denervations were carried out within the first 20 days after birth, and seemed to cease at about 20 days of age, irrespective of when it was elicited.

It seems then that, in the rat at least, there is a "critical period" for collateral sprouting of intact cutaneous nerves into denervated skin, which ends about 20 days postnatally. The sprouting which can be evoked before this time however does not occur uniformly from the still-innervated field. The remaining branch of a D.C.N. showed a distinct preference for the denervated skin of its "parent" dermatome, rather than an adjacent one. When an entire dermatome was left with its innervation intact, but the adjacent dermatomal nerves were cut at 10 days of age, we detected no sprouting, i.e. there was no significant extension of the dermatomal field into the adjacent denervated skin. It seems then that the "domain" character of peripheral nerve fields suggested by previous findings in the salamander (Diamond et al., 1976, Science 193: 371-377) may apply in the mammal too. In contrast, regenerating axons appear to be unaffected by these spatial restrictions, and even in the adult rat will readily invade "foreign" nerve territories.

1516 VISUAL CAPACITIES OF MONOCULARLY DEPRIVED CATS AFTER REVERSE LID SUTURE AND ENUCLEATION OF THE NON-DEPRIVED EYE. K.R. Jones*, M. Berkley, P. Spear, L. Tong. Depts. Psychol., Florida State U., Tallahassee, FL 32306; U. of Wisconsin, Madison, WI 53706.

Behavioral studies of mature cats after monocular occlusion during the first few months of life have shown that vision through the deprived eye is very poor. These results are consistent with electrophysiological studies of visual cortex of such animals which have shown that few neurons (<11%) can be activated via the deprived eye.

More recent studies of the cat have suggested that the number of neurons activated via stimulation of the deprived eye may be increased (e.g., to a maximum of 40%) after enucleation of the nondeprived eye. The degree to which this procedure affects the visual capacities of the deprived eye, however, is not known.

To determine if there is a behavioral correlate of the reported electrophysiological changes after enucleation, kittens were monocularly deprived by lid suture starting at 7-11 days of age. Ten to fourteen months later, the lid-sutured eye was opened and the animals permitted another 6-9 months of normal binocular visual experience before behavioral testing began. All cats were tested monocularly on light-dark, 0 vs. + and grating acuity tasks.

The cats learned all the tasks easily with the nondeprived eye and showed little, if any, transfer to the deprived eye. Acquisition of the discriminations using the deprived eye was very poor. Only two of the four monocularly deprived cats were able to give a grating acuity estimate and they were far below normal (<1.0 c/d). The other two cats never achieved a sufficiently high level of performance when tested with the deprived eye to yield an acuity estimate.

When stable performance levels were reached in each eye, the eyelids of the nondeprived eye were sutured closed and testing of the deprived eye continued. Some small improvements in performance were noted in all four cats but no change in the acuity thresholds for the two animals which yielded acuity estimates. After 3 weeks of testing, the nondeprived, now lid-sutured, eye was removed and testing continued. Another small performance increment at low spatial frequencies was noted but again no change in the grating acuity estimates. At the conclusion of the behavioral testing, single unit studies of visual cortex confirmed earlier reports of an increase in the number of driveable neurons after enucleation when compared to nonenucleated monocularly deprived cats.

These results suggest that while enucleation of the experienced eye increases the number of neurons driveable through the deprived eye, it produces only a small performance improvement and little, if any, change in the acuity threshold of the deprived eye.

Supported by EY 00953, 01916.

1517 PLASTICITY IN THE CORTICOSPINAL TRACT AFTER EARLY LESIONS OF THE MEDULLARY PYRAMID. K. Kalil and T. Reh*. Dept. of Anatomy and Neurosciences Training Program, Univ. of Wis., Madison, WI 53706.

In the newborn hamster the pyramidal tract is still in the process of descending through the medulla and into the spinal cord. Thus, plasticity in this pathway at early postnatal ages can be studied by interrupting the corticospinal fibers in the medulla before they have established connections in the spinal cord.

In normal animals, the growth of the corticospinal pathway was plotted by injecting [^{3}H] poline unilaterally into the sensorimotor cortex of 1 to 8 day old hamsters. Autoradiographs showed few labeled fibers in the medullary pyramid at 2 days of age. By 5 days labeled fibers could be traced in the white matter to thoracic levels of the spinal cord but the dorsal horn of the cord was labeled only at cervical levels. At day 8 the pyramidal tract had reached lumbar levels but had invaded the dorsal horn only as low as the mid-thoracic cord.

In another series of experiments, animals ranging from 2 to 14 days of age received discrete unilateral lesions of the medullary pyramid several mm. rostral to the decussation of the tract. After survival times of 2 to 12 weeks, the sensorimotor cortex ipsilateral to the lesion was injected with [^{3}H] proline and the brains processed for autoradiography. Our results show no labeled axons below and ipsilateral to the lesion, but instead reveal a massive and abnormal decussation of the pyramidal tract several mm. rostral to the lesion. These aberrant fibers follow an abnormal course medial to the spinal trigeminal nucleus and descend through the brainstem to terminate in the dorsal column nuclei, the spinal trigeminal nucleus and in the dorsal horn of the cervical spinal cord. These terminations occur in precisely the same pattern observed in normal animals and in the correct side of the brain. This new pathway develops in animals in which the pyramidal tract was transsected as late as 8 days. Since results in the normal animal show that the pyramidal tract is fully developed at medullary levels by this age, it seems likely that the aberrant fibers represent new growth of the severed pyramidal tract fibers rather than redirection of later arriving corticospinal axons. Further, although the trajectory of the pathway is completely abnormal, the fibers are able to establish their normal pattern of terminations.

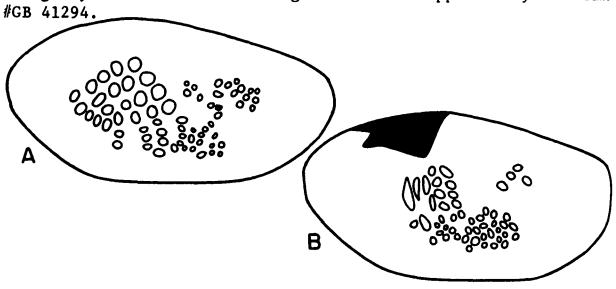
(Supported by NIH grant NS 14428-01.)

1518 EFFECT OF NEOCORTICAL DAMAGE ON THE ORGANIZATION OF THALAMIC PROJECTIONS. H. Killackey, J. Dames* and R. M. Akers. Dept. Psychobio., Univ. of Calif., Irvine, CA 92717.

Previous work in our laboratory has demonstrated that the thalamocortical projections to the rat somatosensory cortex exhibit considerable plasticity. Cauterization of vibrissal follicles at birth results in the development of aberrant projections associated with the damaged vibrissae. In the present experiment we have examined the possibility that the organization of thalamocortical projections may be altered by central, as well as by peripheral damage.

Newborn rats received electrolytic lesions of the posterior somatosensory cortex on the day of birth or on postnatal day 5 and were allowed to survive until at least two weeks of age. Following perfusion with 10% glycerol, their brains were removed, sectioned at 30 µm in a plane tangential to the neocortical surface, and processed for localization of succinic dehydrogenase activity.

Comparison of the reconstructed pattern of thalamocortical afferents in an undamaged hemisphere (A) and an experimental hemisphere (B) reveals that lesions of somatosensory cortex on the day of birth produce marked alterations in the pattern of thalamocortical afferents. First, the area occupied by the projections associated with the mystacial vibrissae is reduced. Second, discrete clusters within a given row tend to be fused. Finally, when individual clusters of afferents are present, they are reduced in size as compared with those in normal animals. Neocortical lesions on postnatal day 5 do not have the same effects. In such cases the organization of thalamocortical projections not directly damaged by the lesion is essentially normal. The present results suggest that the development of thalamocortical afferents may be influenced both by the state of the periphery and the integrity of their central target tissue. Supported by NSF Grant #GB 41294.



1519 EARLY POSTNATAL DEVELOPMENT OF NORMAL AND ABNORMAL UNCROSSED OPTIC PATHWAYS IN ALBINO RATS. Peter Land and R. D. Lund. Dept. of Biol. Struct. and Neurol. Surgery, Univ. Wash., Sch. Med. Seattle, WA 98195.

After unilateral enucleation of newborn rats, the uncrossed optic pathway from the remaining eye exhibits an expanded distribution throughout subcortical visual centers. In this study, we removed one eye from albino rats within 2 hours after birth. The remaining eye was injected with ^{3}H-proline 0 hours to 9 days after enucleation. A comparable series of unoperated littermates received uniocular isotope injections. The animals were perfused after 24 hours and the brains were sectioned and processed for autoradiography.

A substantial crossed projection to thalamic and midbrain visual centers is detectable at 1 day postnatal in both groups of animals, although the projection to the medial portion of the dorsal lateral geniculate nucleus (dLGN) is quite sparse. In day 1 control animals, there is a diffuse uncrossed projection to dLGN, which primarily is distributed in the anterolateral part of the nucleus. The uncrossed projection to the superior colliculus (SC) is close to background at this time. Between 6 and 9 days postnatal, the normal uncrossed projection to dLGN becomes localized much as in adult animals and the projection to SC is more clearly focused anteromedially in the stratum opticum.

The uncrossed projection to SC in enucleated animals is already distinct from controls by 2 days, with grain levels above background throughout the stratum griseum superficiale. However, the ipsilateral projection to dLGN cannot be distinguished from normal until day 4. The density of the abnormal projections appears to increase throughout the time period studied. The rapid appearance of the abnormal ipsilateral projection in SC suggests that at least some of the fibers contributing to it must arise from axons already present ipsilaterally at birth rather than solely from aberrant axons which have grown from the optic chiasm after enucleation. (Supported by USPHS Grants EY-05185 and EY-00596.)

1520 RIGHT HEMISPHERE SPEECH FOLLOWING CALLOSOTOMY. Joseph E. LeDoux,* Bruce T. Volpe,*Charlotte S. Smylie,*Donald H. Wilson,*Michael S. Gazzaniga. Dept. of Neurology, Cornell Medical College, NYC 10021

Language and speech are typically located in the left cerebral hemisphere. Under certain conditions, however, all or part of these processes appear in the right hemisphere. We have been studying case P.S., who was observed to have bilateral comprehension skills, but only left hemisphere speech immediately after section of the corpus callosum. Now,two and one-half years postoperatively,his right hemisphere is acquiring the capacity to speak. The patient can verbally describe visual information exclusively presented to his right hemisphere. This recently acquired ability to verbally describe left visual field stimuli is not attributable to the reinstatement of interhemispheric visual transfer. This is demonstrated through several control tests. First,while he was able to name objects and words presented to either visual field,he was unable to state whether two simultaneously presented stimuli,one in each field,were the same or different. Second,when line drawings of finger postures were presented to one visual field,only the contralateral hand was able to mimic the postures. Third,when one of a pair of homonyms was presented to either hemisphere,for example "coat" or "cote," he could name appropriately. Subsequent to each naming trial he was asked to spell the articulated word;following left hemisphere naming,the words were correctly spelled. Yet,following right hemisphere naming of "cote" he spelled "c-o-a-t." Presumably the right hemisphere said "cote" and on the basis of this information,the more robust and competent left hemisphere speech system took over and spelled "c-o-a-t." If his capacity to name left field objects were attributable to visual transfer from the right hemisphere to the left,he should have performed differently in the above conditions. Further,he performed above chance when asked to name objects in his left hand and,in addition,on certain trials,in which he was seemingly unable to name the object,correct naming was detected through a sensitive microphone. After naming the object at a level that was undectable by the unaided ear,he was unable to respond to the question,"What is in your hand?" Presumably the right hemisphere had named the object and the left hemisphere, which did not have access to the amplified vocalization,had responded to the probe. These observations, which clearly suggest, for the first time,the unequivocal presence of expressive speech in the right hemisphere of a split-brain patient,have implications for notions of neural plasticity and volitional control of conscious activities in man.

Aided by USPHS Grant No.25643 awarded to M.S. Gazzaniga.

1521 QUANTITATIVE DEMONSTRATION OF AXOSOMATIC SYNAPSE SPROUTING IN THE NEONATAL RAT INDUCED BY DENDRITIC DEAFFERENTATION. Nicholas J. Lenn, Viviana Wong* and Geoffrey Hamill*. Depts. Neurol. and Ped., Carnegie Labs. of Embryol., Sch. Med., UCD, Davis, CA 95616.

When one or both habenulae are removed from a rat in the first days following birth, several changes in the synaptology of the interpeduncular nucleus result (Lenn, J. Comp. Neurol., 1978). One of these changes, based on these qualitative observations, was an increase in the number of axosomatic synapses from their low normal number. This increase was evident in axosomatic synapses containing spherical and flattened vesicles.

We have reassessed this phenomenon quantitatively by preparing 10 normal and sham-operated animals and 5 lesioned animals with uni- and bilateral habenula lesions. Neuronal perikarya were randomly selected from the central portion of the interpeduncular nucleus on low power electron micrograph maps (x100). These perikarya were then photographed at moderate magnification (x16,000), and each apparent synapse was photographed at high magnification (x30,000). The only selection made was an attempt to approximately equalize the numbers of large and small perikarya included in the sample as judged by their size on the microscope screen at low magnification. Axosomatic synapses were then recognized in the photographs and counted. The perimeter of each perikaryon was measured. The results were expressed by several statistics of which the most meaningful appears to be synapse number per 100 microns of neuron circumference.

In the control animals the range of synapses per 100 microns of neuron circumference was 6.38 to 10.35, with a mean of 7.99. In the operated animals the range of this statistic was 8.72 to 16.67, with a mean of 12.63. This 50% increase was significant as judged by a two-tailed Student t test ($p < .001$). The endings forming the synapses contained pleomorphic vesicles including at most one or two true flattening vesicles in almost all cases. Occasional endings had all spherical vesicles, or prominent numbers of flattened or large granular vesicles. The synaptic contacts were more asymmetrical with more spherical vesicles, varying to symmetrical for endings with prominent flattened vesicles.

It is concluded that the qualitative observation was correct although the magnitude of the effect is much smaller than had been apparent from the qualitative study. These observations confirm this unusual example of synaptic plasticity, namely an increase in axosomatic synapses following deafferentation of the dendritic portion of the neurons in the interpeduncular nucleus.

Supported in part by grants HD NS 08658 and RR 00169.

1522 REGENERATION OF PRIMARY OLFACTORY NEURONS AND "GLOMERULARIZATION" WITHIN THE FOREBRAIN OF NEONATALLY BULBECTOMIZED MICE. R. R. Levine*, G. A. Monti Graziadei* and P. P. C. Graziadei. Dept. Biol. Sci., Unit 1, Florida St. Univ., Tallahassee, FL 32306.

After sectioning the olfactory nerve, olfactory neurons undergo retrograde degeneration. Stem cells within the olfactory neuroepithelium exhibit increased mitotic activity, differentiate into mature receptor neurons and replace the degenerated receptor units (Graziadei and Monti Graziadei, in Neuronal Plasticity, Raven Press, 1978). Fibers from the reconstituted olfactory neurons reinnervate the olfactory bulb where they reform functional synaptic relationships in the glomerular neuropil. This study was designed to determine the extent of the plasticity of the olfactory neurons and their ability to regenerate in the absence of their primary neural target, namely the post-synaptic processes within the glomerular layer of the olfactory bulb.

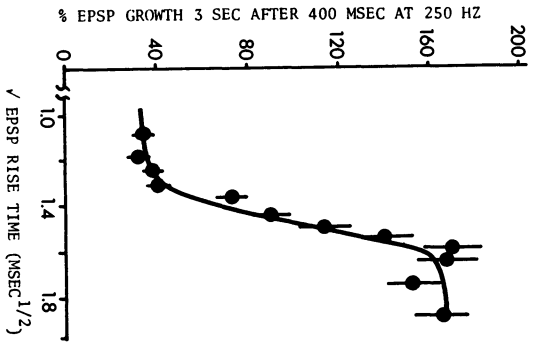
Neonatal (1 - 6 days) mice were subjected to complete unilateral bulbectomy (including accessory olfactory bulb and anterior olfactory nucleus) and sacrificed after periods ranging from 1 - 120 days. Material was processed routinely for light and electron microscopy and several results were noted. By 30 days following surgery, reconstituted olfactory nerve fibers were observed crossing the cribriform plate into the cranial cavity where they ended in glomerular-like formations in close proximity to cerebral tissue. The cerebrum itself appeared to migrate forward to fill the gap created by bulbectomy. In many cases there was a proliferation of cells from the ependymal lining of the cerebral vesicle that surrounded the new fibers and glomeruli. Most striking, however, were our observations of "glomeruli" directly enmeshed within populations of large cell-bodied neurons within the protruding cerebrum. Longer term survivals indicate that this "glomerularization" persists and that there may be a relationship between the length of survival and degree of glomerularization in the forebrain. EM analyses revealed clearly recognizable pre- and postsynaptic elements and synaptic contacts within these forebrain glomeruli, reminiscent of those seen in the in tact olfactory bulb. Thus, despite the loss of its primary target, olfactory axons penetrate an "unfamiliar" cellular matrix and form new synaptic relationships. (Supported by NSF Grant BNS 77/16737 to P.P.C. Graziadei and by NIH Training Grant 5-T-32 NS 07010).

1523 SHORT-TERM SYNAPTIC MODULATION IN THE MEDIAL AND LATERAL COMPONENTS OF THE PERFORANT PATHWAY. Bruce L. McNaughton. Dept. of Psychology, Dalhousie Univ., Halifax, N. S. CANADA B3H 4J1.

As indicated by their discrete pattern of heavy metal accumulation (e.g., Zimmer & Hjorth-Simonsen, J.Comp.Neur. 1975, 161, 71-102), the terminals of the medial and lateral perforant pathways to the fascia dentata appear to represent two biochemically distinct populations. This evidence is not necessarily incompatible with the evidence (Steward, J.Comp.Neur. 1976, 167, 285-314) that there may be a continuously ordered mapping from the medio-lateral axis of the entorhinal cortex onto the proximo-distal axis of the granule cell dendrites.

It was shown previously (McNaughton and Barnes, J.Comp.Neur. 1977, 175, 439-454) that the extracellularly recorded synaptic responses of these two components could be differentiated on the basis of waveform, the more laterally elicited responses having a slower rise time.

The present experiments show that while a continuous range of EPSP rise times can be recorded with varying stimulus locations in the angular bundle, the magnitude of short-term synaptic modulations following either single pulses or brief high-frequency trains, differs in a discontinuous fashion when plotted as a function of EPSP rise time. This result indicates that the two components are physiologically discrete. Under pentobarbital anaesthesia, the lateral pathway shows a dynamic range of synaptic efficacy of at least 170% of baseline whereas the medial pathway has a range of less than 40% for the equivalent conditioning input.



1524 AUTORADIOGRAPHIC MAPPING OF RETINOTECTAL FIBERS IN NORMAL GOLDFISH AND AFTER REGENERATION FOLLOWING NERVE CRUSH. Ronald L. Meyer* (SPON: C. R. Hamilton). Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The distribution of optic terminals was mapped in normal fish and in fish after regeneration of the optic nerve. In both groups, the nasal or temporal hemiretina was lesioned just prior to injection of ^{3}H-proline into the vitreous. Autoradiography on normal fish showed label only in tectal regions appropriate to the intact retina. In fish labeled after optic nerve crush and regeneration, the distribution of terminals was found to be orderly after long recovery periods, but diffuse at early stages. At 1 mo, the label was found over nearly the entire tectum, whereas at 4 mos, grains were restricted to topographic regions similar to that in normal fish. In another study, a mediolateral mid-dorsal incision through the tectum in normal fish disrupted all fibers coursing to dorsal tectum posterior to the lesion, so that label was found only in lateral regions of the posterior tectum. After regeneration of the optic nerve, however, a similar incision failed to produce such a well defined zone of denervation even after long recovery, although there was a marked lateral to medial decrease in the gradient in the posterior tectum. Apparently, the accuracy of termination improves with time, but pathway errors remain. (Supported by the Caltech Hixon Fund and USPHS grant MH-03372 to R. W. Sperry.)

1525 CHANGES IN Ia FIBER PROJECTION TO MOTONEURONS IMMEDIATELY FOLLOWING SPINAL CORD TRANSECTION. S.G. Nelson, T.C. Collatos*, A. Niechaj* and L.M. Mendell, Dept. Physiol., Duke Univ. Med. Ctr., Durham, NC 27710

In previous experiments we have shown that transection of the spinal cord at T13 or L5 6 to 8 hours before recording resulted in enhancement of Ia evoked individual EPSPs recorded in homonymous motoneurons. These data were obtained in anesthetized cats using the spike triggered averaging technique in medial gastrocnemius (MG) motoneurons with comparison made to similar data in intact anesthetized preparations. In the present experiments done using identical techniques, EPSPs were recorded in several MG motoneurons prior to spinal cord transection at either T13 or L5. Following transection uncommonly large EPSPs (> 400 μV) were observed but only after several hours had elapsed. EPSPs exhibiting the largest amplitude increases had brief rise times; the Ia terminals producing them may be located proximally on the motoneuron. Procaine block followed by transection at that level resulted in no change in EPSPs in cells recorded continuously throughout this procedure. We conclude that enlargement of EPSP amplitude is not an immediate consequence of loss of descending input.

The large EPSPs in these preparations were observed both in slow and in fast motoneurons. Their rise times and half widths clustered near the origin of the shape index curve (half width vs. rise time), suggesting a selective enlargement of EPSPs produced by Ia terminals synapsing proximally on the motoneuron. Input resistance of these motoneurons was within normal limits.

Immediately following transection, single Ia fibers evoked EPSPs in virtually all homonymous motoneurons in contrast to the projection frequency of 78% previously reported by Scott and Mendell (J. Neurophysiol. 33, 679-692, 1976) in cats with intact spinal cords. No lower projecting type Y afferents (Scott and Mendell, ibid) were observed following transection in contrast to cats with intact spinal cords in which about 1/3 of all Ia fibers were type Y. Furthermore, a Ia fiber classified as type Y before transection exhibited the characteristics of a type X fiber (projecting to virtually all motoneurons) following transection. Subject to uncertainty due to sampling, we speculate that the increased connectivity (which can occur without increases in amplitude) may be immediate. It could represent either increased invasion of Ia terminal branches or perhaps activity of hitherto "silent" synapses. The link between cord transection and these increases is not presently known.

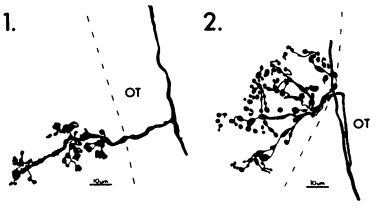
(Supported by NIH.)

1526 REVERSIBLE REDUCTION IN THE ACTIVITY AND AMOUNT OF CHOLINE ACETYLTRANSFERASE IN HYPOGLOSSAL NEURONS DURING THE RETROGRADE (AXON) REACTION. D.H. Park, G.F. Wooten, T.H.Joh and D.J. Reis, Laboratory of Neurobiology, Cornell Univ. Med. College, New York, NY 10021; and (GFW) Dept. of Neurology, Univ. of Washington, St. Louis, MO

A reversible reduction in the activity and amount of the neurotransmitter synthesizing enzymes, tyrosine hydroxylase (TH) and dopamine-β-hydroxylase, probably a consequence of reduced enzyme biosynthesis (Ross et al, Neuroscience Abstr. 3, 431, 1977) characterizes the response of central dopaminergic and noradrenergic neurons to axonal injury. We sought in this study to establish if lesions of cholinergic neurons will also reduce reversibly in the parent cell body the activity and amount of the specific enzyme required for acetylcholine biosyntnesis, choline acetyltransferase (CAT). The cholinergic hypoglossal nerve was transected unilaterally distal to the cricoid cartilage in anesthetized rats. At various days thereafter the animals were killed and the hypoglossal nucleus assayed for CAT and also for acetylcholinesterase (AChE), the enzyme degrading the transmitter, and compared with unoperated controls. Between 24-72 hours after surgery, CAT activity fell to 52% of control, recovering to prelesion levels 4-5 weeks later at a time of reinnervation of the tongue. The activity of AChE was reduced in parallel but with a smaller magnitude (to 80% of control). Immunotitration with a specific antibody to CAT prepared from rat caudate nucleus demonstrated the reduction in CAT activity was entirely due to reduced accumulation of enzyme molecules. Morphologically, signs of chromatolysis in hypoglossal neurons was minimal. These results further support the view that a reduction in neurotransmitter biosynthetic enzymes may be a hallmark of the retrograde reaction in all neurons. Since the retrograde reaction in hypoglossal neurons is associated with a net increase in protein biosynthesis (Watson, J. Physiol. (Lond.), 180, 741, 1965), the reduced accumulation of CAT protein may reflect reordering of patterns of protein biosynthesis favoring production of proteins for reconstitution of regenerating axons at the expense of those required for the function of the cell in neurotransmission. (Supported by NIH grants HL 18974 and MH 24285.)

1527 ABNORMAL AXONAL GROWTH IN THE DORSAL LATERAL GENICULATE NUCLEUS. J.A. Robson and C.A. Mason, Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago, Illinois 60637.

Dorsal lateral geniculate laminae A and A1 of the cat normally receive inputs from different eyes. However, removal of one eye during the first postnatal week induces growth of axons from the intact eye into the denervated laminae. This abnormal growth has been shown to occur predictably in the binocular segment of the nucleus and the region of these translaminar sprouts contains large cells that have not undergone denervation atrophy. Sprouting has also been shown in monocular regions of denervated lamina A, but this does not occur in all experimental animals nor is there evidence that it is associated with surviving large cells. To determine if the abnormal retino-geniculate axons invading the region of large cells differ from those invading the region lacking large cells, the structure of these axons in the binocular and monocular regions of the nucleus has been studied light and electron microscopically. To reveal the axons we filled them with horseradish peroxidase using a method described previously (Science, 1978). There, we demonstrated that translaminar sprouts between laminae A and A1 arise as preterminal and ultraterminal branches of axons that also contribute to their normal target laminae. These sprouted branches give rise to terminals that in all respects resemble those of normal retino-geniculate axon terminals. In comparison, axonal sprouts in the monocular segment of lamina A are not translaminar; they arise directly from the optic tract or as branches of axons in the optic tract. Some have terminals similar to those of normal retino-geniculate axons. That is, many terminals have a crenulated appearance and occur singly or in clusters (Fig. 1). Ultrastructurally these terminals also resemble normal retino-geniculate axon terminals and they form similar patterns of synaptic contacts. However, other sprouted axons in the monocular segment are different (Fig. 2); they branch profusely and give rise to many terminals, most of which have smooth contours and a spherical appearance. Furthermore, these terminals are typically surrounded by glia; make few synaptic contacts; and contain few, if any, synaptic vesicles.



Supported by USPHS Grants NS05407, NS11869, NS14271, NS14283, EY02374.

1528 LESIONS IN OCCIPITAL CORTEX OF RAT LEAD TO SECONDARY LOSS OF CELLS IN OTHER CORTICAL REGIONS. Mark R. Rosenzweig, Edward L. Bennett, Hiromi Morimoto* and Marie Hebert *. Dept. Psych. and Laboratory of Chemical Biodynamics, UC-Berkeley, Berkeley, CA 94720.

We reported previously that lesions confined to occipital cortex result in significant loss of weight and DNA in other intact regions of cerebral cortex (Will et al., J. comp. physiol. Psychol., 1977, 91, 33-50). In subsequent experiments involving about 400 rats we have investigated how magnitude of remote loss varied with (a) cortical region, (b) size of lesion, (c) time elapsed between lesion and sacrifice, and (d) post-operative environment, either enriched (EC) or impoverished (IC). Somesthetic cortex, although adjacent to the occipital area, showed only about 2% loss of DNA while remaining dorsal cortex and ventral cortex lost 6-8%; hippocampus showed no change. Magnitude of remote loss increased directly with size of lesion. There was not a consistent difference between losses in DNA of EC rats (EC-lesion vs EC-sham) and IC rats (IC-lesion vs IC-sham), so post-lesion enriched experience did not help to protect against secondary loss contrary to a hypothesis suggested in Will et al. The secondary loss was somewhat greater in rats sacrificed 95 days after lesion than in rats sacrificed 35 days post-lesion. This apparent progressive loss is being investigated in further experiments; it may provide a model for studying progressive loss that has been reported in some clinical cases. Although the EC experience did not protect against remote loss of cells, it did improve subsequent learning behavior. Furthermore, social grouping did not have this effect, so inanimate features of the enriched environment are important in recovery of function.

This research was supported by grants from the Easter Seal Foundation and ADAMHA grant R01 MH26704. It also received support from the Division of Biomedical and Environmental Research of the U.S. Department of Energy through the Lawrence Berkeley Laboratory.

1529 NEURONAL GROWTH IN ADULT NEOCORTEX INDUCED BY STIMULATION AND CONDITIONING. L. T. Rutledge, Mary Ann Werz* and Marilyn E. Conlon*. Dept. of Physiol., Univ. of Mich., Ann Arbor, Mi. 48109

Neuronal ultrastructure was studied following long-term unilateral electrical stimulation of a suprasylvian gyrus in adult cats. For one group (conditioned), brain stimulation (CS) was paired with foreleg shock (US). For another group (unconditioned), brain stimulation was unpaired with foreleg shock. Daily brain stimulation via indwelling cortical electrodes consisted of 20 2-sec trains of 0.5 msec biphasic pulses at 50 Hz. Constant current levels were 0.4 to 1.1 mA. Unstimulated cats served as controls. Bilateral tissue samples from homotopic cortical areas of the suprasylvian gyri of each cat were prepared for electron microscopy.

Brain stimulation, with and without conditioning, produced neuronal growth in both the stimulated and contralateral cortex as indicated by the presence of axonal and dendritic growth cones and filopodia. Qualitatively, growth cones and filopodia were more numerous on the side contralateral to stimulation. Systematic measurements of various dendritic and axonal processes were made. In cortical layer I, cross-sectional areas of dendrites, on both sides of the brain, were larger in the two stimulated groups compared with normals. The two sides of the brain in the unconditioned group did not differ. However, for the conditioned group, dendritic cross-sectional areas were larger on the side contralateral to stimulation. Preliminary data from one animal show that conditioning produced localized changes within the cortical layers. Axonal terminals containing round vesicles were more numerous, but smaller, in layer I, and more numerous in the upper part of layer II, contralateral to stimulation. These data indicate the development of new axonal terminals. Other data indicate the formation of new synaptic contacts. In layer I, the number of symmetric and asymmetric contacts on dendritic shafts was greater contralateral to stimulation. Furthermore, the number of spines and asymmetric contacts on spines was greater in the upper part of layer II, contralaterally.

In these experiments stimulation induced the growth of both dendritic and axonal processes in the adult central nervous system. Cortical stimulation combined with a conditioning paradigm produced more neuronal growth contralaterally than did stimulation alone. Marked growth restricted to certain portions of the cortical layers argues for specificity of plasticity in these experiments.

1530 EXCITATORY AND INHIBITORY POSTSYNAPTIC POTENTIALS DURING HETEROSYNAPTIC POST-ACTIVATION POTENTIATION IN HIPPOCAMPUS. John M. Servey, Ulrich Misgeld* and Manfred R. Klee*. Dept. of Neurobiology, Max Planck Institute for Brain Research, Frankfurt am Main, F.R.G.

In the pyramidal layer of field CA3 of guinea pig hippocampal slices (300-400 µm thick, 37°C) the orthodromic extracellular population spike from single shocks (0.1 to 0.5 cps) to both mossy fiber (mf) and Schaffer collateral (Sch) regions was potentiated, sometimes for more than 3 hr, after tetanic stimulation (usually 20 cps for 10 sec) to either region. Potentiation was usually enhanced by a second tetanus.

During intracellular recording from pyramidal cells orthodromic stimulation elicited only rarely "pure" excitatory postsynaptic potentials (EPSP; 5%) or inhibitory postsynaptic potentials (IPSP; 25%) but mostly mixed EPSP/IPSP (70%). If potentiation means an increase in orthodromic population spike, the question arose, what changes occur in the IPSP? In most cells with mixed EPSP/IPSP, after tetanus the EPSP progressively increased and the IPSP decreased, while the resting membrane potential remained unchanged. In contrast, in other cells with mixed EPSP/IPSP or apparently pure IPSP, the IPSP increased, while the EPSP remained unchanged. But we observed that, if we repeated the tetanic stimulation, after the second tetanus the EPSP increased (or even developed in cells formerly showing no EPSP), while the IPSP was drastically reduced.

A possible explanation for these data is that after the first tetanus the impaled cell and its synapses are not changed, while the response of most neighboring cells is potentiated. The resulting increased recurrent inhibition, through common inhibitory interneurons, would increase the recorded IPSP. After the second tetanus the impaled cell and/or its synapses are also altered, so that the recorded EPSP increases and the IPSP decreases. This would account for both types of post-tetanic response seen in different cells. Finally, the idea that the potentiation is heterosynaptic (i.e., not restricted to the tetanized synaptic input) is strengthened by the observation that any change in PSPs which occurs after tetanus to one input is seen in response to both inputs.

1531 SPECIFIC NEURITIC PATHWAYS AND ARBORIZATIONS FORMED BY FETAL MOUSE DORSAL ROOT GANGLION CELLS WITHIN ORGANIZED SPINAL CORD EXPLANTS IN VITRO. Neil R. Smalheiser, Edith R. Peterson* and Stanley M. Crain. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

In cultures of fetal mouse spinal cord cross-sectional explants with attached, NGF-enhanced, dorsal root ganglia (DRGs), focal DRG or dorsal cord stimuli evoked characteristic 'PAD'-like synaptic network responses restricted to dorsal horn regions (Cr. & Pet., Br. Res. 79,'74). Stimuli to nearby ventral cord regions, on the other hand, failed to evoke these dorsal horn responses, indicating that few stray collaterals of the primary afferent DRG input were present in the ventral cord. To trace these DRG fibers directly, orthograde horseradish peroxidase, Golgi-like labeling of DRG cells was carried out (at 0.5-5 wks in vitro) in over 60 co-cultures with: attached cord cross-sections, separate longitudinal cord strips, isolated explants of dorsal and ventral regions, and superior colliculus explants. HRP (Type II, 20% in saline) was iontophoresed extracellularly into DRGs via two-four 8 µm pipettes, with 100 msec pulses, 2/sec (~ 3 µa) for 4-12 hrs, then incubated for 0.5-5 hrs, fixed, and processed with Hanker-Yates reagent (modified from Hanker et al., Histochem. J. 9,'77). Scores of fibers (~ 0.3µ-3µ) were filled, up to 3 mm. Fiber growth, ramification, and arborization types in cross-sectional cord explants with attached DRGs resembled those seen in situ (e.g. Proshansky and Eggar, Neurosci. Lett. 5,'77). Similar preferential invasion and ramification of dorsal cord regions was observed in co-cultures with isolated dorsal and/or ventral cord explants. In longitudinal cord strips, HRP-labeling confirmed electrophysiologic evidence (Cr. & Pet., Soc. Neurosci. Abstr. 1,'75) that DRG fibers entered directly into dorsal regions and not via the adjacent ventral regions on the same facing edge; this was observed even within a day or two after arrival of DRG neurites at the cord explant. In repeated attempts with a truly inappropriate target, superior colliculus explants, we have observed only rare, sparse DRG arborizations, in contrast to those seen in nearby cord targets in the same culture. This was not due to deficits in the collicular tissue in vitro; for example, preliminary HRP injections of retino-collicular co-cultures have shown elaborate arborizations in the tectal explants. These studies set limits to the extent of aberrant DRG growth and terminal arborizations within the developing CNS, and they provide a baseline for exploring underlying specificity mechanisms in vitro. (Supported by grants 5T5 GM 1674 from NIH, NS-12405 from NINCDS, BMS75-03728 from NSF; N.R.S. is in the Medical Scientist Training Program at Einstein.)

1532 MONOAMINE INVOLVEMENT IN THE DEVELOPMENT OF THE POST-DECAPITATION REFLEX IN RATS. Sonya K. Sobrian, Martin A. Rogers* and Byron A. Campbell. Dept. Pharmacol., Sch. Med., Howard University, Washington, D.C. 20059 and Dept. Psych., Princeton University. Princeton, N.J. 08540.

The post-decapitation reflex (PDR) is a series of coordinated movements which follows decapitation in the cervical region. Selective depletion of brain and spinal cord norepinephrine (NE) by 6-hydroxydopamine (6-OHDA) treatment altered the development of this reflex in Sprague-Dawley rat pups. Subcutaneous injections of 50 ug/g of 6-OHDA on postnatal Days 0-3 significantly reduced cortical and cerebellar NE concentrations 67% and 57%, respectively, while brain stem NE was increased 55%. In the spinal cord the reduction in NE was 30% of control values, while dopamine levels remained unchanged. This injection schedule also increased the latency and decreased the duration and intensity (bilateral kick frequency) of the PDR in 10-25 day old pups. The incidence of pups exhibiting the reflex was 100% in both groups at each of these ages. However, at 30 days of age, the PDR was abolished in 6-OHDA treated pups; recovery was not evident at 90 days of age. Moreover, the PDR was absent only in pups receiving 6-OHDA during the first 2 or 4 postpartum days (Day 0 = birth). Injections of 6-OHDA on Days 4 and 5 or Days 8 and 9 significantly altered the latency, duration and intensity without reducing the incidence of the PDR in 15 and 30 day old rats. Further evidence for the involvement of NE in the PDR was indicated by the reinstatement of the reflex in 6-OHDA pups by acute treatment of 30 day old animals with clonidine (2 mg/kg, i.p.). The latency, duration and intensity of the response, however, were still significantly different from controls. Amphetamine (4 mg/kg) and l-DOPA (100 mg/kg) were ineffective, while apomorphine (8mg/kg) produced an attenuated PDR in 42% of the rats tested. Depletion of central serotonin concentrations by neonatal 5,7-dihydroxytryptamine treatment reduced the intensity of the PDR without significantly altering the other parameters in 30 day old rats.

Several tasks of motor coordination were also affected by neonatal lesions of NE terminals. 6-OHDA treated pups, 30-35 days of age, failed on a task of hind limb support when suspended, took significantly longer to cross balance beams of various widths and to remount a moving treadmill.

These data indicate that NE neurons of descending bulbospinal pathways are essential for the development and maintanence of the PDR and that dopamine and serotomin may influence several aspects of this reflex.

1533 THE DEVELOPMENT OF PLASTICITY IN THE HIPPOCAMPUS. Timothy J. Teyler and Charles Duffy*. Neurobiology Program, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

It has been well established that environmental influences can have a marked effect on the development of structure and function in the nervous system. These influences are also reflected in the plasticity exhibited by the developing brain. The normal development of neuronal response plasticity has, however, been neglected. In this experiment we have examined the development of several forms of monosynaptic response plasticity in the normally developing rat hippocampus, using a combination of electrophysiological and light microscopic techniques.

Four types of plastic change in electrophysiological response properties were examined in the dentate gyrus of rats as a function of development. Animals of 7, 14, 30, 60 and 210 days of age were used.

The monosynaptic junction between the perforant path fibers and dentate granule cells was examined at each age for frequency potentiation, post-tetanic potentiation, long-term potentiation and habituation. Electrophysiological measures were taken from the in vitro hippocampal slice preparation. The electrophysiological development of plastic processes was compared to the morphological development of dentate granule cells at each age, using rapid Golgi techniques.

Frequency potentiation and long-term potentiation exhibited a developmental progression between 7 and 210 days postnatal. Post-tetanic potentiation remained constant across this period. Except for the youngest group, the degree of habituation increased as a function of age. Thus, most forms of plastic response processes followed a developmental time course.

The anatomical data indicate that the dendritic field of the rat dentate gyrus granule cell layer is not fully mature until well into the first year of life. Four stages of dendritic development are described: (1) The establishment of primary dendrites, (2) The formation of dendritic spines, (3) The elaboration of the dendritic arborization, and (4) The reintensification of spine density.

These results may indicate that the mechanism(s) underlying post-tetanic potentiation is different from that underlying the other forms of response plasticity in that it does not show a developmental time course. These results do not allow us to draw conclusions regarding the neuronal mechanisms underlying response plasticity. The development of response plasticity was seen to be correlated with synaptogensis, spine formation, dendritic arborization and the reorganization of dendritic input patterns.

1534 THE STIMULUS FOR COLLATERAL NERVE SPROUTING IN SKELETAL MUSCLE. C. D. Tweedle and J. J. Kabara*. Dept. Anat. and *Biomech., MSU, E. Lansing, MI 48824.

It is known that, following partial denervation of skeletal muscle, collateral sprouting of the remaining nerve supply occurs. Therefore, there must be a stimulus from the denervated muscle fibers or degenerating nerve to elicit the neuronal outgrowth. Our experiments indicate that lipid extracts of rat gastrocnemius muscle that has been denervated for nine days will elicit histological evidence of significant nerve sprouting when injected into normal rat muscle (tongue). Control muscle lipid extracts or lipid extracts from normal or degenerating nerve have no such activity, indicating that the stimulus for sprouting is from the denervated muscle and is lipophilic in nature. Analysis of the denervated gastrocnemius muscle shows increases in both non-polar and polar lipids. Biological activity was found in the glycolipid and phospholipid fractions.

Preliminary evidence also indicates that extracts from the endplate region of denervated muscle have more biological activity than extracts from the non-endplate region. Data to date indicate that at least two lipophilic substances isolated from denervated muscle can elicit nerve sprouting.

(Supported by NSF grant #BNS 76-81406.)

1535 CYTOARCHITECTONIC ALTERATIONS IN MOUSE CORTICAL BARRELS FOLLOWING DIFFERENT PATTERNS OF EARLY VIBRISSAL DAMAGE. K.L. Valentino*, T.A. Woolsey and A.J. Wilson*. (SPON: W.M. Landau). Dept. of Anat. and Neurobiol., Washington U. Sch. Med., St. Louis, MO 63110.

In mice the large vibrissae on the face project to the posteromedial barrel subfield (PMBSF) in the contralateral SmI cortex. The vibrissae and PMBSF barrels are arranged in 5 rows labeled A-E. Within each row the vibrissae and barrels are numbered from 1 up to 8. Barrels or vibrissae in different rows having the same number are described as arcs. If the vibrissae are lesioned, the PMBSF barrels can be altered. When Row-C vibrissae are lesioned on different postnatal days (PND), there is a "critical period" after which the peripheral damage does not alter the cortex. Areal losses in Row-C barrels are "compensated" for by enlargement of barrels in Rows B and D, and there is a caudal to rostral gradient in the cortex for this compensation (Woolsey and Wann, '76).

The present experiments were designed to examine this gradient of "compensation". Vibrissae in Row B or D, arcs 2 or 5 and other combinations including all vibrissae were lesioned in animals on PND's 1-6. On PND-30 the animals were sacrificed, the brain embedded, sectioned serially at 75 µm tangential to the pia stained with thionin, the PMBSF's drawn using a camera lucida and the areas of each barrel measured with a computer.

The results are: (1) Lesions of Row B or Row D produced the most severe cortical alterations when the vibrissae were damaged on PDN's 1 and 2. There is a critical period on PND-5 after which no cortical changes are produced by the peripheral damage. The row zones did not show evidence of segregation into barrels until PND-4, and there was "compensation" by the adjacent rows. (2) Animals with lesions of vibrissae in arcs 2 or 5 show the same "critical period", but the arc zones become segregated into barrels earlier-on PND-2. In several cases, there was a clear indication of the caudal to rostral gradient in that across an arc the Row-E barrel was more severely effected than the Row-A barrel. In all cases, there was areal "compensation" within the barrel row by the more medially placed barrels.

These data confirm our earlier observations with respect to a "critical period" and provide direct evidence for a caudal to rostral gradient of "compensation" in the cortex. There is a second lateral to medial gradient of compensation within PMBSF barrel rows. The organization of barrel neurons into rows, presumably by segregation of the thalamo-cortical afferents, dominates over the segregation of neurons within a row into single barrels. This finding agrees with single unit studies in which neurons in the supragranular layers have been shown to respond to several vibrissae which are commonly within a particular row.

Supported by NIH Grants NS10214 and EY01255.

1536 SOME BEHAVIORAL CONSEQUENCES OF NEURAL TRANSPLANTS IN THE RAT BRAIN. Robert B. Wallace, Gopal D. Das*, Joanne Bell* and David Maheux*. Dept. of Psych., Univ. of Hartford, West Hartford, Ct. and Dept. of Biol. Sci. Purdue Univ., West Lafayette, Ind.

Embryonic neural tissue obtained from the forebrain of 17 day rat embryos was transplanted into the midline cerebellum of 10 day old Long-Evans hooded rats. The animals were allowed to survive for behavioral testing and were then sacrificed and the brains processed for Nissl stain and Golgi-Cox impregnation.

These heterotopic transplants had displaced much of the host cerebellar tissue and in terms of cytology and cytoarchitecture, had retained the characteristics of their region of origin. Anatomically all transplants were continuous with the host brain tissue.

These animals were tested on a series of behavioral tasks and compared with normal animals of the same age and strain. There were no differences between the groups in emotionality as assessed by the King Emotionality Scale. General activity, ataxia, tremor, dysmetria, and rearing behavior were measured in an open field situation. There were no differences between the two groups on the first four measures but the transplant animals did have more abnormal rears than the control subjects.

Results on an elevated runway revealed no substantial differences between the groups.

Activity as measured in activity wheels did reveal differences with the transplant animals being significantly more active than the control subjects.

These data were then contrasted with results from a previous study (Wallace and Altman, 1969) where two groups of Long-Evans hooded rats received neonatally 8 or 10 x 200r of x-irradiation focally delivered to the cerebellum. This experimental treatment reduced by approximately 80% the number of granule cells in the internal granular layer. These animals were tested at ages comparable to the subjects in the present investigation.

In terms of emotionality and the measures assessed in the open field, the x-ray animals were in all instances more impaired than the control and transplant material from the present study. Only with regard to rearing behavior did we note any comparability between the transplant and the x-ray animals. Activity as assessed in the wheels with the x-ray subjects showed them to be significantly less active than the control subjects of the present study.

Although our results are tentative at this point and it is clear that much additional work has to be done, it is suggested that heterotopic tissue transplants might well possess some capacity to maintain reasonably normal levels of function. It is further suggested that transplanted tissue might produce a juvenilization effect in the host animals.

1537 SYNAPTOGENESIS IN RAT LUMBAR SPINAL CORD AND RECOVERY OF FUNCTION. Eric D. Weber* and Dennis J. Stelzner (SPON: James A. Horel). Dept. of Anatomy, S.U.N.Y. Upstate Medical Center, Syracuse, N.Y.

Mid-thoracic spinal cord transection produces dramatically different behavioral results which depend upon a rat's age at the time of surgery. If complete spinal transection occurs on or prior to the twelfth postnatal day, spinal shock is minimal and recovery maximal. If the lesion is produced at 15 days of age or later in development little recovery occurs and the classical picture of paraplegia is observed. (Brain Research 125:241-255; 1977). The present study was initiated to determine whether the synaptic development in the gray matter of the normal, developing spinal cord differs before and after this critical period for behavioral recovery.

The L6 segments from ten groups of animals, 0 - 30 days of age, taken at three day intervals (four animals/group) were studied by light microscopy. Areal measurements of the gray matter were made using an integrating X - Y tablet interfaced to a computer. Cell size, cell density, and area of neuropil were evaluated in the lateral portions of the intermediate gray matter, laminae VI and VII. This region was selected for study since it is an interneuron pool which matures relatively late in ontogeny. (Exp. Neurol. 31: 337-357; 1971). Electron microscopic analyses of synaptogenesis were performed on material from the same region in animals 3, 12, 15, 21, and 30 days old using similar morphometric methods while taking note of vesicle, junctional, and mitochondrial morphology.

A 60% increase in area of neuropil paralleled a linear increase of comparable magnitude, in area of the gray matter until 15 days of age when both curves reached a plateau. Cell size remained constant ($\approx$20 μm^2 in plane of nucleolus) throughout development so could not have contributed to the increase in area of gray matter. Areal measurements of the size and counts of the number of vesicle containing profiles demonstrated a 50% increase in density of axon terminals between 3 and 12 days of age and a steady decline thereafter. The size of vesicle containing profiles in laminae VI and VII remained constant at a small value ($\approx$0.37 μm^2) until 12 days of age, showed rapid growth to 0.55 μm^2 between 12 and 15 days of age, followed by a more moderate increase in sectional area after 15 days. This may indicate that the addition of new vesicle containing profiles ceases at about the time a growth in their size commences. These results suggest that synaptogenesis in this region of the lumbar spinal cord proceeds rapidly between birth and 15 days of age but only very slowly thereafter.

We interpret our results to indicate that recovery is maximal after spinal cord injury if the damage occurs before synaptogenesis is complete. If synaptic development is complete at the time of injury, little recovery of function results.

Supported by Grant # NS 10579 and NS 10496

1538 THE EFFECT OF ENVIRONMENTAL DEPRIVATION ON THE GROWTH OF THE MOUSE PURKINJE CELL DENDRITIC TREE: A MORPHOMETRIC GOLGI ANALYSIS. Gary M. Weiss* and J.J. Pysh. Dept. Anat. Northwestern Univ. McGaw Med. Ctr., Chicago, IL 60611.

Previous studies of the effects of functional deprivation on neuronal development have been largely confined to the visual system. Since the isoplanar nature of the dendritic tree makes the cerebellar Purkinje cell uniquely suited for morphometric analysis, we chose to investigate in Golgi-Kopsch preparations the effect of environmental deprivation on the late postnatal development of the Purkinje cell dendritic tree.

Littermates of C57/DBA hybrid mice were placed in either a deprived or enriched environment after weaning at 18 days of age until sacrifice at 35 days of age. Deprived animals were kept together in a small cage, where their movements were severely restricted, allowing them to move about only enough to gain free access to food and water. In contrast, enriched animals were raised in a large Habitrail cage with exercise toys which allowed running and climbing ad lib. In addition, on a daily basis, enriched animals were trained to swim and walk a tight wire and encouraged to exercise.

Body and brain weights were reduced significantly 15.5 and 3.0% in the deprived group. Histometric analysis of sagittal sections of the vermis revealed a significant reduction of 10.7% in the molecular area in deprived animals and no change in the area of the granular layer. Morphometric analysis of Golgi sections revealed a significant reduction in deprived animals of 9.6% in dendritic field areas and 9.2% in total dendritic length of Purkinje cells. This reduction was consistent in all lobules of the vermis.

Purkinje cells of deprived mice did not appear to be merely immature cells reflecting a slower rate of development because they had a normal branching density for their age. The above environmental effects on cerebellar development might result from a decrease in the length of parallel fibers and/or the amount of glia which could reduce the thickness of the molecular layer and hence the area available for Purkinje cell dendritic growth.

These data offer the first evidence that the development of the Purkinje cell dendritic tree may be alterable by environmental manipulation.

(Supported by NIH Grant #NS 10657-01).

1539 LONG TERM POTENTIATION IN THE LESION-INDUCED CROSSED TEMPORO-DENTATE PATHWAY OF THE RAT. R. Wilson* and O. Steward. Depts. of Neurosurgery and Physiology. University of Virginia School of Medicine, Charlottesville, VA 22901

Unilateral entorhinal cortical lesions disrupt the normal ipsilateral entorhinal cortical (IEC) projection to the dentate gyrus (DG) of the rat hippocampal formation and induce marked alterations in the terminal organization of surviving afferents to the partially denervated DG (sprouting). Among these changes is a proliferation of terminals of the normally sparse crossed temporo-dentate pathway originating in the contralateral entorhinal cortex (CEC). As part of an effort to ascertain the extent to which this lesion-induced crossed pathway reproduces the electrophysiological properties characteristic of the normal IEC projection, we compared their capacities for long term potentiation (LTP). Stimulation of the IEC with short, high frequency (8-12 pulses, 400 Hz) stimulus trains induces dramatic, long lasting increases in the amplitudes of the DG population EPSP (a measure of summed synaptic currents) and population spike (a measure of DG granule cell discharge). Comparison of population EPSP amplitudes to population spike amplitudes at several stimulus intensities previous and subsequent to delivery of the potentiating trains indicated that the two parameters increased in parallel during LTP such that the increased synaptic drive induced greater granule cell discharge. In experiments in animals with long-standing IEC lesions, delivery of identical short, high frequency stimulus trains to the CEC likewise produced LTP of the synaptic response elicited via the sprouted crossed temporo-dentate connections. No evidence of potentiation of the contralaterally elicited population spike was obtained, however, indicating the increased synaptic drive associated with LTP in the lesion-induced crossed temporo-dentate pathway did not, in contrast with LTP in the normal ipsilateral pathway, result in an enhancement of granule cell discharge. The results suggest that the lesion-induced and normal connections possess similar synaptic capacities, but that the enhanced synaptic input has different consequences for granule cell discharge in the case of the lesion-induced crossed pathway. (Supported by USPHS RESEARCH GRANT #1 ROl NS12333 to O. Steward).

1540 MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL STABILITY OF THE MAUTHNER CELL FOLLOWING AXOTOMY BY SPINAL CORD TRANSECTION. Steven J. Zottoli and Donald S. Faber. N.Y.S. Res. Inst. on Alcoholism, 1021 Main Street and Dept. Physiol., SUNY at Buffalo, Buffalo, N.Y. 14203, U.S.A.

In order to explore the potential of the Mauthner cell (M-cell) system for studies of neuronal plasticity, we have analyzed its behavioral, morphological and electrophysiological responses to axotomy performed by spinal cord or bulbar transections at different levels rostral to the anterior edge of the dorsal fin. M-cell function was evaluated by the presence or absence and extent of the sound-evoked startle response it initiates. There were no such responses involving distal trunk and tail musculature in 171 trials conducted from 1-232 days postoperatively (n= 16; prior to axotomy the same fish had a response rate of 80%) although the characteristic movements of the jaw, operculi, fins and eyes were present. In contrast, in most fish examined coordinated swimming behavior returned within 2-7 months. Another 30 experimental fish were sacrificed at different postoperative times for morphological investigations. In no case was there definitive light microscopic evidence of M-cell chromatolysis or other retrograde reactions. Furthermore the Mauthner axon maintained a "normal" appearance 3-4 mm rostral to the level of transection while in the immediate region of the cut abnormal myelin profiles were generally observed for up to 8 months after transection. More caudally this myelin became fragmented and gradually disappeared. These findings indicate a lack of functional and morphological M-axon regeneration and further suggest a maintenance of normal M-cell function proximal to the cut. Electrophysiological experiments on similarly axotomized fish (1 day to 1 yr postoperatively) confirmed that the M-cell retains a high degree of stability in its membrane properties and input connectivity despite an appreciable axonal truncation and the consequent loss of output connections. Specifically, the cell could be activated orthodromically by ipsilateral VIIIth nerve stimulation and antidromically by spinal cord stimulation above but not below the level of transection, and no physiological signs typical of other axotomized neurons (e.g., dendritic spikes or selective deafferentation) were found. The hypotheses that this apparent lack of an axon reaction is due to a minimal trophic influence exerted upon the M-cell by the efferent connections removed by axotomy and/or a stabilizing influence of the predominantly electrotonic afferent inputs to this neuron are presently being studied further. (Supported in part by NIH Grant No. NS-12132)

PSYCHO-PHARMACOLOGY

1541 EFFECTS OF INTRACRANIALLY ADMINISTERED PENTYLENETETRAZOL ON ELECTROCORTICOGRAM AND CONCOMITANT MOTOR ACTIVITY IN THE FREELY MOVING RAT. Bruce J. Albala and Tibor Palfai*
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Pentylenetetrazol (PTZ) is often utilized as both a diagnostic as well as a research tool in clinical or model epilepsy. A number of publications indicate that when applied intracranially the drug induces epileptogenic foci with charateristics comparable to grand mal seizures. However, because most intracranial animal preparations are anesthetized or restrained during electrical recording, no data are available on the effects of intracranially administered PTZ on concomitant motor behavior.

Work in our laboratory indicates that PTZ, (in concentrations up to 50 %) fails to elicit overt motor convulsions when administered intraventricularly in freely moving rats. That the absence of convulsions are not due to faulty injection procedures is suggested by the fact that while the distribution of ^{3}H-PTZ in the brain following intracranial (I.C.) or intraperitoneal (I.P.) injections are comparable, the amount of ^{3}H-PTZ is significantly lower following the I.P. administration. Electrocorticograms (ECoG) recorded following I.P. or I.C. injection of PTZ indicate brain seizures that are similar qualitatively on a number of measures. Overt convulsions however occur only after I.P. but not after I.C. infusion. These data suggest a dissociation between neural activity measued by ECoG and motor behavior following the intraventricular administration of PTZ.

1542 ANTAGONISM OF ETHANOL NARCOSIS IN MICE BY LOW LEVEL HYPERBARIC TREATMENT WITH HELIUM-OXYGEN. R. L. Alkana and R. D. Malcolm, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

Preliminary studies in this laboratory suggest that hyperbaric treatment with pure oxygen or 20% oxygen-80% helium at 3-6 atmospheres absolute (ATA) antagonizes ethanol depression without accelerating ethanol metabolism. The present experiments further investigated the effects of low level helium-oxygen hyperbaric treatment on ethanol sleep-time in mice. Drug naive, C57 Bl/6j mice were injected i.p. with 3.2 g/kg ethanol (20% v/v). Upon loss of their righting reflex, animals were placed in a hyperbaric chamber and the atmospheric pressure was brought to 1, 6, 8, or 10 ATA with helium-oxygen mixtures adjusted to maintain an oxygen partial pressure approximating 0.20 ATA. Control animals were treated similarly and exposed to air (1 ATA) or 20% oxygen-80% helium (1 or 6 ATA). Immediately after return of their righting reflex and decompression of the chamber, 20 µl blood samples were obtained from the tail vein for gas chromatographic ethanol determination. Mean ± S.E. sleep-times in minutes or blood ethanol concentrations upon awakening in mg/deciliters are shown in parenthesis with the respective oxygen content of the mixture. Hyperbaric helium-oxygen significantly reduced sleep-time at 6 ATA ($\bar{X}_{3.41\%O_2}$ = 8.2±0.8; $\bar{X}_{20\%O_2}$ = 10.3±2.6) and 8 ATA ($\bar{X}_{2.60\%O_2}$ = 8.8±2.3) when compared to 1 ATA controls ($\bar{X}_{air}$ = 18.0±3.0; $\bar{X}_{20\%O_2}$ = 17.7±2.9) ($p<0.05$; 1-tailed t-test). The 10 ATA treatment did not significantly reduce sleep-time ($\bar{X}_{2.05\%O_2}$ = 17.2±3.6). Blood ethanol concentrations were significantly higher at 6 ATA ($\bar{X}_{3.41\%O_2}$ = 388±12) and 8 ATA ($\bar{X}_{2.60\%O_2}$ = 381±10) when compared to 1 ATA controls ($\bar{X}_{air}$ = 350±7; $\bar{X}_{20\%O_2}$ = 334±8) ($p<0.05$; 2-tailed t-test). No significant difference was seen at 6 ATA ($\bar{X}_{20\%O_2}$ = 342±11) or 10 ATA ($\bar{X}_{2.05\%O_2}$ = 360±8). The present findings replicate the ethanol antagonism demonstrated in previous hyperbaric studies with pure oxygen and 20% oxygen-80% helium. Furthermore, these results indicate that antagonism occurs at elevated atmospheric pressures even when the oxygen partial pressure is not raised above normal (0.20 ATA). Although the mechanism of hyperbaric-induced ethanol antagonism remains unknown, these and previous results suggest that the antagonism is not due to elevated partial pressures of oxygen, nor to enhanced ethanol metabolism. Further studies are necessary to clarify the mechanism of the hyperbaric antagonism and to assess its potential in the treatment of acute ethanol overdose.

1543 ESCAPE DEFICITS AFTER INESCAPABLE SHOCK: NEUROCHEMICAL CONDITIONING OR SENSITIZATION. H. Anisman*and L.S. Sklar* (SPON: Jane Stewart). Department of Psychology, Carleton University, Ottawa, Ontario.

Exposure to inescapable shock produces deficits of subsequent escape performance. These deficits are thought to be due to difficulties in maintaining sustained vigorous responding owing to depletion of norepinephrine and dopamine. However, the escape deficits have been found to be long lasting, whereas the stress-induced amine changes are relatively transient. It is suggested that the neurochemical changes induced by stress are subject to conditioning or sensitization. Thus, upon reexposure to stress the extent and rapidity of the amine reduction is accentuated. In support of this notion it was observed that L-Dopa (400-600 mg/kg) administered prior to either inescapable shock or testing, eliminated the escape deficits otherwise observed. Moreover, pairing α-methyl-p-tyrosine (125 mg/kg) or bis (4-methyl-1-homopiperazinylthiocarbonyl) disulphide (FLA-63)(40 mg/kg) with as few as 1 or 5 shocks,which have no effect on their own, disrupted performance 24 hours later. The drug plus 5 shock combination had effects which persisted for 7 days. These effects could not be attributed to long term effects of the drug treatments on catecholamine levels or turnover. It is posited that pairing of catecholamine depletion with stress results in the augmentation of subsequent stress elicited amine changes. The relevance of these data to "learned helplessness" are critically discussed.

1544 LONG-LASTING INFLUENCE OF STRESS ON THE BEHAVIORAL EFFECTS OF AMPHETAMINE AND HALOPERIDOL. S.M. Antelman*, A.J. Eichler*, C.A. Black* and G. McCloskey* (SPON: D.J. Edwards). Dept. of Psychiatry and Psychobiology Program, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

We have recently begun to investigate the related questions of whether moderate stress can produce effects similar to those observed following repeated treatment with amphetamine (AM) and whether such effects might be long-lasting. Since repeated AM treatment is known to produce a progressive augmentation of stereotypy, we first examined whether chronic administration of a moderate tail-pressure (TP) stress to adult male rats could likewise enhance the stereotypy seen following a subsequent dose of AM. We now report that when TP sufficient to induce eating (approximately 80-100 psi) was applied daily for a total of 56 mins. spread over a 15 day period it markedly enhanced AM induced sniffing (3 mg/kg) when this was examined 24 hrs after TP had been discontinued.

In order to determine whether the effects of stress persisted we also conducted tests two weeks after discontinuation of our stressor. This time a 72 hr period of food deprivation was used as the stressor. It's effects were measured 15 days later (at which time body weights and food intake were no different from controls) on both AM (4 mg/kg)-induced sniffing and the ability of haloperidol (0.8 mg/kg) to attenuate TP induced eating. Animals that had received the food-deprivation stress 15 days earlier showed significant enhancement of AM stereotypy and a marked (significant) diminution in the ability of haloperidol to reduce eating precipitated by TP.

These results indicate that moderate stress can produce effects similar to those obtained with AM. By inference they also suggest that many of the effects of AM (and presumably other stimulants as well) may be attributable to its capacity to mimic the influence of stress on the organism. The ability of our stressors to enhance the effect of a dopamine (DA) agonist such as AM while retarding the influence of the DA antagonist, haloperidol, suggests that moderate stress can have a long-lasting, sensitizing influence on DA function. Such an influence could be of considerable importance in a stress-related progressive disorder such as schizophrenia.

Supported by USPHS grant MH 24114 to S.M.A.

1545 LACK OF EFFECT OF SELECTIVE SEROTONIN DEPLETION ON BODY WEIGHT AND AMPHETAMINE ANOREXIA. L. A. Baez, R. A. Browning and M. Cusatis. Department of Psychology and School of Medicine, Southern Illinois University, Carbondale, Ill. 62901.

Intraventricular administration of 5,7-dihydroxytryptamine (5, 7-DHT) in rats has been reported to enhance the intake of food and water and to increase body weight (Saller & Stricker, Science, 192, 385, 1976). Moreover, the intraventricular administration of parachlorophenylalanine (PCPA) was reported to produce hyperphagia and elevated body weight (Breisch, et al., Science, 192, 382, 1976). These findings have suggested an inhibitory role for serotonin (5-HT) neurons in regulatory functions. In the present study this question has been examined further by assessing the influence of chronic serotonin depletion on body weight and ingestive behavior in both Long-Evans and Sprague-Dawley rats. Animals were treated with protriptyline (20mg/kg; i.p.) followed two hours later by an intraventricular injection of 5,7-DHT (200 µg) or vehicle. After behavioral testing was completed, all animals were sacrificed and forebrains were assayed for norepinephrine (NE) and 5-HT content. Body weight data from four separate groups of animals are presented below in table form. Numbers in parentheses indicate the number of animals in each group.

	Time after	Weight ± S.E.M.	
Strain	Treatment	5,7-DHT	Vehicle
Long-Evans	24 days	376±9(6)	363±6(10)
Long-Evans	24 days	359±12(10)	377±10(10)
Sprague-Dawley	30 days	326±8(7)	337±5(7)
Long-Evans	1 year	580±19(6)	578±17(7)

In addition, body weight data were obtained for eight weeks, at weekly intervals, in both male and female Long-Evans rats. Again, there were no significant differences between the treated and untreated rats within each sex category. 5,7-DHT treatment also failed to alter the anorexigenic properties of (+)-amphetamine. Forebrain 5-HT was depleted in all groups of treated animals, with values ranging from 66% to 85% depletion relative to vehicle-injected animals. NE was not affected significantly in any group. The present study, then, failed to produce any evidence of increased body weight in animals chronically depleted of brain 5-HT with 5,7-DHT. The lack of a body weight effect in animals at a broad range of post-treatment intervals as well as the lack of change in (+)-amphetamine-induced anorexia, casts serious doubt on the hypothesis that 5-HT neurons exert a major inhibitory influence on ingestive behavior and body weight regulation.

1546 EFFECTS OF NALOXONE ON ACTIVITY AND REACTIVITY IN THE RAT. Gary G. Berntson, Timothy C. Champney*, Thomas S. Paulucci*, and J. Michael Walker. Dept. Psychol., Ohio State University, Columbus, Ohio 43212.

The exogenous administration of the opioid peptides, enkephalins and endorphins, can induce a variety of behavioral effects. The role of these peptides in natural behavioral regulation, however, is not well understood. While stress has been shown to elevate brain levels of opioid peptides and to produce parallel increases in pain thresholds, the tonic activity of these peptide systems is less clear. We have previously shown that naloxone (an opiate receptor blocker) administered to rats in a non-stressful situation decreases pain thresholds as measured by the tail flick test, suggesting some degree of tonic activity in these systems. To further examine the potential tonic behavioral effects of the opioid peptides, we examined the effects of naloxone on a number of measures of activity and reactivity in the rat. We found that naloxone (2mg/kg SC) significantly reduced activity, as measured by an electronic activity counter, for a period of at least one hour after injection. Comparable decreases in activity were seen when naloxone was preceeded by 1) no treatment, 2) a 3 min. warm water (25°C) swim, and 3) a stressful 3 min. cold water (0°) swim. There was no interaction between the stress condition and the decline in activity. In a second set of experiments, naloxone was found to have no effect on the magnitude of the acoustic startle reflex, while it decreased inter-stimulus activity in the same experiment. These data provide further evidence that tonic activity exists in opioid peptide systems, and that these systems may participate in natural behavioral regulation.

1547 A COMPARISON OF THREE ANIMAL MODELS FOR SCHIZOPHRENIA. R.L. Borison, H.S. Havdala* and B.I. Diamond. Ill. St. Psych. Inst. and Anesthes. Dept., Mt. Sinai Hospital, Chicago, IL 60612 & 60608

Amphetamine-induced stereotyped behavior is the well accepted animal model for schizophrenia because it can be blocked by antipsychotic agents and amphetamine abuse in man produces a schizophreniform-like paranoid psychosis. Amphetamine is not alone in these properties, as we have proposed that another central nervous system (CNS) stimulant, phenylethylamine, may actually provide a more accurate model for schizophrenia (Life Science 21: 117, 1977). We now report a comparison of the behavioral effects in rats of amphetamine and phenylethylamine with another CNS stimulant, cocaine. The repeated daily administration of either of these three agents, in a dose which is per se subthreshold for eliciting stereotypy, produces stereotyped behavior which increases in intensity until plateauing after approximately 3 - 5 weeks. This stereotypy is characterized by head-swaying, forepaw treading and stereotyped sniffing in animals. In animals receiving cocaine a significant amount of locomotor activity is also present. The latency to onset of these behaviors was from 2 to five minutes after injection and the duration of phenylethylamine stereotypy was 30 minutes, whereas that for cocaine and amphetamine was over two hours. The administration of the potent dopamine blockers haloperidol or pimozide completely block stereotypy induced by either phenylethylamine or d-amphetamine, while markedly antagonizing the actions of cocaine. Clozapine, a dopamine blocker with few extrapyramidal effects, selectively blocked phenylethylamine and cocaine behavior, while having no significant action upon d-amphetamine. Thioridazine, another antipsychotic agent with a low index of extrapyramidal side-effects, selectively blocked only phenylethylamine stereotypy. Two alpha-adrenergic blocking agents, phentolamine and phenoxybenzamine, antagonize phenylethylamine stereotypy, whereas only phentolamine antagonizes cocaine and neither affects d-amphetamine behavior. A serotonin blocker, methysergide, selectively blocks only phenylethylamine stereotypy. Although all three compounds, phenylethylamine, d-amphetamine and cocaine can precipitate toxic paranoid schizophrenic-like syndromes in man, only phenylethylamine-induced behavior, as shown in our animal model, can be effectively blocked by a range of antipsychotic agents.

1548 OPIOIDS AND PRESSING FOR HYPOTHALAMIC INTRACRANIAL STIMULATION (ICS) IN RATS. Michael A. Bozarth and Larry D. Reid, Depts. of Psychol., Concordia University, Montreal, Quebec, Canada, and Rensselaer Polytechnic Institute, Troy, NY, USA, 12181.

This presentation summarizes the results of several studies testing the effects of various opioids on pressing for ICS. Rats were prepared with a chronically indwelling bipolar electrode. Histological analyses, after behavioral testing, have confirmed that sites of ICS were in the lateral hypothalamic region, e.g., medial forebrain bundle, Forel's fields, zona incerta.

After at least 5 days post surgery, each rat was trained to press a lever to obtain ICS in a typical ICS-apparatus where each lever depression resulted in a single train of ICS (60 Hz sine waves; .3 sec duration; individualized intensities, but always less than 50 µA, rms).

After stable pressing for ICS was established (2 trials/day), 65 rats were given morphine (10 mg/kg/day, s.c.) for 9 days and tested 2 and 4 hr later. After 6 days of tests without drugs, one group was again tested with morphine (10 mg/kg/day) across 13 days while other groups received equianalgesic doses of other opioids: leverphanol (2 mg/kg), methadone (8 mg/kg), codeine (120 mg/kg), and nalorphine (10 mg/kg). These multiple tests provide an opportunity for a drug to produce an increment in pressing if the drug has that capability. Additionally, there have been tests with pentazocine (40 mg/kg), naloxone (1 and 10 mg/kg), and nalorphine (10 mg/kg) before and after opioid administration.

Morphine in a variety of doses (including the standard 10 mg/kg dose), levorphanol, and methadone produced reliable increments in pressing. Neither nalorphine, pentazocine, nor naloxone led to reliable increments in pressing following initial injections of these agents. Pentazocine did, however, lead to reliable increases in pressing among rats that had previously received considerable quantities of either morphine or pentazocine. Nalorphine produced only slight increments in pressing even subsequent to considerable exposure to opioids. Naloxone has not reliably modified pressing for hypothalamic ICS among opioid-free subjects, but did decrease the pressing of those rats whose pressing rates were augmented by opioid administration. Because the drugs that human beings are apt to abuse are those that increased pressing for ICS in rats and because previous opioid-use seems to potentiate human abuse of pentazocine as well as increasing pressing for ICS under pentazocine in rats, it is suggested that drugs which increase pressing for ICS have a high abuse potential.

1549 TAIL PINCH-STRESS ACTIVATES NIGROSTRIATAL DA NEURONS AND BEHAVIOR: THE REVERSAL OF BOTH EFFECTS BY CERVICAL PROBING. A.R. Caggiula*, L.A. Chiodo*, S.M. Antelman* and C.G. Lineberry* (SPON: C. Malsbury). Departments of Psychology, Psychiatry and Pharmacology. University of Pittsburgh, Pittsburgh, PA 15260.

Non-painful tail pinch and related manipulations can reliably induce or potentiate a variety of biologically significant, active motor responses, including eating, gnawing, maternal behavior and male copulatory behavior in rats. Comprehensive pharmacological analyses strongly suggest that tail pinch-induced behavior is critically dependent on unimpaired function of nigrostriatal dopamine (NSDA) neurons (Antelman & Caggiula, 1977).

We now report evidence for a direct relationship between tail-pinch and NSDA function. That is, mild, undulating pressure (1-2 min.), when applied to the tail of urethane anesthetized female rats in a manner which almost invariably induces behavior in awake animals, increased, and often doubled the firing rate of extracellularly recorded units in the zona compacta of the substantia nigra, the origin of the NSDA pathway. In addition to confirming York's (1976) recent report that tail pinch increases cell discharge in the pars compacta, our data more clearly implicate the NSDA in this effect since cells responding to tail pinch exhibited spontaneous firing rates (1-7 Hz) within the range reported by Bunney and Aghajanian as characteristic of this system, and their baseline activity could be suppressed by i.v. amphetamine (1-2 mg/kg) and subsequently reinstated by haloperidol (.1-.2 mg/kg).

We have also obtained evidence which suggests that tail pinch-activation of NSDA neurons is directly related to its behavioral effects, by showing that a stimulus which suppresses tail pinch-induced behavior also blocks its effect on NSDA activity. The stimulus used was vaginal-cervical pressure, since this stimulus inhibits active motor responses (Komisaruk, 1974), and blocks tail pinch-induced feeding in awake animals (Naggar and Szechtman, in preparation and our own unpublished observations). A reciprocal relationship between tail pinch and cervical probing is further supported by the finding that, just as tail pinch-behavior is reduced by drugs which block DA function, the same drugs potentiate some of the behavioral effects of cervical stimulation (Crowley et al., 1977).

In the present study, pressure against the cervix, induced by a glass rod, completely blocked the activation of presumed NSDA neurons if applied concurrently with tail pinch, or brought their firing rates back to baseline if applied after the onset of tail pinch. Continuation of the tail pinch after removal of the rod again activated the cell whereas cervical stimulation, by itself, had little effect.

This study appears to represent the first demonstration that stress can increase the firing of verified NSDA neurons.

1550 BLOCKADE OF INTRACRANIAL SELF-STIMULATION BY HIGH DOSES OF AMPHETAMINE IS LINKED TO THE OCCURRENCE OF HYPERTHERMIA. Robert J. Carey. VAH at Syracuse, Syracuse, NY 13210 USA

Many studies have shown that low doses of d-amphetamine facilitate but high doses suppress responding for rewarding brain stimulation. The low dose facilitative effects of amphetamine are generally considered supportive of, and consistent with, a large body of evidence which implicates brain catecholamines in the mediation of brain stimulation reward. While high doses of amphetamine have a predominant dopaminergic action any attempt to relate this dopaminergic effect to brain stimulation reward is obscured by the disruptive response stereotypy syndrome evoked by a high dose of amphetamine. Interestingly, recent studies on the thermoregulatory dysfunction produced by a high dose of amphetamine indicate that it may be possible to administer a high dose of amphetamine without eliciting the stereotypy syndrome since the stereotypy syndrome emerges only when the ambient temperature is sufficiently high to trigger a hyperthermic response. Accordingly, the present study compares the effect on brain stimulation reward of d-amphetamine when testing is conducted under a normal versus a cooled laboratory environment. Seven rats with medial forebrain bundle electrode implants which supported self-stimulation were administered high (5-10 mg/kg) doses of d-amphetamine. In one set of tests the chamber temperature was cooled to 12° C. in order to prevent hyperthermia. In other tests, however, the chamber temperature was at the normal laboratory temperature of 24° C. which was conducive to an amphetamine-induced hyperthermic reaction. When hyperthermia was prevented by chamber cooling the rats given amphetamine not only responded for brain stimulation but also acquired differential response rates to stimuli which signalled stimulation versus non-stimulation. In contrast, when tested at 24° C. hyperthermia invariably developed and the rats failed to respond differentially to the presence or absence of brain stimulation. Also, the response rates of the hyperthermic rats particularly at the 10 mg. dose level became erratic and unreliable. Thus, the interference with intracranial self-stimulation by high doses of amphetamine appears to be closely linked to the occurrence of hyperthermia.

1551 STIMULUS-ELICITED INVESTIGATION IN THE AMPHETAMINE-TREATED GERBIL AS A MODEL SYSTEM FOR PSYCHOTIC DISORDERS. MaryLou Cheal. Neuropsychology Lab., McLean Hospital, Belmont, MA 02178.

Amphetamine-induced stereotypies reported in the gerbil (Cheal et al., Behav. Biol., in press) are analogous to stereotypic behaviors emitted in human amphetamine psychotics and schizophrenics. Following injections of large doses of d-amphetamine, gerbils (like other species) develop repetitive motor patterns and social behavior ceases. Another analogy to schizophrenic behavior would be the demonstration of "passive" attention. In spite of a lack of overt interest in surroundings, schizophrenics may later report events to which they had appeared oblivious at the time. Bleuler (Dementia Praecox, Internat. U. Press, 1950) called this behavior "passive" attention in contrast to active attention, or seeking of stimuli. An analogous test for "passive" attention was made in amphetamine-treated gerbils using stimulus-elicited investigation. In this paradigm, a novel stimulus elicits investigatory behavior in a series of one minute trials. Following the initial response on the first trial, the investigation habituates on the second trial. Habituation on the second trial has been exhibited with intervals between the two trials from 60 sec to 2 weeks indicating a long-term memory process (Cheal, J. Biol. Psychol., in press). Gerbils were given single injections of d-amphetamine (4.0-6.0 mg/kg) and were observed 45 min later during one trial with a novel object. Due to competing stereotypies, on this trial an average of less than 2 sec was spent investigating the stimulus. Twenty four hours later, when given a second trial, little responding occurred, indicative of habituation. The effect was not due to residual effects of amphetamine as gerbils treated on Day 1 with amphetamine, but not exposed to the novel stimulus, showed normal investigation on Day 2.

This model system was used to study the gerbils' gradient of response to the location of an object following a low dose of amphetamine (1.0 mg/kg) that did not elicit stereotypies. Normally, when the gerbil has habituated to the novel object, investigation can be renewed, or dishabituated, by moving the object 45° or 90°. The amphetamine-treated gerbils demonstrated dishabituation only if the object was moved 90°, not 45°.

The data indicate that an amphetamine-treated gerbil displays behaviors analogous to those observed in human amphetamine psychotics and schizophrenics and that the neurochemical changes caused by the drug treatment result in a broadened generalization gradient of localization of an object.

(Supported by the Scottish Rite Schizophrenia Research Program, N. M. J., U. S. A. and by the Biomedical Research Support Program, D. R. R., N. I. H.)

1552 ESTROGEN MODULATION OF SPIROPERIDOL-INDUCED CATALEPSY AND AMPHETAMINE STEREOTYPY IN THE RAT. L.A. Chiodo* and A.R. Caggiula* (SPON: D. Asdourian). Psychobiology Program, Depts. of Psychology and Pharmacology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Evidence from both the clinical and experimental animal literature suggests that sex and/or hormonal condition can significantly influence the behavioral consequences of drug-induced alterations of brain DA activity. For example, women have been reported to show a greater degree of motor disturbances after antipsychotic drugs. The possible role of gonadal hormones in this difference is suggested by the finding that the higher incidence of chlorpromazine-induced catalepsy displayed by female, when compared to male rats, was abolished by ovariectomy (Mislow and Fridhoff, 1973).

In the present study, the potentiating influence of estrogen on neuroleptic-induced catalepsy in female rats was confirmed under conditions in which both drug and hormonal effects were clearly specified. That is, ovariectomized (OVX) female rats that received estradiol benzoate (EB) (100 ug/kg s.c.) 48 h before the test showed significantly longer durations of catalepsy than OVX/oil controls in response to 250 ug/kg of the specific DA receptor blocker, spiroperidol. Catalepsy was tested at 14 time points (7 hours) by measuring the step-down latency after the animal's hind quarters were placed on an elevated, wooden block.

Although these results suggest that estrogen may exert a behaviorally relevant influence on DA function, its effects are likely to be neither simple nor direct. That is, EB's potentiation of catalepsy, obtained at some doses of spiroperidol, was actually reversed at others. While it is possible that hormone-induced alterations in peripheral drug metabolism may have contributed to some of these changes, the additional finding that EB drastically altered the short-term (5-10 min.) development of catalepsy in response to intraventricularly administered spiroperidol (20 ug/25 ul) suggests a central mechanism as well.

The foregoing suggests that estrogen's effect on spiroperidol-induced catalepsy is critically dependent on the dose of the drug. An additional study indicates that the type of drug, and perhaps the topography of the behavior being tested, are also decisive factors. That is, the same EB treatment greatly increased the intensity of amphetamine (3 mg/kg)-induced stereotypy, when compared to OVX/oil treated controls.

These results point to the importance of taking hormonal conditions into account when assessing drug effects on DA function and behavior, and suggest a potentially fruitful new direction of research into endocrine modulation of neurotransmitter function.

1553 ACTIVITY OF Δ^9-THC SIDE CHAIN ANALOGUES. H. Dix Christensen, W. C. Goad*, M. Perez-Reyes* and M. E. Wall*, Dept. Pharm., OUHSC, Okla. City, OK 73190, Dept. Psych. UNC, Chapel Hill, N.C. 27514 and RTI, Research Triangle Park, N.C. 27709.

The relative potency, any unusual physiological or behavioral effects, and an estimate of relative safety were determined for a series of Δ^9-THC analogues with side chain hydroxylation. The profile of activity was determined using 130 CF-1 male mice, 29.2 ± 0.3 gms, by a slight simplification of Irwin's procedure (Psycho pharm. 13, 222, 1968). In addition, rectal temperature was recorded at the end of each observational period of control, 10, 20, 30, 45, 60, 90 and 120 minutes post administration intravenously through a tail vein. The vehicle consisted of 5% ethanol, 5% Emulphor EL620 and water, which was inactive at 0.3 ml/10 gms, with 0.1 ml/10 gms the normal injection volume. The relative potency was determined by the minimum dose that would cause a significant decrease in spontaneous activity with marked depression.

Compound	Decrease in activity with depression				Relative Potency	Minimum Lethal Dose
	Dose mg/kg	Onset	Peak Minutes	Duration		mg/kg
Δ^9-THC	4.0	1	15	100	1.0	24
11-OH-Δ^9-THC	2.0	1	8	60	2.0	>48
1'a-OH-Δ^9-THC	24.0	5	15	20	0.17	?
1'b-OH-Δ^9-THC	64.0	10	45	80	0.06	?
2'-OH-Δ^9-THC	16.0	0.5	10	45	0.25	32
3'-OH-Δ^9-THC	0.4	0.5	10	60	10.0	16
4'-OH-Δ^9-THC	16.0	0.5	10	60	0.25	48
5'-OH-Δ^9-THC	24.0	1.5	10	45	0.17	64

The relative potency varied slightly if hypersensitivity to tactile stimuli, crouched posture and gait, a sensorimotor response such as visual placing or coordination, or respiratory arrest were compared. At the depression dose, all of the compounds had a maximum decrease of about 4° in rectal temperature. This occurred at 25-30 minutes with essentially normal temperature for all except Δ^9-THC at 120 minutes. If death occurred it was within the first 30 min. by respiratory arrest. No long term behavioral effects were observed from a single dose. The hydroxylated side-chain Δ^9-THC analogues did not form a progressive series of responses. The two 1'epimers were relatively inactive with a slow onset, the 4'OH and 5'OH analogues had a change in their properties having an ataxia gait rather than the Δ^9-THC narcotic type gait. 2'OH Δ^9-THC was the most similar to Δ^9-THC. The potency of the 3'OH analogue may be due to an extra ring created by hydrogen bonding of the 3'OH to the 5 oxygen. Pathological examination indicated no gross or significant light microscopic findings. Supported in part by Medsera Inc. C7704.

1554 EVIDENCE FOR PHARMACOKINETIC AND PHARMACODYNAMIC TOLERANCE TO PENTOBARBITAL FOLLOWING BRIEF CHRONIC PENTOBARBITAL ADMINISTRATION TO RATS. R.L. Commissaris* and R.H. Rech. Dept. of Pharmacol., Mich. State Univ., East Lansing, Mich. 48824

Female Sprague-Dawley rats, trained on the rotarod (RR), were fed ground chow containing pentobarbital (PB; 2.0 mg/g chow) and were given twice-daily i.p. injections (30 mg/kg PB) for 6 days. Controls were given ground chow and distilled water injections. On day 7 the animals were injected i.p. with various test doses of PB and tested on the RR 5, 15, 30, 60 and every 30 minutes thereafter until recovery (180 seconds on RR) or sacrificed at various times post-injection by decapitation and assayed for PB in brains and sera by gas chromatography. Chronic PB treatment significantly reduced the duration of RR disruption following all i.p. test doses of PB as compared to controls. Using the time to 50% recovery of RR, we found that chronic PB treatment resulted in a significant shift to the right of the dose-response curve for PB-induced RR disruption. Body levels of PB 35 minutes after administration of 30 mg/kg PB (time to 50% recovery in chronic rats) were lower in the chronic PB-treated animals than in controls, indicating an enhanced removal of the drug due to the chronic treatment. Measuring the body levels of PB in these groups at their respective times to 50% recovery of RR following 20 mg/kg PB, we found that chronic PB-treated rats had significantly higher levels of PB in brains and sera as compared to controls (time to 50% recovery in controls = 330 minutes). This data indicates a decrease in central sensitivity to PB following chronic PB treatment. Thus, there is evidence for both pharmacokinetic and pharmacodynamic tolerance to PB following brief chronic PB administration. (Supported by NIDA Contract ADM-45-74-146.)

1555 Reversal and Prevention of Acute Morphine Induced Catalepsy by Phenytoin in Naive Rats. Susan L. Cookson* and J. Douglas Mann. Department of Neurology, University of North Carolina, 27514

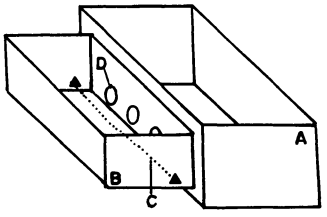
Catalepsy is a state of prolonged motor immobility, waxy rigidity, and an apparent increase in alertness despite reduced responsiveness to stimuli. Morphine induced catalepsy in rats has been well described, though the neurochemical basis of this phenomenon is poorly understood. Chronic administration of morphine results in depletion of calcium in brain, possibly producing instability of endplate regions and increased neurotransmitter release. To determine if altered calcium metabolism is important in acutely induced morphine catalepsy, phenytoin, an agent known to decrease calcium conductance and increase membrane stability was administered to rats prior to, or following, intravenous morphine sulfate.

A chronic indwelling venous catheter was placed in adult albino rats, not previously exposed to morphine, two days prior to experimentation. Groups consisting of six animals were tested for catalepsy using a standard foot/bar test (Kuschinsky and Hornykiewicz, Eur. J. Pharmacol. 19: 119-122, 1972), a test for duration of sustained motor immobility. Experimental protocol consisted of the intravenous administration of: 1. morphine sulfate in saline, 1 mg/kg (a dose adequate to produce catalepsy for more than two hours); 2. phenytoin 35 mg/kg (all phenytoin was administered in a carrier of 40% propylene glycol and 10% ethanol); 3. morphine followed fifteen minutes later by phenytoin; 4. phenytoin followed fifteen minutes later by morphine; 5. saline alone; 6. phenytoin carrier alone; 7. phenytoin carrier followed by morphine; and 8. morphine followed by phenytoin carrier. Within fifteen minutes of administration, phenytoin was markedly effective in reversing morphine induced catalepsy ($p < .001$). Phenytoin also prevented the appearance of catalepsy when given fifteen minutes prior to morphine ($p < .001$). Administration of phenytoin, carrier alone, or saline alone did not produce significant changes in motor activity. Additionally, phenytoin carrier or saline had no effect on the expected cataleptic response when given in combination with morphine.

Morphine induced catalepsy in rats is both prevented and reversed by phenytoin. Morphine and phenytoin may have opposing effects on calcium linked neurotransmitter release at the presynaptic membrane. While phenytoin reduces membrane calcium conductance, morphine may produce increased calcium flux with each depolarization, resulting in enhanced release of neurotransmitter. This view is consistent with the previously reported increased turnover of dopamine in association with morphine catalepsy.

1556 EFFECT OF CHRONIC LOW LEAD EXPOSURE ON THE DEVELOPMENT OF EXPLORATORY BEHAVIOR IN RAT PUPS. Kevin M. Crofton*, Douglas H. Taylor*[1], and Richard J. Bull. U.S. E.P.A., Cincinnati, OH 45268; [1]Miami Univ., Oxford, OH 45056

Exploration away from the mother was studied in male offspring of female rats which had been administered lead (200 mg/l) via their drinking water from two weeks prior to breeding until their pups were weaned. The mean blood lead levels of the lead exposed pups at 21 days of age was 36 ug/100 ml ± 3.4 S.E. Pup exploration away from the dams was monitored continuously (see figure below), beginning when the pups were 10 days old and ending when they were 21 days old. No significant differences were noted in the time of eye opening between the control and experimental groups. A statistically significant delay in the development of exploratory behavior was noted between the lead treated and the control animals. Additionally, the activity levels of lead treated pups were depressed, and significantly different, from the control pups from day 14 through day 19. By day 20, and through day 21, the activity levels of the treated and untreated pups were no longer significantly different. The lead induced modification in behavior noted in this experiment correlates temporally with biochemical and morphological indications of delayed cerebral cortical development as evidenced by delayed increases in cytochrome content and synaptic development (McCauley and Bull, Fed. Proc. 37:2764, 1978).



A - Home Cage
B - Exploration Cage
C - Infrared Monitor
D - Exploration Holes

1557 ANTAGONISM OF METHYLPHENIDATE-INDUCED STIMULATION BY SPIROPERIDOL. Michele A. Cusatis and Luis A. Baez. Dept. Psych., S. Ill. U., Carbondale, IL 62901

It has been reported that methylphenidate-induced stimulation can be effectively antagonized by pretreatment with reserpine but not by AMPT pretreatment; the reverse is true of arousal behavior produced by amphetamine (Scheel-Kruger, Psychiat. Neurol. Neurochi., 1972). These findings have been taken to reflect differential release of stored versus newly-synthesized catecholamines, respectively. This apparent difference in mechanism of action led us to investigate the possible existence of other dissimilarities in the neurochemical actions of these stimulants.

Previous research in our laboratory has demonstrated that a very low dose of the dopamine receptor blocking agent, spiroperidol, can prevent the development of amphetamine-induced locomotion and stereotypy (Baez, Kerns & Smith, Eur. J. Pharm., 1977). These data are consistent with other evidence suggesting that amphetamine influences behavior primarily through an interaction with dopamine neurons. The present study was designed to determine whether spiroperidol would also block the development of the behaviors generally elicited by methylphenidate.

Adult male Long-Evans rats were placed in stabilimeters and allowed to habituate for 30 minutes. Following habituation, animals were injected with a dose of spiroperidol (0.0, 0.03125, 0.25 or 1.0 mg./kg. i.p.). Thirty minutes later methylphenidate was administered (0.0, 5.0, 10.0 or 30.0 mg./kg. i.p.). A total of eight animals received each spiroperidol-methylphenidate combination, and animals were used only once.

Locomotion and stereotypic behaviors were observed for 2 hours after methylphenidate injection; observations were made of each animal during a one minute period every five minutes for a total of 24 such periods in a two hour session. Within these one minute periods, observations were scored at 10 second intervals in a dichotomous presence/absence fashion. The behaviors observed were: walking, rearing, sniffing, licking, nosing, head nodding, gnawing, grooming and jaw opening. Locomotion was scored as stabilimeter crossings.

Scores were subjected to factor analysis and the resulting factors were used as input data for analysis of variance tests. Both the intermediate and high doses of spiroperidol were effective in preventing the appearance of methylphenidate-induced locomotion and stereotypy, even with the highest dose of methylphenidate examined. These data suggest that methylphenidate produces its behavioral stimulation via an enhancement of dopaminergic synaptic transmission. In this respect, methylphenidate and amphetamine appear to be functionally similar.

1558 GABA BLOCKADE: THE EFFECTS OF PICROTOXIN ON BIDIRECTIONAL ACTIVE AVOIDANCE, LOCOMOTOR ACTIVITY AND STRAIGHT ALLEY PERFORMANCE Robert E. Davis, David Wirtshafter, Karen Asin and Ernest Kent Dept. of Psych., Univ. of Ill. at Chicago Circle, Chicago, Ill.

Picrotoxin (Pic), a putative GABA antagonist, has been previously reported to depress bar pressing for electrical stimulation of the lateral hypothalamus (ESB-LH). To determine if this deficit was specific to ESB-LH, we examined the effects of this compound on bidirectional active avoidance (BAA), locomotor (LA) activity in the jiggle cage (JC), open field (OF), and closed field (CF), and straight alley performance (SA, after 8 and 24 hrs deprivation, early and late in training). Additionally, we studied the effects of prior amphetamine (Amph, 2 mg/kg),lysergic acid diethylamide (LSD, 10 ug/kg), diazepam (Diaz, 10 mg/kg), methysergide (MS, 10 mg/kg) and cinanserin (Cin, 10 mg/kg) administration on Pic-induced behavioral changes.

BAA acquisition was impaired by pretreatment with picrotoxin (.75 and 1.4 mg/kg: 50 trials/day, 200 trials over 16 days). However, this appears to be a performance deficit since animals trained under Pic immediately performed at control levels when switched to saline. BAA performance was maximally depressed by 1.5 mg/kg Pic. This deficit could be reversed by prior administration of Ampt, LSD or Diaz, but was unaffected by MS or Cin.

Locomotor activity was also depressed by picrotoxin in a dose dependent manner. The three testing environments yielded different dose-response relationships. LA exhibited in the OF or CF was more sensitive to the depressant effects of Pic than was activity in the JC. Rearing in the open field was also severely reduced reduced. Amph reversed the activity activity decreases in the JC but was not tested in the other environments. Both LSD and MS did not influence the Pic induced LA deficit.

Picrotoxin (up to 2 mg/kg) was not effective in depressing well trained approach responses in the SA under 24 hour food deprivation conditions. Yet, if the deprivation level was lowered or if animals were tested during the early trials of training, running speed was decreased in a dose dependent manner.

This evidence suggests that Pic produces a general behavioral depression which is reversed by environmental and pharmacological manipulations resulting in increased arousal. These picrotoxin-induced behavioral changes are similar to those seen after antagonism of dopamine systems by haloperidol and may be mediated through similar biochemical mechanisms. However, distinct functional mechanisms can not be clearly established, since Pic may be simultaneously influencing a number of diverse behavioral and biochemical systems.

1559 CAUDATE LESIONS CHANGE THE BEHAVIORAL EFFECTS OF MORPHINE IN CATS. I. de Andrés*,J.R.Villablanca and Ch.E.Olmstead. Depts. Psychiat., Anat. and MRRC, UCLA, CA. 90024

The behavioral effects of low doses(1-3mg/kg,i.p.) of morphine SO_4(MS)were evaluated on cats with bilateral caudate aspirations (BAc N:8)and intact controls(N:6). Comparisons were made with cats with removals of both cerebral hemispheres(diencephalic,N:4),the caudate unilaterally(UAc N:2),frontal cortices(BFr N:3)or the septum (SEp N:2). MS was administered 2-6 times per animal at at least 15 day intervals.Observations were made via a one-way mirror in a sound attenuated chamber for 1 hr prior and 5 hrs after MS. Two min samples were videotaped every 15 min and selected items(i.e., basic postures and discrete behaviors)were later quantified using an event recorder.Intact animals showed a 3-phase response: I)2-4 min after MS they had licking and swallowing which usually ended in retching and vomiting; II)For the next 10-20 min depending on the dose, they adopted a sitting posture or, less frequently,they crouched or laid down attentively with fixed gaze and pinna movements,Mydriasis was present; III)Usually without changing their posture they began to look around,both laterally and up and down with intervals of fixed gaze,pinna movements and vocalizations as if they were exploring or tracking imaginary objects.After the first hour, with doses larger than 1 mg/kg,they showed lateral and forward forepaw and back and forth body movements.The head movements peaked between 2-3 hrs and the amount was dose dependent.These events had clearly attenuated after 4 hrs when cats usually laid down but remained alert.UAc,BFr, and SEp cats showed,with slight differences,similar behavior patterns.BAc cats were indistinguishable for the I and II stages.However, stage III was different.They showed increased unspecific motor activity.Most started walking incessantly and occassionally stopped briefly and looked around or exhibited fixed gaze.Upon stopping some were extremely restless with head and body movements.Panting was frequent.Both the latency to onset of locomotor activity and its duration were dose dependent.Compared to intacts the BAc had a significant decrease in the frequency of head movements, the duration of fixed gaze episodes and total sitting time.Although histological studies are not completed,a direct correlation appears to exist between amount of"looking around"and the amount of remaining caudate tissue.Diencephalic cats did not show these head movements. In conclusion:1)we saw no feline"morphine mania";2)caudatectomy seems to eliminate the marked apparently visually determined behavior of intacts;3)the results suggest that the forebrain and, particularly, the striatum mediates these morphine behavioral effects. This contrasts with our findings relative to the action of amphetamine following similar brain lesions.A 16 mm film will be presented. (USPHS grants HD-05958, MH-07097, HD-94612 and Fellowship of MEC, Spain)

1560 DIFFERENTIAL EFFECTS OF METHYLPHENIDATE AND AMPHETAMINE ON THE ORIENTING BEHAVIOR OF GERBILS. J. Diaz,R. Bien* and A. McClelland*; Depts of Neurology and Psychiatry,NPI,Sch Med,UCLA,LA,CA 90024.

Numerous studies indicate that stimulants improve attention in hyperactive children as well as in normal children (Rapoport et al, Science,199,560,1978) and adults (Sroufe and Stewart, N Eng J Med, 289,407,1973). This increase in attention span can be produced by various stimulants in spite of the different central pharmacological actions of these drugs. The purpose of the present study is to examine the effects of low doses of two stimulants on the orienting behavior of an active animal--the Mongolian gerbil (Meriones Unguiculatus).

Twenty-three adult gerbils were divided into three groups: a vehicle group (n=8), a methylphenidate group (n=8), and an amphetamine group (n=7). Each animal was tested once a week for six weeks in a plexiglass shuttle box. For each 15 minute session the number of crossings, as well as the number of rears and the time spent rearing, was recorded by trained observers. For the fifth session, the animals were injected thirty minutes prior to the testing with either saline, or methylphenidate (1 mg/kg, ip), or d-1 amphetamine (1 mg/kg, ip).

Prior to drug administration, there were no differences between the groups. The administration of either amphetamine or methylphenidate just before testing produced opposite effects on orienting behavior. The gerbils which received methylphenidate consistently spent more time rearing than the vehicle treated animals ($t=5.52$, $p<.001$): whereas, the gerbils which received amphetamine consistently spent less time rearing than the control animals ($t=10.75$, $p<.001$). In addition, during minutes four through seven of the testing session, animals given methylphenidate reared more often than the control animals ($t=3.36$, $p<.001$) and animals given amphetamine reared less often than control animals ($t=8.06$,$p<.001$). In contrast to orienting behavior, the number of crossings for the two drug groups was not different than that for the control group. There were no differences between the groups one week after drug administration.

There is mounting evidence that the attentional improvements produced by small doses of stimulants, once thought to be a peculiarity of a hyperactive organism, may instead be a general characteristic of normal central arousal and inhibitory systems. Even though this may be the case, the effects of methlyphenidate and amphetamines on the orienting behavior of gerbils are different than those observed in the orienting behavior of the rats given the same drug treatments (Bryan and Ellison, Psychopharm,43,1975). Since rats have a much lower activity level than gerbils, these data suggest there may be fundamental differences in central systems between animals that have normally high activity levels compared with those that do not.

1561 HUMAN FEMALE CHRONIC MARIJUANA USE AND ENDOCRINE FUNCTIONING. Rhea L. Dornbush, Robert C. Kolodny,* Joan E. Bauman,* and Sandra K. Webster.* Reproductive Biology Research Foundation, St. Louis, MO 63108.

The relationship between chronic marijuana use ($\bar{x}$ = 4x/week) and reproductive hormones was evaluated by comparing 26 women (18-30 years of age) who used marijuana with 16 age-matched controls. All subjects were in good general health, did not use oral contraceptives, and were not using other drugs. Subjects were evaluated by physical examination, personal interview, daily written self-reports of drug use, mood, sexual activity, and intercurrent health problems; and blood samples obtained on cycle days 1, 5, 11-19, 25, and 30 (analyzed for estrone, estradiol, progesterone, testosterone, luteinizing hormone (LH), follicle-stimulating hormone, and prolactin). Most subjects (37 of 42) were studied for two complete menstrual cycles. Cycle length was significantly shorter for marijuana-using women (26.8 ± 2.3 days vs. 28.8 ± 2.3 days). Prolactin levels were consistently and significantly lower and testosterone was consistently and significantly higher in marijuana users on all sampled days during the menstrual cycle. There were no significant differences in other hormone measurements. These data do not support the female animal data, which -- although sparse -- suggest depressed fertility via the mechanism of suppression of ovulation. In the current study there were no statistically significant differences in the number of anovulatory cycles or LH peaks.

1562 CHANGES IN TELENCEPHALIC SELF-STIMULATION RATE RESULTING FROM PRIOR SELF-STIMULATION EXPERIENCE AND FROM CHRONIC HALOPERIDOL. David C. Douglin* and Robert B. Glassman, Dept. Psychol., Lake Forest College, Lake Forest, IL 60045

Demonstrations of neuroplasticity may shed light on phenomena of drug tolerance or on dyskinesias that arise spontaneously or as a result of long term drug treatments. Nine rats were prepared with intracranial self-stimulation (ICSS) electrodes at telencephalic (TEL) points (medial frontal cortex, MFC, or caudate nucleus, CN) and 7 of these animals also had successful placements in more caudal (CDL) points (lateral hypothalamus, LH, substantia nigra, SN, midbrain reticular formation, RF). Daily 10-minute sessions were carried out with TEL ICSS either immediately before or after a 10-minute session of CDL ICSS. Parameters were: 0.1 msec negative pulses at monopolar electrode; 100 pulses/sec; 0.2 sec train; TEL current 0.55 ma or 0.74 ma in different rats; CDL 0.2 or 0.45 ma. TEL ICSS was initially slow and steady (138 barpresses/10 min, average of first 5 sessions over all rats) and, in each animal, gradually rose during about 15 daily sessions, leveling off at 453 barpresses/10 min. CDL ICSS showed no such changeability. The longterm increase of TEL ICSS appeared associated (a) with a phase, occurring approximately days 7-12, when cumulative records showed an upturn within a session and (b) with an increased tendency for behavioral seizures, following TEL ICSS, suggesting kindling. Changes in reward value of hippocampal stimulation have previously been attributed to kindling (Campbell et al, Neurosci Abs 535, 1976).

Administration of haloperidol in the drinking water of these rats for a month (0.013 mg/ml) caused a sharp drop in TEL ICSS; following withdrawal, ICSS recovered to predrug level in 3 cases, to 50% above predrug level in one case, and to below (half) predrug rate in 5 cases (3MFC, 2CN). In four of these last cases, reduction of current intensity on 1-3 occasions led to a clear rise in ICSS rate. Since similar probes were not run before drug it is hard to say yet whether this represents a drug-induced heightened sensitivity of the stimulated points. We have a small amount of data for the CDL points during and after drug treatment. Of 5 rats, all showed less decrease in CDL ICSS than TEL ICSS during drug; 4 returned to predrug ICSS rate the day after withdrawal. The rat that had shown the most barpresses during drug pressed at greater than the predrug rate after withdrawal. Eichler et al (Neurosci Abs 1225, 1976; 1400 1977) have reported increased ICSS, following chronic spiroperidol, for points in frontal cortex, SN, and far--but not near--LH.

Supported by the Illinois Department of Mental Health and Developmental Disabilities.

1563 PLATELETS-PROTEIN PHOSPHORYLATION IN SCHIZOPHRENICS WITH AND WITHOUT TARDIVE DYSKINESIA. Y. H. Ehrlich, I. Jackson*, M. V. Reddy*, L. G. Davis and E. G. Brunngraber. Univ. of Mo.-Columbia, Sch. Med. at The Missouri Inst. Psychiatry, St. Louis, Mo. 63139

Several lines of investigation indicate that long-term treatment with neuroleptic drugs results in the development of receptor supersensitivity in the central nervous system. Our animal studies have indicated that the latter may involve alterations in the phosphorylation of membrane-bound proteins, presumably of synaptic origin (Life Sci. in-press). Such changes may occur, therefore, in the brain of chronic schizophrenics in-general, and in patients demonstrating symptoms of tardive dyskinesia, in-particular.

Human platelets and lymphocytes have cyclic AMP-generating systems as well as protein kinases that phosphorylate membrane proteins in a cyclic AMP-regulated fashion. These cells may serve, therefore, as a model for investigating the possible involvement of these enzymatic systems in mechanisms underlying certain mental disorders. We have found that membrane fractions of platelets, RBC and leukocytes prepared from as little as 7 ml of blood are sufficient for examining phosphorylative activity and obtaining the pattern of endogenously phosphorylated proteins (Reddy et al, in-prep.). In the assay, membranes are incubated with radioactive ATP in the presence and absence of cyclic AMP. The reaction products are separated by SDS-gel electrophoresis and specific phosphoproteins are identified by autoradiography of dried gel-slabs (Ehrlich et al, Nature 265: 238, 1977; Neurochem. Res. 2: 533, 1977). In a preliminary study, membrane preparations from platelets of nine female schizophrenic patients, 5 with and 4 without symptoms of tardive dyskinesia, were examined. Endogenous phosphorylative activity directed towards specific proteins in the preparations was found to be greater in 4 of these samples than in the other five. These 4 were then identified as patients with tardive dyskinesia. Quantitative analysis of the phosphorylation of a specific band, indicated that in spite of great variability in the pattern of protein and protein phosphorylation of individual samples, the difference between patients with and without tardive dyskinesia was statistically significant. It should be emphasized, however, that further research is required to determine whether protein phosphorylation systems are involved in mechanisms related to the development of this syndrome. Supported by intramural funds from the Missouri Institute of Psychiatry.

1564 CHRONIC AMPHETAMINE ADMINISTRATION PRODUCES A SENSITIZATION OF SNIFFING AND A TOLERANCE FOLLOWED BY A RETURN OF LICKING BEHAVIOR. A.J. Eichler*, S.M. Antelman* and C.A. Black* (SPON: G. Werner). Psychobiology Program and Dept. of Psychiatry, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

Although chronic administration of amphetamine is known to produce a progressive sensitization of the drug's ability to induce stereotypy, individual components of the stereotypy syndrome (e.g., licking and sniffing) have not been studied in this way. Since it has been suggested that amphetamine-induced licking and sniffing are subserved by different brain dopamine (DA) pathways, this hypothesis was tested in the present studies by examining the profile of these behaviors during chronic amphetamine administration.

Rats were injected i.p. daily for 65 days with amphetamine sulfate (2,4,8 or 12 mg/kg), and both stereotyped licking and sniffing were rated twice weekly. Licking behavior, which was seen following acute injection of the 8 and 12 mg/kg doses, virtually disappeared by the 14th injection day. This tolerance persisted until the 24th injection day when licking behavior gradually began to return. At about this same time, licking also started to occur to doses which failed to elicit this behavior acutely (i.e., 2 and 4 mg/kg).

Stereotyped sniffing displayed a considerably different pattern than licking. No tolerance occurred at any time. Rather, sensitization became apparent beginning on the 17th day of amphetamine injection. Sniffing intensity increased until day 35, whereupon it stabilized until drug treatment was discontinued. This enhanced response to amphetamine involved both the maximum sniffing intensity as well as the duration of the behavior.

The dissimilar profiles of licking and sniffing to chronic amphetamine support the notion that these behaviors involve different brain DA systems. The return of licking behavior following tolerance may also reflect an interaction with norepinephrine (NE) systems since: (1) assays of brain catecholamines one day following termination of amphetamine treatment revealed a significant depletion (approximately 20%) of cortical NE at all doses of the drug and (2) at a point where tolerance to licking had occurred, this behavior could be reinstated by administration of beta NE receptor blockers (propanolol or alprenolol; 6 mg/kg).

Supported by USPHS grant MH 24114 to S.M.A.

1565 SILASTIC TUBING SYSTEMS FOR CONTINUOUS DRUG ADMINISTRATION: CONSTRUCTION AND APPLICATIONS. Michael S. Eison, Gaylord Ellison, and Harris H. Huberman*. Dept. Psychology, UCLA, Los Angeles, CA 90024

Simply constructed, inexpensive, and easily implanted continuous slow-release systems for drug administration can add a much needed dimension to the study of drug effects upon brain biochemistry and behavior. We have developed two such systems based upon the passive diffusion of drug across the semipermeable walls of silastic tubing reservoirs and have used these systems in our research on the effects of continuous amphetamine intoxication on rat behavior and neurochemistry.

One such system is a non-refillable silicone pellet that when filled with 50 mg d-amphetamine base in polyethylene glycol vehicle releases drug for at least 10 days. We recently reported a reliable behavioral syndrome observed in rats implanted with these pellets: initial hyperactivity followed by prolonged stereotypy lasting 48-72 hrs,followed by a late phase of exaggerated social behaviors. We have suggested that the late phase may serve as an animal model of amphetamine psychosis. Implantation of this pellet also induces progressive catecholamine (CA) depletion and alterations in tyrosine hydroxylase (TH) activity. Two days after implant (during maximum stereotypy), brainstem and diencephalic norepinephrine (NE) and striatal dopamine (DA) are significantly depleted; brainstem DA is significantly elevated at this time. Changes in CA levels are paralleled by similar trends in TH activity in these regions;the most pronounced change in TH occurred in the caudate nucleus (48% of control values). Five days after implant (when stereotypies break and animals exhibit exaggerated social behavior), NE is depleted in the brainstem, diencephalon, caudate, and frontal cortex; DA is depleted in the caudate and frontal cortex but remains elevated in the brainstem. Striatal TH remains at 50% of control values. In assays done 110 days after a 7 day period of implantation, CA levels return to control values while caudate TH remains uniquely depressed.

A second silastic system used in the course of our research is a refillable loop with which one can maintain desired levels of drug available for release over longer periods of time; such implants have proven viable for 30 days. Although the time course of behavioral changes observed in loop animals (11 mg/kg/loop twice daily) is somewhat different from that seen in pellet rats, the sequence of changes is remarkablely similar. Brain amphetamine levels of approximately 3.0 ug/g were found after 2 days and 5 days of loop administered drug. Construction of these systems will be discussed more fully at this poster session.

1566 ALTERATION IN STIMULANT-INDUCED MOTOR FREQUENCIES BY CLOZAPINE. Everett H. Ellinwood, Jr., and M. Marlyne Kilbey. Dept. Psychiat. Behav. Neuropharm. Sect., Duke Univ. Med. Ctr., Durham, NC 27710.

Use of a new sensitive motility transducer, which allows reduction of movement to power spectrums, has demonstrated a number of drug-induced changes which cannot be quantified will less sensitive observation methods. Pimozide, a neuroleptic which has little presynaptic activity, blocks 6 mg/kg amphetamine effects in a linear dose-response curve. Clozapine induces a biphasic response potentiation at lower doses and suppression at higher doses. At the same 20 mg/kg dose that maximally potentiates amphetamine stereotyped motility, clozapine blocks direct dopamine agonists and stimulants that act through "reserpine-dependent" storage pools of dopamine (e.g., 40 mg/kg cocaine and 12 mg/kg methylphenidate). The data are consistent with a strong presynaptic (synthesis activation) and moderate postsynaptic activity of clozapine. Examination of the more unique features of the pharmacologic profile of clozapine may provide important clues as to the nature of certain psychotic processes.

1567 EVIDENCE OF DOPAMINERGIC SUBSENSITIVITY AND HALLUCINATORY BEHAVIORS DURING THE LATE STAGES OF CONTINUOUS AMPHETAMINE INTOXICATION. Gaylord Ellison, Michael Eison, Melvin Lyons*, Linda Nelson, and Erik Nielsen*. Dept. Psychology, UCLA, Los Angeles, CA 90024 and University of Copenhagen, Denmark.

Following 4-5 days of continuous amphetamine intoxication produced by implantation of slow-release silicone pellets rats and monkeys enter a late stage characterized by heightened startle responses, socially abberant behaviors (in rats) and hallucinatory episodes (in monkeys) involving distress vocalizations, parasitosis, fleeing responses, and frequent orienting responses. At about the same time fluorescence studies of dopamine fibers in the caudate of rats reveal swollen axons. Rats show a subsensitivity to the motor stereotypies evoked by amphetamine or apomorphine injections just after amphetamine pellet removal, whereas daily injections of the same amount of amphetamine produce heightened stereotypies. Thus, continuous amphetamine intoxication both evokes hallucinatory episodes and has a selective neurotoxic effect on caudate dopamine fibers.

1568 DIFFERENTIAL EFFECTS OF NALOXONE, PENTAZOCINE, CYCLAZOCINE, NALORPHINE, AND MORPHINE ON INTRACRANIAL SELF-STIMULATION IN THE RAT. R.U. Esposito, J.O. Jacobson*, S. McLean* and C. Kornetsky, Boston University School of Medicine, Boston, MA. 02118

Previous research in our laboratory has demonstrated that drugs known for their ability to produce euphoria in man (e.g. morphine, cocaine, amphetamine) will cause a significant lowering of the reinforcing threshold for self-stimulation behavior in rats. (Esposito & Kornetsky, Science, 195:189, 1977; Esposito, Motola & Kornetsky, Pharmac. Biochem.& Behav., in press, 1978). The present report represents an attempt to determine if our method for assessing self-stimulation thresholds could differentiate between opioid drugs with varying capability for producing morphine-like subjective effects. Accordingly, the effects of pentazocine (5-30 mg/kg), cyclazocine (0.01-1.0 mg/kg), and nalorphine (4-16 mg/kg), on self-stimulation thresholds to the medial forebrain bundle were measured. The effects of morphine (1-8 mg/kg) and the narcotic antagonist naloxone (1-4 mg/kg) were also assessed on the same procedure.

Morphine, as reported previously, caused a marked lowering of the threshold, while naloxone had no significant effect. Pentazocine yielded significant threshold reductions which were, however, not as large as those produced by morphine. Nalorphine and cyclazocine generally yielded modest reductions at low doses, and threshold increases or response suppression at higher doses. These results are discussed within the context of the subjective effects and abuse liability associated with these agents.

(Supported by NIDA grant DA 00377 and Research Fellow, NRSA Biological Science Training Program, MH15189, NIMH - RUE and Research Scientist Awardee MH 1759 - CK)

1569 GENETIC DIFFERENCES IN DOPAMINE-MEDIATED SPONTANEOUS AND DRUG-ELICITED BEHAVIORS IN INBRED MOUSE STRAINS WITH DIFFERENT NUMBERS OF MIDBRAIN DOPAMINE NEURONS J. S. Fink, T. H. Joh and D. J. Reis, Laboratory of Neurobiology, Dept. Neurology, Cornell Univ. Medical College, New York, N.Y. 10021

Mice of the BALB/cJ strain have 25% more midbrain dopamine (DA) cells than mice of the CBA/J strain. This difference is reflected in a correspondingly higher activity of tyrosine hydroxylase (TH), the rate-limiting enzyme for dopamine (DA) biosynthesis, in the major terminal field of this DA cell group, the caudate nucleus (Nature 264:654, 1976). We sought to determine if these strains also differ in: (a) TH activity in the mesolimbic and mesocortical terminal fields, (b) a spontaneous behavior (investigatory exploration) which is dependent on DA release in these terminal fields, and (c) the behavioral responses to the presynaptic DA agonist d-amphetamine (AMPH) and the postsynaptic DA agonist apomorphine (APO). TH activity was measured in the frontal and parietal cortices and in micropunches of the nuc. accumbens and olfactory tubercle. TH activity was also measured in the region of the A10 perikarya, the source of DA afferents to the mesocorticolimbic terminal fields. BALB/cJ mice had 64%, 62%, 115% and 23% (all $p < 0.05$) greater TH activity in the nuc. accumbens, olfactory tubercle, frontal cortex and A10 area, respectively, than CBA/J mice. TH activity in the predominantly noradrenergic parietal cortex was the same in the two strains. In the exploration test, mice of the BALB/cJ strain showed more investigation of a novel object than CBA/J mice. AMPH produced locomotion (2.0-5.0 mg/kg) and stereotypy (5.0-20.0 mg/kg) at lower doses in BALB/cJ mice than in CBA/J mice. In contrast, APO (2.0-10.0 mg/kg) produced stereotypy at lower doses in CBA/J than in BALB/cJ mice. A greater number of midbrain DA neurons and higher TH activity in forebrain DA terminal fields in BALB/cJ mice correlates with greater exploratory behavior, increased responsiveness to a presnaptic DA agonist and decreased responsiveness to a postsynaptic DA agonist. Genetically-determined differences in the number of midbrain DA neurons are reflected in differences in spontaneous and drug-induced behaviors which are dependent on DA release or receptor activation in these terminal fields. (Suported by grants NS 03346, NS 06911 and MH 24285).

1570 THE ROLE OF CATECHOLAMINERGIC RECEPTORS IN THE HYPOTHALAMIC CONTROL OF LORDOTIC BEHAVIOR IN THE OVARIECTOMIZED-ESTROGEN PRIMED RAT. Mark M. Foreman* and Robert L. Moss. Dept. of Physiol. and Obst.-Gyn., Univ. Tex. Health Science Center, Dallas, Texas, 75235.

Catecholaminergic effects upon lordosis behavior were studied in ovariectomized(OVX)-estrone primed rats by infusing dopaminergic, α- and β-adrenergic receptor stimulants and blockers via unilateral, 23 gauge stainless steel cannulae implanted into either the medial preoptic area(MPOA,N=44), arcuate-ventromedial area(ARC-VM,N=44) or lateral hypothalamic area(LHA,N=13). In the first experiment, OVX rats were primed with 100-250μg of estrone 48 hrs prior to testing to maintain low preinfusion receptivity(mean lordosis to mount ratio, L/M=.164). The infusion of 200 or 800ng/0.5μl doses of dopamine; norepinephrine; epinephrine; dopaminergic receptor stimulant, apomorphine; α-adrenergic receptor blockers, phentolamine or phenoxybenzamine; and β-adrenergic receptor stimulant, isoproterenol significantly increased lordotic behavior compared to vehicle($p < .005$). Conversely, infusions of dopaminergic and β-adrenergic receptor blockers, haloperidol and propranolol, as well as α-adrenergic receptor stimulant, methoxamine in identical dosages depressed lordotic reflex. None of these agents had any effect when infused into the LHA.

A second experiment evaluated catecholaminergic effects on sexual behavior in OVX rats primed with a 350μg of estrone to maintain high preinfusion receptivity(mean L/M=.884). MPOA or ARC-VM infusions of dopaminergic receptor blockers, haloperidol and α-flupenthixol; βadrenergic receptor blocker, propranolol; or α-adrenergic receptor stimulant, methoxamine(1.0μg/0.5μl) significantly depressed mating behavior compared to vehicle response($p < .001$). However, MPOA or ARC-VM infusions of apomorphine, isoproterenol, phentolamine, or phenoxybenzamine had no significant effect.

A third experiment evaluated the hypothalamic interactions between catecholamines and luteinizing hormone-releasing hormone(LRH) upon lordotic behavior. Comparisons were made among lordotic responses to 0.5μl MPOA and ARC-VM infusions of LRH(50ng); LRH(50ng)-haloperidol(1.0μg); LRH(50ng)-propranolol(1.0μg); LRH(50ng)-methoxamine(1.0μg) and vehicle in OVX rats primed with 100-250μg estrone. Infusions of LRH into either area significantly increased lordotic behavior($p < .001$); whereas the addition of haloperidol, propranolol or methoxamine abolished this response.

These results suggest that MPOA and ARC-VM areas contain neurons which contribute to the efferent pathway mediating lordotic responses and can amplify sexual behavior in response to dopaminergic, β-adrenergic and LRH receptor stimulation and α-receptor blockade. The dopaminergic and β-adrenergic blockade or α-adrenergic stimulation can depress either estrogen- or LRH-facilitated mating behavior. Supported by NSF Grant PCM76-10015.

1571 PIMOZIDE-INDUCED EXTINCTION OF SELF-STIMULATION: A REWARD DEFICIT. K.B.J. Franklin* and S.N. McCoy* (SPON: R. Hirsh). Dept. Psych., McGill Univ., 1205 McGregor, Montreal, PQ, Canada H3A 1B1.

When a rat pretreated with the DA antagonist pimozide is given an opportunity to self-stimulate it commences responding in its usual manner but ceases responding after a few minutes. This cessation of responding is thought to represent extinction through loss of the rewarding properties of brain stimulation but a similar behavior pattern might be the result of excessive fatiguability or some other non-motivational deficit.

It was reasoned that if the pimozide-induced loss of responding were due to loss of reward, it should be possible to bring responding under stimulus control, while if the extinction-like responding were due to a non-motivational deficit, stimulus control should be lost.

Two groups of rats were trained on a schedule brain stimulation reward in which 6 min of VI 15 sec alternated with 6 min of FR 4. After 5 sessions of training the schedule was changed so that the last 3 min of each component was unreinforced. For 1 group the FR 4 component was always signalled by a flashing light (correlated group) and for the other group the flashing light was presented independently of the VI-FR schedule (uncorrelated group). Thus in the correlated condition, after a period of no-light (VI), the flashing light signalled the recommencement of reward on FR 4. In the uncorrelated condition the flashing light had no consequences. After 6, 30-min sessions on the VI, EXTN, FR, EXTN schedule all animals were run to extinction in the absence of the light signal. For half the animals in each group, 'extinction' was produced by pimozide (.25 mg/kg, 4h), for the other half by reduced brain stimulation.

When the flashing light was introduced animals in the correlated group immediately recommenced responding at a high rate and then again extinguished. Animals in the uncorrelated group emitted small bursts of responding at various times following light onset. The same pattern of responding was seen when extinction was induced by pimozide as when induced by vehicle and reduced brain stimulation. It was concluded that responding under pimozide was under stimulus control and that pimozide blocks the rewarding effect of brain stimulation.

1572 EFFECTS OF HALOTHANE ON SCHEDULE-INDUCED BEHAVIOR IN THE RAT. Joseph Garfield* and Ennio Vivaldi* (SPON: R.W. McCarley) Dept. of Anesthesia, Peter Bent Brigham Hosp., and Dept. of Psychiatry Harvard Medical School, Boston, Ma. 02115

Potency of inhalational anesthetic agents is generally expressed as an ED_{50} for not withdrawing from a painful stimulus. This quantal measure neglects the continuum of behavioral effects from the awake state to onset of Stage III (surgical) anesthesia. Hence, comparisons based on the ED_{50} alone can be misleading.

We sought a measure of graded behavioral effect that would obviate this problem by quantitating the degree of narcosis.

Four Long-Evans rats were trained to bar-press for evaporated milk on a fixed-ratio-10 schedule (FR-10). Dose-response curves were obtained in each animal by measuring response rates at several ascending subanesthetic halothane concentrations (0.10-0.34%v/v) until responding ceased. Halothane was chosen because it is a widely used inhalational anesthetic agent, with potent effects on performance at sub-anesthetic concentrations (Cook et al., ASA Abstracts, 625, 1977). Sub-anesthetic concentrations were administered by incremental liquid injection, with gas chromatographic monitoring. Each rat was studied three times, at one-week intervals, to determine whether tolerance developed.

Results: No order effect was seen, indicating that no tolerance occured. On the other hand, the dose-response curves revealed a clear-cut rate-dependency effect. Two animals, with low control rates, had initial depression of response rates at low concentrations, elevations approaching control at mid-range, and, at higher concentrations, depression again until responding ceased. This effect was much less pronounced in those animals with higher control rates. Rate-dependency occurs with "excitatory" agents (amphetamines) as well as "depressants" (barbiturates), but has not been reported with an inhalational anesthetic agent. We postulate that the rate increases we observed in our animals may reflect second-stage anesthetic excitement owing to depression of inhibitory control centers. This is supported by the observation in man that Babinski responses appear during the excitement stage of general anesthesia. We plan further study of this phenomenon using fixed-interval schedules, a more sensitive measure of rate-dependency. Schedule-induced behavior using positive reinforcement is a sensitive index of graded anesthetic effect.

Supported in part by GM 23794, NIGMS.

1573 SEROTONERGIC MECHANISMS IN AGGRESSION. Judith L. Gibbons, Murray Glusman, Gordon A. Barr, Wagner H. Bridger, and Sarah F. Leibowitz. Dept. Psychiatr. Columbia Univ. Col. of Physicians & Surgeons, New York, NY 10032 and Dept. Psychiatr. Albert Einstein Col. Med., Bronx, NY 10461.

Several neurotransmitters appear to be involved in the regulation of aggressive behavior. Among these is serotonin (5-hydroxytryptamine, 5-HT) which may be inhibitory to certain types of aggressive behavior, including predatory aggression. Consistent with this hypothesis are studies indicating that reduction of brain 5-HT activity via tryptophan hydroxylase inhibition, destruction of the dorsal and median raphe nuclei of the brainstem, or a tryptophan-free diet, induces or facilitates muricidal behavior (mouse killing) in rats. In the present investigation further pharmacological manipulation of serotonergic mechanisms were carried out and their effects on muricidal behavior in the rat were determined. Injections of l-tryptophan (25, 50, and 100 mg/kg) inhibited muricide in killer rats in a dose related manner, and at the highest dose significantly elevated whole brain 5-HT and 5-HIAA levels. Similarly, the serotonin reuptake inhibitor, fluoxetine (3.3, 10, and 30 mg/kg) and the 5-HT receptor agonist, quipazine (3, 6, and 12 mg/kg) also inhibited muricide by significantly increasing latencies to attack and kill mice. Conversely, fenfluramine, which is neurotoxic to cells of the raphe nuclei and decreases brain 5-HT (Harvey et al, J. Pharm. exp. Ther., 202, 1977, 581-589) induced mouse killing behavior in nonkiller rats tested 3 days after a single injection of 27 mg/kg fenfluramine. These results provide further evidence for an inhibitory role of 5-hydroxytryptamine in aggressive behavior.

This investigation was supported in part by training grants 1 T32 MH 15174-01 (JLG) and MH 06418 (JLG and GAB) from the National Institute of Mental Health, by an NIH Research Grant MH 22879 and an Alfred P. Sloan Fellowship to SFL, and by funds from the Whitehall Foundation (SFL).

1574 ASSOCIATIVE AND NONASSOCIATIVE EFFECTS OF D-LYSERGIC ACID DIETHYLAMIDE (LSD) ON PAVLOVIAN AVERSIVE AND APPETATIVE CONDITIONING IN THE RABBIT. I. Gormezano* and J. A. Harvey. Dept. of Psychology, University of Iowa, Iowa City, Iowa 52242.

Previous investigations from our laboratories, employing Pavlovian aversive conditioning of the rabbit nictitating membrane response, revealed that LSD produced enhanced acquisition of conditioned responses (CRs). Maximal enhancement occurred at 30 nmol/kg LSD. This study examined whether LSD would also produce enhancement in Pavlovian appetative conditioning of the rabbit jaw movement response. For purposes of contrast two experiments were conducted each involving 10 days of acquisition training to tone and light conditioned stimuli (CSs) at a CS-UCS interval of 800 msec. For one experiment, involving conditioning of the jaw movement response the unconditioned stimulus (UCS) consisted of a 1 cc injection of water over a 300 msec interval into the oral cavity via a permanently indwelling cheek fistula. Sinusoidal jaw movement responses, representing components of the swallowing reflex, were recorded as CRs when they occurred during the 800 msec CS-UCS interval. The second experiment involving conditioning of the nictitating membrane response, utilized a 3 ma, a.c. shock of 100 msec duration as the UCS. Extension of the membrane was recorded as a CR when it occurred during the CS-UCS interval. Animals were injected intravenously with equal volumes (0.4 ml/kg) of vehicle or LSD (30 nmol/kg) thirty minutes before each daily conditioning session. Under these experimental conditions LSD produced a significant enhancement in rate of acquisition of both the conditioned jaw movement and nictitating membrane response relative to vehicle controls. Enhanced acquisition occurred to both the auditory and visual CSs. Moreover, the enhancement in rate of acquisition produced by LSD was reflected not only in the greater overall level of conditioned responding to the CSs but also in a fewer number of trials required to initiate the first CR and to reach a criterion performance of 10 successive CRs. To control for possible nonassociative effects of LSD on the acquisition of CRs, separate groups of rabbits received explicitly unpaired presentations of CSs and UCSs under vehicle and LSD injections. These latter experiments revealed that LSD did not alter nonassociative responding to tones and lights, nor did it alter the base-rate of responding. In summary, LSD produces enhanced acquisition of CRs in both appetative and aversive Pavlovian conditioning preparations, which cannot be attributed to nonassociative effects such as sensitization, pseudoconditioning or altered base rate of responding. This study was supported by USPHS, NIDA, Grant Number DA01759.

1575 TRANSMITTER GATING, ADAPTATION, AND REBOUND IN EXAMPLES OF SENSORY PROCESSING, REINFORCEMENT, AND MOTOR CONTROL. Stephen Grossberg. Dept. Math., Boston Univ., Boston, MA 02215.

Competition between parallel channels whose signals are gated by chemical transducers occurs in cortical sensory processing, motor agonist-antagonist commands, and the control of net incentive motivation, among other topics. This talk discusses a physiological mechanism that can be derived from behavioral considerations, in which intracellular habituation and adaptation, antagonistic rebound in response to novel arousal increments, underaroused and overaroused motivational syndromes, and analgesic effects occur, among others. For example, the mechanism suggests why reducing J units of shock to J/2 units of shock is less rewarding than reducing J/2 units of shock to 0 units, and generalizes this result to predict when reducing J_1 units to K_1 units will be less rewarding than reducing J_2 units to K_2 units. It also suggests how a mismatch between actual and expected sensory data can generate short term memory reset across a field of cortical feature detectors to begin a search for a correct sensory code of the data. The model suggests that a sudden increment in nonspecific arousal, gated by a catecholaminergic transmitter, can do this by driving an antagonistic rebound that causes STM reset. The transmitter model appeared in J. Theoret. Biol., 1969, 22, 325 and is reviewed in Prog. in Theoret. Biol., 1974; the rebound mechanism is derived and applied to problems about reinforcement in Math. Biosci., 1972, 15, 253; about attention and discrimination learning in Internatl. Review of Neurobiol., 1975; and about cortical reset and parallel code search in Biol. Cybernetics, 1976, 23, 187.

1576 MATERNAL CONSUMPTION OF ETHANOL, BARBITAL OR CHLORDIAZEPOXIDE: EFFECTS ON THE BEHAVIOR OF THE OFFSPRING. R. Adron Harris and Jeff Case* (SPON: D. Easton). Dept. of Pharmacol., Univ. of Missouri School of Med., Columbia, MO 65212.

Female Long-Evans rats were given solutions of barbital (1 mg/ml) or chlordiazepoxide HCl (CDP, 1 mg/ml) as their sole drinking fluid. Neither of these treatments reduced fluid consumption as compared to the control rats receiving tap water (H_2O group). Another group was given 5.5% v/v ethanol in a liquid diet; the control group was pair-fed with a diet in which sucrose was substituted isocalorically for ethanol (sucrose group). All treatments were begun 1 week before breeding and were continued until the pups were weaned at 28 days of age. At 21 days of age pups were trained to avoid footshock in a one-way shuttlebox avoidance test and were retested at 28 days of age. At 90 days of age, the male offspring from each litter were trained to press a bar 20 times (FR20) in order to receive a food pellet and their response rates during 20 min. daily sessions were recorded for 15 days (see Pharmacol. Biochem. Behav. 6:371, 1977). Data are summarized:

		Body Weights			
Treatment	Litter Size	7 days	14 days	21 days	28 days
Barbital (vs. H_2O)	NC	↓*	↓*	NC	NC
CDP (vs. H_2O)	NC	NC	NC	NC	NC
Ethanol (vs. Sucrose)	NC	↓*	↓**	↓**	NC
Sucrose (vs. H_2O)	NC	↓**	↓**	↓**	↓**

	Avoidance Latencies		FR20 Rates
	Acquisition	Retest	
Barbital (vs. H_2O)	↑**	↑**	↓**
CDP (vs. H_2O)	↓**	NC	↓**
Ethanol (vs. Sucrose)	↓**	NC	NC
Sucrose (vs. H_2O)	NC	NC	↑ *

*, $p<0.05$; **, $p<0.01$; NC, no significant change

The effects of ethanol on avoidance behavior resembled those of CDP but not those produced by barbital. These alterations in avoidance responding may be due to effects of the drugs on development or to direct effects of the drugs on behavior since the pups were ingesting the drug (from the milk and/or from the drinking solution) when these tests were conducted. The suppression of FR20 responding in the barbital and CDP groups must be attributed to long-lasting developmental effects of the drugs as the drug solutions were removed about 70 days before these tests. These results indicate that exposure to ethanol, barbital or CDP (in utero and post partum) alters the ability of pups to perform certain behavioral tests. These experiments demonstrate the usefulness of shock-avoidance and food-reinforced behaviors in the study of behavioral teratology. Supported in part by grants from the Pharmaceutical Manufacturers Association Foundation to R.A. Harris.

1577 IPRINDOLE IS A POTENT ENHANCER OF SPONTANEOUS AND KCl-INDUCED EFFLUX OF NOREPINEPHIRINE FROM RAT BRAIN SLICES. Edith D. Hendley. Dept. Physiol. & Biophys., Univ. Vermont, Burlington, VT 05401.

Iprindole is an atypical tricyclic antidepressant whose clinical efficacy has heretofore not been attributable to inhibition of amine uptake, nor to enhancement of amine metabolism in the brain. This lack of effect of iprindole on amine mechanisms has seriously questioned the amine hypothesis of affective disorders. We have confirmed that iprindole is not a very potent inhibitor of ^{3}H-l-norepinephrine (^{3}H-l-NE) uptake in slices or homogenates of rat cerebral cortex (ID_{50} = 3.8 uM). However, we also found that this agent enhanced spontaneous as well as KCl-induced efflux of ^{3}H-l-NE from rat cerebral cortical slices, at doses of iprindole as low as 10 picomolar.

McIlwain slices (0.4 x 0.2 x 0.2 mm) of rat cerebral cortex were preloaded in vitro with ^{3}H-l-NE in the presence of nialamide (10 uM) and tropolone (10 uM), inhibitors of the enzymatic degradation of NE, then rinsed thoroughly with warm fresh Ringer. Efflux was determined by incubating, at 37°C for 5 to 15 min, 10 mg of washed slices in 10 ml of Krebs HCO_3 Ringer containing nialamide and tropolone, and varying doses of iprindole from 1 picomolar to 10 micromolar. The small amount of tissue in a large volume of incubating medium minimized the effects of reuptake on the measurement of efflux. The radioactivity remaining in the slices after incubation at 37°C was compared with that in the slices incubated at 0°C, and the difference was used as an estimate of the efflux. The effects of iprindole were determined by comparison with no-iprindole controls.

Efflux under the conditions described above was essentially totally dependent on temperature. As high as 50 mM KCl caused a loss of only 4% of the radioactivity in the slices at 0°C as compared with 70% loss at 37° (maximal efflux in this system). Iprindole increased spontaneous efflux as well as efflux induced by the addition of KCl (6.25 to 25 mM) as long as control efflux (no iprindole present) was not maximal. The shape of the dose-response curves was biphasic over the wide range of doses of iprindole used, both with respect to spontaneous efflux and to KCl-induced efflux. Significant increases in both types of efflux (20-30% above controls) were noted at 10 picomolar iprindole, and maximal enhancement (140% above controls) was observed at 10 uM iprindole. These findings emphasize that although iprindole is not as potent as the other tricyclic antidepressants in inhibiting NE uptake, its marked potency in enhancing NE efflux would increase the availability of NE during nerve transmission, in accordance with the amine theory of affective disorders.
Supported by USPHS grant MH 25811.

1578 TREATMENT OF TARDIVE DYSKINESIA AND ITS UNDERLYING MECHANISM. Chuong C. Huang, Richard I.H. Wang* and Luca Alverno*. Wood VA Center and Dept. of Psychiatry, Medical College of Wisconsin, Milwaukee, WI. 53193.

The population of Tardive Dyskinesia (T.D.) patients has increased as the long term neuroleptic chemotherapy is continued. We have seen the development of new cases. In a sample survey at this VA Center, 75 chronic schizophrenia patients who had been on antipsychotic medication for years were interviewed. 21 of them shown evidence of Tardive Dyskinesia symptoms. (28%) 18 T.D. patients were studied on inpatients on the clinical pharmacology ward with double-blind controlled method. They were randomly assigned to 3 treatment groups receiving different medications--placebo, alpha-methyldopa and reserpine. The T.D. movements of each patient were evaluated every day by the same observer. They were also videotaped once before medication and once while on medication. Those patients who received alpha-methyldopa or reserpine shown reduction of T.D. symptoms. Severe rebound phenomenon were observed after the discontinuation of alpha-methyldopa medication. No placebo effect was seen. Reserpine is considered as a dopamine depleting agent and alpha-methyldopa is considered as false transmitter. The results of this study are consistent with the hypothesis that the T.D. symptom is a receptor hypersensitive phenomena to dopamine. Gradual desensitization phenomenon were seen in 5 cases after the discontinuation of antipsychotic medication despite of the fact that they have T.D. symptoms for many years. It is concluded that the T.D. symptom is not an irreversible phenomena, its underlying cause is the receptor hypersensitivity to dopamine caused by many years use of antipsychotic medication. The treatment of T.D. depends on desensitization processes. Reserpine appears to be helpful in receptor desensitization processes. Alpha-methyldopa does not appears to be an useful agent for the treatment of T.D. because its effects are the same as the increase of antipsychotic medication.
The authors would like to express their gratitude to Dr. Leitschuh, his colleague and many others who made this study possible.

1579 EFFECTS OF DIAZEPAM ON THE SUPPRESSION OF SCHEDULE-CONTROLLED AND SCHEDULE-INDUCED BEHAVIOR DURING SIGNALLED AND UNSIGNALLED SHOCK. N. Hymowitz* (SPON: B. Natelson). New Jersey Medical School, Newark, NJ 07103.

The effects of diazepam on the suppression of food-maintained leverpressing and schedule-induced licking during signalled and unsignalled response-independent electric-shock delivery were studied in the rat. For each behavior, two measures of response suppression were obtained; (1) the suppression of responding during the preshock stimulus (conditioned suppression) and (2) the suppression of the total number of responses emitted in the components of a multiple schedule associated with signalled and unsignalled shock (differential suppression). Differential suppression was influenced by the intensity of shock and the dose of diazepam. Depending upon the intensity of shock, some doses of diazepam "released" from suppression responses which were emitted primarily during signalled shock; other doses primarily influenced responding during unsignalled shock. The intraperitoneal injections of diazepam (1.00 mg./kg-10.00 mg./kg.) had no effect upon the conditioned suppression of schedule-controlled and induced responding. Responding remained suppressed during the preshock stimulus throughout the study. The data fail to support findings which suggest that benzodiazepines further enhance the suppression of responding due to response-independent shock. The data also do not lend themselves to analysis according to the rate-dependent effects of drugs. There did not seem to be a consistent relationship between the control rates of pressing and licking and the effects of the drug on the enhancement of schedule-controlled and-induced responding during signalled and unsignalled shock. Rather, the findings support a safety signal analysis of response suppression and suggest that diazepam primarily affects that aspect of response suppression due to the failure to discriminate when the noxious event is not available.

1580 EEG AND BEHAVIORAL EFFECTS OF BUPRENORPHINE, A NEW NARCOTIC AGONIST-ANTAGONIST, IN THE RAT. Sarala Kareti* and J. Edward Moreton. Dept. Pharmacol. and Toxicol., Univ. of Maryland Sch. of Pharm., Baltimore, MD 21201.

Rats were prepared with chronic cortical and temporalis muscle electrodes for continuous recording of the electroencephalogram (EEG) and electromyogram (EMG) and intermittent observations of overt behavior for two days before and two days after acute intravenous administration of physiological saline or buprenorphine hydrochloride at doses of 0.3, 1.0, 3.0, 10, and 30 mg/kg. Generally, narcotic agonists such as morphine produce a biphasic behavioral and EEG response of an initial phase of behavioral stupor associated with high-voltage EEG slow-wave activity (slow bursts) followed by a secondary phase of behavioral arousal and low-voltage EEG activity. Sleep and REM sleep then reappear. These agonistic effects can be prevented by administration of the pure narcotic antagonist, naloxone. In the case of morphine (10 mg/kg) each of these phases lasts about 1-1½ hours with the appearance of REM sleep at about 3-4 hours. It might be expected that partial agonists would have either agonist or antagonist effects depending upon the dose used. The present study investigated the agonist-antagonist effects of buprenorphine with respect to duration of stupor, duration of arousal, latency to REM sleep and increase in EEG spectral power during behavioral stupor. The duration of the stuporous behavior was an inverted U-shaped function of the doses from 0.3 to 10 mg/kg. 0.3 mg/kg produced EEG and behavioral stupor lasting about 15 minutes; increasing the dose to 1 mg/kg produced effects lasting from 2-3 hours while further increases in dose to 3 or 10 mg/kg decreased the duration of stupor to less than 30 minutes. The duration of the secondary phase of behavioral arousal was 3-4 hours at the 0.3 mg/kg dose and 15-20 hours at 3 to 10 mg/kg. On the other hand, a high dose of 30 mg/kg produced only about 2 hours of arousal. The latency to REM sleep showed a similar dose-effect curve with a latency of about 5 hours at 0.3 mg/kg and 25 hours at 10 mg/kg. In contrast, REM sleep appeared within 3-4 hours after 30 mg/kg. Computer derived EEG spectral power between 0-10 Hz also showed an inverted U-shaped relation to doses from 0.3 to 10 mg/kg. Maximal power output corresponding to maximal EEG synchrony and intensity of behavioral stupor occurred at 1 mg/kg. These findings demonstrate a dose-dependent interaction of the agonist and antagonist effect of buprenorphine; it has narcotic agonist action at low to intermediate doses while it manifests antagonists action, blocking its own agonist effects, at higher doses. (Supported by NIDA Grant DA 01050)

1581 EFFECTS OF DRUGS ON RESPONSE RATE AND "REWARD VALUE" IN CAUDATE AND HYPOTHALAMIC SELF-STIMULATION. Ernest W. Kent Dept. Psychol. U. of Illinois at Chicago Circle, Box 4348, Chicago, Ill. 60680.

Rats working for electrical stimulation of the lateral hypothalamus or caudate were trained to a two-lever titration paradigm which provided measures of response rate and "reward value" (intensity at reset) of the stimulus. When stimulus intensity is manipulated, lateral hypothalamic animals show high and variable response rates which, together with reward values, follow changes in stimulus intensity in the expected manner. Caudate animals, in contrast, show low and extremely stable response rates which are relatively independant of stimulus intensity above a minimum threshold for responding. Their reset intensity choices however indicate that reward value follows stimulus intensity in the usual fashion, and their response rate may be raised with drugs, indicating it is not physicaly limited.

When stimulus intensity is held constant, the effects of drugs on response rate and reward value may be observed. Hypothalamic animals in our sample showed a characteristic pattern of response to a variety of drugs. Among these effects were increases in response rate with amphetamine, and decreases with haloperidol, apomorphine, and picrotoxin. Reward value was increased by amphetamine (but the effect was not time-locked to the effect on rate), and picrotoxin, and decreased by apomorphine. Caudate animals on the other hand appeared to fall along a continuum with drug response type "A" at one end having a response profile very similar to the hypothalamic animals, and type "B" at the other end which showed no effect of amphetamine, haloperidol or picrotoxin on response rate. Type "B" animals show increases in reward value with apomorphine and haloperidol. Animals falling between these extremes show smaller effects, in one direction or the other, even with large doses. The only drug which was found to affect both response rate and reward value in the same fashion in all animals was Lioresal (Baclofen) which depressed rate and enhanced reward value.

It is not excluded that more than one type of hypothalamic animal may be encountered as coordinates are varied, and our sample to date may represent nigro-striatal bundle (NSB) stimulation at the hypothalamic level. It is tempting to speculate that the "A" and "B" drug response profiles in caudate animals may represent varying degrees of driving in elements pre- and post-synaptic to the NSB. In many cases, a depression in rate together with a rise in reward value appeared to represent a loss of contingency between response and reward effect rather than a physical incapacitation. An interpretation of these results will be presented.

1582 HALOPERIDOL CLASSICAL CONDITIONING - PARADOXICAL RESULTS. James J. King*, Stanley R. Schiff* and Wagner H. Bridger, Albert Einstein College of Medicine, Department of Neuroscience, Bronx, New York, 10461.

Male Long-Evans hooded rats were randomly assigned to conditioning groups (haloperidol 0.1, 0.2, or 0.6 mg/kg as treatment for experimental session) and pseudo-conditioning control groups (saline as treatment for experimental session, followed by drug injection 45 min to 1½ hr after animal has been returned to its home cage-one group for each drug dose).

The experimental sessions were conducted each day and consisted of (1) transfer rat to injection cage, (2) expose to tone (CS) for one min while scoring activity by observing the number of crossings (forepaws crossing into separate quandrants of injection cage), (3) inject i.p. with treatment (UCS) and immediately place into observation cage, (4) quantify behavior (UCR) for 90 sec intervals at 3 min, 7 min, 11 min, and 41 min post injection and (5) return rat to home cage. The behaviors sniffing, staring (rat immobile with fixed gaze), rearing (both forepaws leaving the floor-time and frequency), crossing and grooming were rated blind using microswitches and automatic counters through a two-way mirror from a sound-proof room.

The animals received 10 days of drug treatment while the 11th day was a saline test day during which conditioning was assessed. Behavioral scores for conditioning and pseudoconditioning control groups were compared using two-tailed tests, with $p < 0.05$ accepted as significant.

Results show significant conditioning of behaviors paradoxical (opposite) to the behavioral effects of haloperidol itself. These include decreased staring, increased crossing, increased rearing, increased sniffing and increased preinjection crossing (as recorded during the 1 min CS presentation). Conditioning seemed to take from 6 to 8 days as evidenced by significant differences in preinjection crossings. Thus, the paradoxical response to haloperidol conditioning suggests there is no significant conditioning of post-synaptic blockade. Rather, the conditioning appears to involve increased pre-synaptic activity.

1583 ROTATIONAL BEHAVIOR AFTER ACUTE AND CHRONIC SYSTEMIC AMPHETAMINE TREATMENT. L. Kokkinidis* (SPON: W.G. Webster). Department of Psychology, University of Saskatchewan, Saskatoon, Sask.,

Circling behaviour elicited by acute and chronic d-amphetamine administration was evaluated in a series of five experiments. Acute systemic treatment with d-amphetamine was found to produce a dose dependent increase in locomotor activity, direction changes as well as circling behaviour in a circular alleyway. Reduction of whole brain dopamine (DA) and norepinephrine (NE) by α-methyl-para-tyrosine (α-MpT) antagonized both the locomotor and circling effects elicited by amphetamine. Potential involvement of NE in mediating the circling response to amphetamine was suggested by the findings that a 50% reduction of whole brain NE by bis (4-methyl-1-homopiperazinylthiocarbonyl) disulphide (FLA-63) successfully attenuated the drug-induced circling, without altering the locomotor excitation. Pretreatment with propranalol, a B-adrenergic receptor blocker, decreased the circling behaviour induced by amphetamine, whereas phenoxybenzamine, an α-adrenergic blocker, had no effect in this respect. Following chronic d-amphetamine treatment the circling elicited by acute drug treatment was abolished. Moreover, as found with FLA-63 and propranalol the number of direction changes were increased. Since tolerance was not observed to the locomotor effects of amphetamine, a behaviour primarily mediated by dopamine activity, the results of the present study are consistent with earlier reports suggesting that tolerance is observed to develop to behaviours which involve a noradrenergic component. The data were also discussed in terms of the relationship between circling and perseverative behaviour, as well as changes in selective attention elicited by acute and chronic amphetamine treatment.

1584 EFFECTS OF ETHANOL ON PUNISHED RESPONDING. G.F. Koob, S.L. Foote and F. E. Bloom. A.V. Davis Center for Behavioral Neurobiology, The Salk Institute, La Jolla, CA 92037.

Rats were trained on a three component multiple schedule consisting of reward, time out and conflict. Responses during the reward component (10 min. period) were reinforced on a random interval (RI) schedule - 25 sec; responses during the time out component (4 min. period) were never reinforced; and responses during the conflict component (4 min. period) were reinforced on a RI-25 sec. with both food and foot shock. Following training each rat received chlordiazepoxide (10 mg/kg) and five doses of ethanol (glucose, 0.5, 1.0, 1.5 and 2.0 g/kg). As already reported by others, chlordiazepoxide produced a significant increase in responding during both the time out and conflict components of the schedule, but no change in the rate of responding during the reward component. Ethanol also produced a dose-dependent increase in responding during the time out and conflict components at low doses (maximum effect: 1.0 g/kg), and significant decreases in responding during the reward component at the higher doses. This decreased responding at 1.5 and 2.0 g/kg was accompanied by signs of motor incoordination.

Pretreatment with 5 and 10 mg/kg of naloxone significantly reversed the release of punished responding observed with 10 mg/kg of chlordiazepoxide and 1.0 g/kg of ethanol. Naloxone by itself (10 mg/kg) failed to alter responding in any of the components of the schedule. These results suggest that ethanol possesses anxiolytic properties similar to the benzodiazepines and that both drugs interact with a naloxone sensitive substrate.

Supported by grant AA 03504.

1585 SERUM PROLACTIN AND CLINICAL STATE IN PATIENTS DURING NEUROLEPTIC TREATMENT AND WITHDRAWAL. Thomas P. Laughren*, Walter Armin Brown
Dept. Psychiat., VA Hosp., Brown Univ., Providence, RI 02908.

Serum prolactin is elevated both acutely and chronically by a variety of neuroleptics. There is little evidence regarding the development of tolerance to the prolactin-elevating effect of neuroleptics or relating prolactin to clinical response. Subjects were 11 male outpatients taking thioridazine (mean dose:212mg/day) or haloperidol (mean dose:17 mg/day) for a mean duration of 4.4 years. Patients continued their usual dose for a 2 week baseline period (chronic), took matched placebos for 4 weeks (placebo), and returned to baseline dose for 4 weeks (acute). An AM fasting blood sample was obtained at each weekly visit along with a Brief Psychiatric Rating Scale (BPRS) and a Profile of Mood States (POMS). Samples from the same subject were measured in the same radioimmunoassay.

The mean prolactin during placebo was 5.8 $\pm$ 0.5 ng/ml and differed from the chronic (13.2 $\pm$ 2.2 ng/ml) and acute (14.8 $\pm$ 2.1 ng/ml) prolactins ($F = 6.8$, $p < .01$). Nine patients had higher mean prolactins during the acute than chronic period (Sign Test, $p < .05$). The percent increases in prolactin from chronic to acute period ranged from 4 to 120 percent (Mean = 42%).

Although mean BPRS and POMS scores did not change across treatment periods, individual patients varied considerably. Chronic prolactin levels did not relate to chronic clinical state, but 3 patients with the lowest chronic prolactins (<6 ng/ml) did show the greatest change in POMS scores ($>50\%$) after drug withdrawal (Fisher Exact Test, $p = .006$). There was a trend for lower prolactins during placebo to be associated with clinical worsening during placebo (BPRS:$p = .067$; POMS:$p = .067$). The 3 patients with lowest prolactins chronically also had lowest prolactins during placebo (<4 ng/ml) and showed the greatest improvement in POMS scores after drug resumption ($p = .006$). Patients showing tolerance effects (i.e., greater serum prolactin levels acutely than chronically) tended to be the same patients who showed clinical worsening after drug withdrawal (BPRS:$p = .067$; POMS: $p = .013$) and greater improvement after drug resumption (BPRS: $p = .013$; POMS:$p = .006$). Prolactin levels, tolerance and clinical state were not related to dose or duration of drug treatment.

Either dopaminergic receptor sensitization or increased drug metabolism could explain the tolerance finding. Alternatively, noncompliance during chronic treatment could have occurred. In either case, lower prolactin levels predicted greater susceptibility to clinical change with either drug withdrawal or resumption.

1586 ENHANCED ^{3}H-NEUROLEPTIC BINDING IN POST-MORTEM SCHIZOPHRENIC BRAINS. Tyrone Lee and Philip Seeman. Pharmacology Department, University of Toronto, Toronto, Canada.

It has been reported in a previous communication (Lee and Seeman, Proc. Neurosci. Soc. 3:443, 1977) that ^{3}H-haloperidol receptors in the caudate nucleus, putamen and nucleus accumbens was significantly higher in post-mortem brains from schizophrenic patients when compared to normal controls. The present report extends the study to incorporate 24 schizophrenic brains and 32 neurologically normal human brains.

Post-mortem normal human brains were obtained through hospital pathologists from people who had died from sudden deaths of non-neurological origin. Schizophrenic brain tissues were contributed by Dr. W.W. Tourtellotte (NINCDS/NIMH Human Neurospecimen Bank, Los Angeles, California), Dr. E.D. Bird (MRC Human Brain Bank, Cambridge, England) and Dr. O. Hornykiewicz (Vienna, Austria). Tissues were homogenized in Tris-buffer, incubated with ^{3}H-apomorphine, ^{3}H-haloperidol or ^{3}H-spiroperidol and the mixture was rapidly filtered through GF/B filters and washed. Specific binding of the radioligand was defined as that amount bound in buffer minus the amount bound in the presence of (+)-butaclamol.

The specific binding of ^{3}H-haloperidol, ^{3}H-spiroperidol and ^{3}H-apomorphine (femtomoles/mg protein) to normal and schizophrenic brain tissues were as follows:

	^{3}H-HALOPERIDOL	^{3}H-SPIROPERIDOL	^{3}H-APOMORPHINE
NORMAL			
CAUDATE	44.4±1.9 (21)	89.1±7.0 (14)	28.7±2.8 (13)
PUTAMEN	49.8±3.3 (21)	87.6±4.1 (12)	26.3±2.7 (13)
N. ACCUM.	37.5 (2)	51.7 (2)	30.5±3.1 (4)
SCHIZOPHRENIC			
CAUDATE	76.8±2.9* (18)	150.2±10.6*(16)	27.7±3.3ΨΨ (7)
PUTAMEN	75.3±4.1* (18)	157.5±13.8*(17)	27.6±2.3ΨΨ (7)
N. ACCUM.	65.5 (2)	117.0±18.6 (4)	20.0±4.6ΨΨ (3)

* $P<0.001$; ΨΨ non-significant.
(Number in parentheses denotes number of human brains assayed, each with 5-20 replicate determinations).

The present data indicate that specific binding of both ^{3}H-haloperidol and ^{3}H-spiroperidol were significantly higher in schizophrenic brains whereas no difference was found for specific ^{3}H-apomorphine binding.

Although most patients had been on long-term neuroleptic therapy, the untreated patients also exhibited significantly higher binding of ^{3}H-neuroleptics.
(Supported by the Ontario Mental Health Foundation and the Medical Research Council of Canada).

1587 EFFECTS OF APOMORPHINE ON AVOIDANCE PERFORMANCE AND ACTIVITY IN DEVELOPING RAT PUPS TREATED WITH 6-HYDROXYDOPAMINE (6-OHDA). Susan V. Lipton* and Bennett A. Shaywitz. Lab. Dev. Neurobiol. Yale Univ. Sch. of Med., New Haven, CT. 06510

The selective depletion of brain dopamine (DA) by intracisternal administration of 6-OHDA in the 5 day old rat pup results in a spectrum of behaviors including hyperactive motor activity between the 2-3 weeks of life, inability to habituate to a novel environment, and impairment of avoidance performance in both T-maze and shuttle box tasks. Administration of d-amphetamine or methylphenidate reduces the hyperactive motor behavior and decreases the prolonged latency of avoidance tasks characteristic of the 6-OHDA treated animal. In order to further explore the effects of selective DA depletion during maturation we have administered the DA receptor agonist apomorphine (APO 0.1 and 1.0 mg/kg) to developing rat pups treated with 6-OHDA at 5 days of age. Such treatment resulted in a marked reduction of brain DA by more than 90% ($p < 0.001$) while norepinephrine concentrations were not significantly altered. APO produced an increase in escape latency at 20 days of age (T-maze) of 158% in normal animals but reduced escape latency by 44% in 6-OHDA treated rat pups ($p < 0.001$). At 26 days of age, APO increased escape latency (shuttle box) by 92% at the low dose and by more than 500% at the high dose in normal animals, but in 6-OHDA treated rat pups APO at both dosages reduced escape latency by 16% ($p < 0.001$). In both normal and 6-OHDA treated pups APO increased activity in a dose dependent fashion and these effects were more pronounced as maturation progressed. Thus APO increased activity by only 40% at 13 days but by 200% at 19 and 26 days ($p < 0.001$). At 19 days of age this effect was more pronounced in normal pups. Exploratory activity as measured in a hole box at 30 days was reduced by APO in both normal and 6-OHDA pups. The effects on stereotyped activity were more complex. High doses of APO produced a six fold increase in rotary activity in both treated and normal pups at 26 days; the low dose was ineffective in normals but 0.1 mg/kg APO produced a marked increase in 6-OHDA pups. At 19 days the low dose APO produced increases in both groups, significantly greater than that observed after high doses. No significant effects were evident at 13 days. Sniffing behavior was increased moderately in treated pups at 19 days and to a lesser extent at 26 days. Our results support the notion that both the decrease in escape latency and the increase in stereotyped activity observed in 6-OHDA rat pups after APO are most parsimoniously explained on the basis of denervation supersensitivity of damaged DA receptors.

1588 THE EFFECTS OF IMIPRAMINE ON SCHEDULE INDUCED AND SCHEDULE DEPENDENT BEHAVIOR. Costas C. Loullis and Matthew J. Wayner. Brain Res. Lab., Syracuse Univ., Syracuse, NY 13210.

Rats were reduced to 80 percent body weight and were exposed to an FI 1 min food reinforcement schedule for 60 min daily until lever presses, licks and water consumption stabilized for at least ten days. During the acute phase, animals were injected with 0.9 percent saline, 2, 4, 8 and 16 mg/kg of imipramine in a counterbalanced design. Following return of all three measures to pre-injection baseline, the same animals were injected with 16 mg/kg of imipramine daily for 8 days. Results of the acute phase revealed a significant decrease in lever presses at 8 mg/kg and a decrease in lever presses and water consumption at 16 mg/kg. Licks were not significantly different from saline baseline at any of the doses used. During chronic administration of imipramine lever presses and water consumption were both significantly depressed from baseline on all 8 days. Licks, however, were not significantly different from saline baseline. These data indicate that imipramine produces differential and dose dependent effects on schedule induced and schedule dependent behavior.

1589 DEGRADATION AND BRAIN PENETRATION OF ^{3}H-MUSCIMOL AFTER SYSTEMIC ADMINISTRATION. A. Maggi* and S.J. Enna. Depts. Pharmacol. and of Neurobiol. and Anat., Univ. Texas Med. Sch., Houston, TX. 77025.

In vitro, muscimol is a potent GABA receptor agonist and, after systemic administration, it induces behavioral changes in laboratory animals and man. These findings have led to the hypothesis that muscimol may be clinically useful as a GABA agonist agent. However, little is known about the metabolism of muscimol and the extent to which it penetrates into the brain after systemic administration. In the present investigation, ^{3}H-muscimol was injected systemically into mice and the amount of tritium in the brain was analyzed both quantitatively and qualitatively. For the study, two types of ^{3}H-muscimol were utilized, methylene side chain ^{3}H (12.1 Ci/mmole, mus-A) and muscimol having a ^{3}H on the 4 position of the isoxazole ring (10.35 Ci/mmole, mus-B). Administration (5 μCi,i.v.) of either mus-A or mus-B into mice results in the accumulation of measurable amounts of tritium in the brain for up to 3 hr after injection. Chromatographic analysis of the brain tritium revealed that 90% of the brain tritium after mus-A and 10-30% after mus-B is volatile. TLC analysis indicates that all of the nonvolatile tritium migrates as authentic muscimol. Coadministration of a high dose of unlabeled muscimol (24 μmoles/kg) or bicuculline (0.75 μmoles/kg) did not affect the accumulation of nonvolatile tritium in the brain. Intravenous administration of ^{3}H-GABA, ^{3}H-mus-A or ^{3}H-phenobarbital indicated that, in terms of the percent of dose administered, muscimol accumulates in the brain no better than ^{3}H-GABA, which is more than an order of magnitude less than ^{3}H-phenobarbital. Determination of chloroform/water partition coefficients revealed that muscimol and GABA have similar coefficients and that they are some 300 times less lipid soluble than phenobarbital. These results suggest that muscimol is rapidly degraded after systemic administration by at least two processes, side chain oxidation and ring cleavage. Furthermore, these findings indicate that very little, if any, unchanged muscimol gains access to brain GABA receptors after systemic administration. Thus, the behavioral and biochemical effects evoked by this agent may be due to some derivative formed after administration. (Supported in part by grants from the Pharmaceutical Manufacturers Association, the Huntington's Chorea Foundation, Merck Sharp and Dohme, USPHS RCDA NS-00335 (S.J.E.) and (A.M.) a Salk Institute-Texas Research Foundation Fellowship.)

1590 OUABAIN-INDUCED CHANGES OF IN VITRO TRANSMEMBRANE LITHIUM DISTRIBUTION IN ERYTHROCYTES FROM DEPRESSED PATIENTS. Alan G. Mallinger, Joan Mallinger,* Jonathan M. Himmelhoch,* John F. Neil* and Israel Hanin. University of Pittsburgh School of Medicine, Western Psychiatric Institute and Clinic, Pittsburgh, PA. 15261.

Lithium (Li^+) was used in this investigation to study cell membrane cation transport in erythrocytes (RBCs) from depressed patients. The transmembrane distribution of this ion was studied both in vivo and in vitro in 13 patients. Interindividual differences of transmembrane Li^+ distribution found during lithium carbonate treatment in vivo were replicated in vitro when RBCs were incubated with Li^+, so that there was a significant correlation between the in vivo and in vitro measures of this parameter ($r = 0.80$, $p<0.001$). However, when in vitro incubations were conducted with the addition of 0.1mM ouabain, the correlation between the in vivo and in vitro measures of transmembrane Li^+ distribution was abolished ($r=0.32$, $p>0.1$). Moreover, ouabain reduced differences in transmembrane Li^+ distribution between individual subjects, thereby causing a significant decrease in the variance of this parameter ($r_{DS} = 0.79$, $p<0.001$).

In order to investigate potential relationships between transmembrane Li^+ distribution and specific clinical features of depression, RBCs from 20 drug-free depressed patients and 14 non-depressed control subjects were incubated with Li^+ in vitro. We found that transmembrane Li^+ distribution values were more homogeneous among bipolar (manic-depressive) patients than among patients with recurrent unipolar depression ($F = 15$, $p<0.01$), patients with secondary depression ($F = 17$, $p<0.01$), or non-depressed control subjects ($F = 19$, $p<0.01$). Moreover, although ouabain substantially reduced the range of transmembrane Li^+ distribution values in both control subjects ($r_{DS} = 0.80$, $p<0.05$) and in patients with unipolar and secondary depression ($r_{DS} = 0.91$, $p<0.001$), it had no overall effect on this parameter in the bipolar patient group ($r_{DS} = 0.24$, $p>0.1$). These findings provide evidence that, with respect to Li^+ transport, the membrane characteristics of cells from bipolar patients may differ from those of other depressed patients or control subjects. Because Li^+ is effective for the treatment of bipolar mood disorders, such a difference in membrane transport characteristics could be an important factor in the action of this drug.

1591 EVIDENCE AGAINST THE CURRENTLY HELD VIEWS ON THE MODE OF ACTION OF LYSERGIC ACID DIETHYLAMIDE. M. K. Menon* and W. G. Clark. Psychopharmacol. Res. Lab., V. A. Hospital, Sepulveda, CA 91343

Using an electromyographic method of Bieger et al (Eur. J. Pharmacol., 18, 128, 1972), LSD in low doses was shown to produce a central antiserotonin effect and a serotonin (5-HT) receptor stimulant effect in higher doses. It also produced hyperactivity in reserpinized mice. This may indicate a dopaminergic activity. Lisurid, a nonhallucinogenic lysergic acid derivative, which is structurally closely related to LSD, also showed all the above effects in similar dose ranges. Hence the hallucinogenic effect of LSD may not be dependent on its effects on 5-HT and/or dopamine (DA) systems alone.

Experiments were performed in mice to bring to light the differences in the central effects of these two drugs. Lisurid, but not LSD, produced a dose-dependent hypothermic response. This effect of lisurid was blocked by haloperidol. LSD, but not lisurid blocked the apomorphine-induced hypothermia. Lisurid, but not LSD, inhibited the alpha-methyl-p-tyrosine-induced depletion of brain DA. The hyperactivity produced by lisurid in reserpinized mice was more effectively blocked by haloperidol than that of LSD. These and other differences in the effects of LSD and lisurid may help one to obtain new insight into the mode of action of LSD.

Supported by the Medical Research Service of the Veterans Administration.

1592 A NEW TEST FOR AGGRESSION IN RATS: DIFFERENTIAL EFFECTS OF d-AMPHETAMINE AND COCAINE. Klaus A. Miczek. Dept. Psychol., Carnegie-Mellon Univ., Pittsburgh, PA 15213.

Psychopharmacological and neurochemical studies of aggression in the laboratory are frequently confronted with the problem of investigating animals that are subjected to very stressful procedures such as isolated housing, food deprivation, conditioning procedures and electric shock applications evoking mostly defensive reactions. A simple test situation has been developed which does not require stressful manipulations in order to generate the species-specific repertoire of agonistic behavior, including attacks, threats, defensive, submissive and flight reactions, in common laboratory rats. The alpha male of 4-member colonies of rats attacks reliably intruder rats. Either resident alpha or intruder rat were injected i.p. with d-amphetamine or cocaine 30 or 10 min before the test. Two observers recorded frequency and duration of agonistic and non-agonistic acts and postures for 5 min after the initial attack. Administration of d-amphetamine to resident alpha rats increased their attacks, threats and pursuits at a very low dose (0.063 mg/kg, i.p.) and decreased them at higher doses (0.5, 1.0 mg/kg), whereas cocaine (0.5-32.0 mg/kg) only decreased these behaviors in a dose-dependent linear manner. At the highest dose of cocaine (32 mg/kg) and d-amphetamine (1 mg/kg), alpha rats also showed more non-agonistic motor activity. Intruder rats, when administered with d-amphetamine, but not with cocaine, were attacked, groomed, and investigated more often by non-treated alpha rats. The defensive upright posture was shown less by amphetamine-treated intruders, but more by those given cocaine. Amphetamine also impaired the immobility reaction and submissive-supine posture and decreased ultrasonic vocalizations in intruder rats. The pattern of drug effects indicates that (1) cocaine selectively decreases the attack component of agonistic behavior, (2) d-amphetamine enhances several aspects of the attack pattern at very low doses, and at higher doses disrupts many elements of attack and defensive behavior and increases escapes, (3) non-treated resident rats alter their behavior as a function of the intruder's drug state, i.e. amphetamine-treated intruders. The resident-intruder situation appears to be a reliable, easy-to-use, and drug-sensitive procedure avoiding the confounding influence of aversive stimulation typically associated with aggression tests in the laboratory.

(This research was supported by USPHS Grant DA 01502.)

1593 EFFECT OF FOREBRAIN NOREPINEPHRINE DEPLETION ON THE EXTINCTION-RELEASING EFFECTS OF CHLORDIAZEPOXIDE. Michael D. Morris*, Frederick Tremmel* and Gerald F. Gebhart. Dept. Pharmacol., U. of Iowa, College of Med., Iowa City, Iowa 52242.

Forebrain norepinephrine (NE) was reduced to 35% of control values by means of bilateral radiofrequency lesions of the noradrenergic dorsal tegmental bundle (DTB). Hypothalamic NE was unaffected and dopamine levels in both hypothalamus and cortex were also unchanged. Both DTB lesion rats (n=21) and sham controls (n=21) were then trained on a straight alley runway task for food reward. Training continued for eight days; six trials were given each day. On the last two days of acquisition, all rats received chlordiazepoxide (CDP; 10 mg/kg as base) so that when CDP was administered during extinction it would not produce dissociation. Following acquisition, eleven of the lesion animals and eleven of the sham animals received CDP on each of four days in which food was no longer given for the runway response (i.e., extinction). The remaining animals (10/group) received vehicle injections at the same 30 min interval prior to each session. The following results were obtained: 1. During acquisition of responding, animals with DTB lesions were retarded in acquiring the running response. This deficit disappeared by the end of acquisition. 2. In the two groups which received vehicle, DTB lesion animals did not perseverate more in extinction than sham animals. This result is contrary to the perseveration of responding during extinction previously reported following selective reduction of forebrain NE (e.g., Tremmel et al., Brain Res. 126: 185, 1977). 3. The finding that CDP elevates extinction-response speeds was replicated for the sham groups. 4. Depletion of forebrain NE attenuated the anti-extinction effect of CDP.

The present results, in combination with previous reports, are interpreted as suggesting that (1) the DTB carries information about frustration and fear to a limbic response-suppression system, (2) that incomplete depletion of forebrain NE (i.e., 65%) leads to compensatory functional reorganization which, under certain circumstances, may lead to increased suppression of ongoing behavior, and (3) that CDP produces its anti-extinction effects by indirectly inhibiting the response-suppression system. Supported by USPHS Grant NS12114 to GFG.

1594 DOSE RESPONSE EFFECTS OF PHENCYCLIDINE ON STEREOTYPED BEHAVIOR IN RATS. Thomas F. Murray* and Akira Horita* (SPON: Lawrence M. Halpern). Dept. Pharmacol., Sch. Med., Univ. of Washington, Seattle, WA 98195.

Phencyclidine (PCD) is a psychotomimetic drug of abuse which has recently been implicated in the production of a schizophreniform psychosis in some users. Because of current interest in the role of dopamine in the pathogenesis of psychotic symptomatology, we investigated the ability of PCD to induce stereotyped behavior in rats. All subjects were observed individually for 1 minute epochs every 5 minutes for a period of 2 hours following i.p. injections of PCD. During each observation period the presence of any of 3 stereotyped behaviors was recorded in an all or none manner. The 3 stereotyped behaviors that were evaluated were backward locomotion, circling, and head swaying. This scoring system has been employed by other authors in the study of amphetamine-induced stereotyped behavior (Psychopharmacology 40:249, 1974). PCD treatment (4-16 mg/kg) induced dose-dependent increases in the mean stereotypy scores for all 3 behaviors. The predominant behavior elicited by low doses was head swaying, while with higher doses circling and backward walking were observed in addition to the head swaying. Pretreatment with either chlorpromazine (2.5, 5.0, and 10.0 mg/kg) or haloperidol (0.25, 0.5, and 1.0 mg/kg) antagonized the PCD-induced (4 mg/kg) stereotyped behavior in a dose-dependent manner. These results indicate that at least some of the behavioral effects of PCD appear to be mediated by dopaminergic systems.

1595 NEUROLEPTIC/DOPAMINE RECEPTORS: BIOCHEMICAL AND AUTORADIOGRAPHIC STUDIES. L. Charles Murrin*, Nikolai Klemm*, and Michael J. Kuhar, Dept. Pharmacology & Exp. Ther., Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

We have carried out in vivo studies of dopamine (DA) receptors in the rat brain using ^{3}H-spiroperidol (^{3}H-SP) as a ligand (Life Sciences 22: 203, 1978). There was a regional distribution of stereospecifically bound ^{3}H-SP consistent with in vitro studies and with known DA innervation of brain regions. The in vivo binding was saturable and was blocked by DA agonists and antagonists, such as (+)butaclamol. Compounds inactive at DA receptors, such as (-)butaclamol, were ineffective in blocking ^{3}H-SP binding. These data suggested that autoradiographic studies of the DA receptor were feasible. In the present work male rats were administered 100 µCi (biochemical studies) or 125 µCi (autoradiographic studies) of ^{3}H-SP via tail vein, were decapitated two hours later and various brain regions were either analyzed for radioactivity or were prepared for autoradiography. Autoradiographic grains were found to be highly localized in specific brain regions. The olfactory tubercles and the nucleus accumbens were heavily labeled, consistent with their innervation by the mesolimbic DA system. The striatum had the greatest grain density of any region studied, as would be expected from its dense dopaminergic innervation. In the striatum the grains were found to be localized over the neuropil rather than over cell bodies with a ratio greater than 2:1. Other areas found to accumulate ^{3}H-SP as reflected in autoradiographic grain densities were parts of the frontal cortex, the claustrum, the nucleus of the stria terminalis, the lateral septum, the central nucleus of the amygdala, the ventral hypothalamus, the nucleus subthalamicus and the substantia nigra (particularly the zona compacta). The accumulation of grains in all of these areas was blocked by prior treatment with (+)butaclamol. Treatment with 1 mg/kg of either d-LSD or pipamperone (a serotonin receptor antagonist) one hour after injection of ^{3}H-SP resulted in a significant decrease in the accumulation of radioactivity in the frontal cortex, but not in the striatum, olfactory tubercles, nucleus accumbens or cerebellum. This confirms the work of others (Nature 272: 168, 1978) and suggests that in the frontal cortex ^{3}H-SP is binding to a significant degree to serotonin receptors. The effects of d-LSD and pipamerone treatments on the distribution of autoradiographic grains in the frontal cortex will be presented. The effects of kainic acid and 6-OHDA lesions of the striatum and the substantia nigra and of electrolytic lesions of the nigro-striatal and strio-nigral pathways on DA receptors will also be presented. Supported by MH 25951, RCDA MH 00053 and Deutsche Forschungsgemeinschaft.

1596 **INDUCTION OF PHYSICAL DEPENDENCE UPON ETHANOL IN RATS USING INTRAVENOUS INFUSION.** **Robert Numan and Anne M. Gilroy*. Dept. Psychol., Univ. Santa Clara, Santa Clara, CA 95053.**

The intravenous (i.v.) method of drug administration, while successfully employed to study many dependence producing substances, has not been adequately investigated for use in animal studies on alcoholism. The present research shows that the i.v. procedure can be safely used to rapidly induce physical dependence upon ethanol in rats.

Male hooded rats were implanted with chronic jugular cannulae and housed in sound attenuating chambers with food and water freely available. During the immediate postoperative period rats were infused with saline (3 ml every 4 hr at rate of 0.3ml/min) until food and water intake stabilized. Following this habituation period ethanol infusions were initiated in experimental rats (n=11) and saline infusions were continued in control subjects (n=6). Ethanol (30% v/v) was administered over a 7-day period (inter-animal range 5-10 days). The mean daily dose ranged from 10g/Kg/day to 14g/Kg/day. This dose was administered in 4-5 fractional doses over each 24-hr period (infusion rate: 0.3ml/min). Following this intoxication phase ethanol infusions were discontinued and rats were observed hourly for 20-hr and thereafter at 8-hr intervals for signs of physical dependence.

All ethanol treated rats showed withdrawal symptoms (moderate to severe, n=9; mild, n=2). Saline treated controls did not show these symptoms. Five days of intoxication was sufficient to produce these effects. The most reliable symptoms are listed below. Parenthetically we include the number of animals showing the symptom, the mean hr of onset during the withdrawal, and the mean hr of peak intensity onset during withdrawal respectively. The symptoms include: spontaneous seizures (n=7, 13.6 hr, 14.0 hr), audiogenic seizures (n=7, 10.9 hr, 11.1 hr), tremors (n=6, 10.2 hr, 11.5 hr), tail stiffening (n=10, 7.3 hr, 10.7 hr), and body rigidity (n=9, 4.8 hr, 12.0 hr). Other symptoms were also observed (e.g., aggression, body shakes, stereotypy, teeth chattering) but were less reliable. All symptoms were greatly reduced by 36 hr of withdrawal. (Supported by NIAAA Grant 1 R03 AA 03451-01)

1597 DIFFERENTIAL TOLERANCE TO EFFECTS OF CHRONIC d-AMPHETAMINE ON AGONISTIC AND NONAGONISTIC BEHAVIOR IN MICE. James M. O'Donnell* and Klaus A. Miczek. (SPON: H. Barry III). Dept. Psychol., Carnegie-Mellon Univ., Pittsburgh, PA 15213.

Assessing tolerance or sensitization to the effects of amphetamine is problematic when a single behavioral parameter is measured. The effects of amphetamine on locomotion and stereotypies illustrate that many elements of an animal's repertoire are interdependent, i.e. amphetamine-induced stereotypies prevent further stimulation of locomotor activity. We investigated the possibility that tolerance or sensitization may develop differentially to the effects of d-amphetamine on various components of an animal's behavioral repertoire. The ethological analysis of agonistic and nonagonistic behavioral elements permitted a comprehensive assessment of the behavioral effects of d-amphetamine during a nine week chronic treatment period in mice that were subjected to periodic resident-intruder tests. Agonistic behavior such as attack bites, threats, pursuits, and tail rattles as well as nonagonistic behavior such as anogenital investigation, walking, rearing, and self-grooming were recorded in male resident mice confronting an intruder mouse. Resident-intruder tests, conducted twice per week, were recorded on videotape for five minutes after the first attack. After an initial dose-response determination of d-amphetamine's effects on agonistic and nonagonistic behavior, mice were injected i.p. daily for four weeks with either d-amphetamine (n=15) or saline (n=15). While maintaining the animals on their respective chronic treatments for five more weeks, the dose-dependent effects of d-amphetamine were redetermined and also, the effects of cocaine were assessed. Our results indicate that (1) tolerance does not develop to the antiaggressive effects of d-amphetamine, (2) d-amphetamine-maintained mice show tolerance to the locomotion stimulating effect of the drug, (3) amphetamine induces stereotypies at a lower dose and to a greater degree in mice maintained on amphetamine than in those maintained on saline, and (4) there was no difference in the behavioral effects of cocaine in animals maintained on d-amphetamine and those maintained on saline. We also observed changes in fighting behavior of saline control animals during the course of the chronic treatment. This emphasizes the necessity of dose-response determinations before and after chronic treatment in order to guard against invalid conclusions about tolerance or sensitization to the effects of d-amphetamine.

(This research was supported by USPHS Grant DA 01502.)

1598 EFFECT OF IPRINDOLE ON MOUSE BRAIN SEROTONIN METABOLISM. Bertha G. Ortega-Corona, José Carranza-Acevedo, Patricia Guzmán-Amaya*, Nora Esparza-Avalos*, and Guadalupe Castro-Osuna*. Subjefatura de Investigación Básica, Instituto Mexicano del Seguro Social, Po. Box 73-032, Mexico City, 73, D.F., MEXICO.

Tricyclic antidepressant drugs (TAD) were found to block transmitter uptake. This finding inspired the older and now classic hypothesis about their mode of action. However, accumulating evidence about the actions of TAD on serotonin (5-HT), norepinephrine(NE) and dopamine (DA) in the brain make natural to consider other hypothesis. In order to shed some light into the mechanisms of action of these TAD, it was investigated the chronic administration of iprindole upon NE, DA 5-HT and monoamine-oxidase (MAO) in the midbrain, cerebral cortex, and cerebellum of the mouse Four groups of 40 animals were administered intraperitoneally with 0, 0.5, 1.0, 2.0 mg/kg/day respectively, during 120 days. Monoamines levels were determinated by Welch and Welch's method and MAO activity was measured by Wurtman's method. Independently of the doses chronic administration of iprindole induced remarkable increase in serotonin levels ($p < 0.001$) in the regions studied. It is important to point out that no changes were observed in DA and NE levels. MAO activity showed a significant inhibition ($p < 0.01$) at the doses used in all the regions. The inhibition showed a direct dose-response relationship in the midbrain. The specific changes observed in 5-HT, in its degradation product and in MAO activity, as well as, in the absence of catecholamine changes, permit us speculate a direct and specific action of iprindole on the serotonin metabolism.

1599 DEVELOPMENT OF IMPROVED TRAINING PROCEDURES FOR THE TWO-BAR DRUG DISCRIMINATION TASK. Donald A. Overton. Dept. Psychiatry, Temple Med. Sch., EPPI, Phila., Pa. 19129.

Many investigators use a drug discrimination (DD) task in which rats must press one lever when drugged and a second lever when not drugged in order to obtain food or water reinforcement. However, as usually implemented, this task has several unfortunate properties--prolonged training is required, asymptotic accuracy of discrimination is low, and no index of the degree of discriminability of a drug is provided. In an attempt to find solutions to these problems, a series of experiments was conducted in which rats discriminated phenobarbital 50 mg/kg ip from saline injections, as follows:

(1) Variations in training compartment design were tested to see if they would increase DD accuracy above that obtained in the usual box in which two bars are mounted side-by-side on one wall. They did not.

(2) Various schedules of reinforcement were used. Markedly different asymptotic accuracies were observed under these schedules. Fixed ratio schedules yielded highest DD accuracies, DRL, tandem VI-FR and VI-approach/avoidance schedules yielded intermediate DD accuracy, and variable interval schedules yielded lowest accuracy.

(3) Several methods of accomplishing the early stages of operant training were compared. Accurate discrimination was much more rapidly achieved if drug was used during early shaping sessions on the 'drug' bar than if shaping was completed without drug. When response-appropriate drugs were used during initial training, many rats showed state dependent learning and pressed the state-appropriate lever from the very beginning of DD training.

(4) DD training using the FR schedule was performed with several doses of phenobarbital in order to develop an index that would reflect the relative degree of discriminability of various training drugs.

The combined use of a ratio schedule and response-appropriate drugs during shaping allowed us to train DDs to asymptotic accuracy in less than 10 training sessions which is considerably faster than previously reported.

1600 RESPONSE TO PENTOBARBITAL IN ALBINO AND PIGMENTED MICE: A WITHIN STRAIN COMPARISON. Regina Pakalnis* and I.S. Westenberg (SPON: A.S. Schwartz). ASU, Tempe, AZ 85281 & VA Hosp., Phoenix AZ 85012.

Albino strains of rodents differ from pigmented strains in their responses to drugs; e.g. when compared with pigmented strains of rats, albinos had lower LD50's and increased sleep-time to pentobarbital (Shearer et al.,1973). Their study was a "between strains" comparison; thus the genetic variable of albinism was confounded with other genetic variables. To eliminate this confounding, we used a "within strain" design comparing albino and pigmented mice of the same inbred strain; animals differed in 1 gene at the C locus but were otherwise genetically identical. We used 7 littermate pairs of coisogenic C57BL/6J-c^{2J} male mice 137-162 days of age (mean = 146) at the start of the experiment. Each pair was from a different litter and consisted of an albino, c^{2J}/c^{2J}, and a pigmented, $+/c^{2J}$, matched for weight. In some litters additional male mice were available and used as controls (4 albino, 2 pigmented). All mice were food deprived 3-4 hours prior to injection. Mice were weighed on the morning of the injection and 3 days after. Experimental animals were injected weekly i.p. with sodium pentobarbital(Nembutal); dosage increased 10 mg/kg/week (to 150 mg/kg as of 5-5-78). Controls were injected i.p. with saline 2 µl/g. After injection littermates were placed together in observation cages. Controls were removed upon experimental animals' loss of righting reflexes (LRR). Frequent checks assured accurate timing of the return of the righting reflexes(RRR). Experimental animals were returned to their home cages after RRR. Between experimental sessions littermates were housed together in the mouse colony.

Median sleep-time (interval from LRR to RRR) was consistently greater for albinos at dosages greater than 50 mg/kg. Sleep-time differences were significant ($p \leq .05$, Mann-Whitney U) at 70,100,110,120,130, and 150 mg/kg and approached significance at 80 mg/kg. The LD50 was 140 mg/kg for both albino and pigmented mice. No significant differences were found for time to LRR. Sleep-time results are consistent with the findings of Shearer et al.(1973), but LD50 data are not. However, their study involved comparisons across several strains of rats while ours was a within strain comparison of mice.

Supported by NIH Grant No. 1 R01 EY 01888-01 and Fight For Sight, Inc., (NY), Grant No. G-599.

1601 AN OPIATE EXCESS MODEL OF CHILDHOOD AUTISM. Jaak Panksepp, Barbara Herman*, and Tom Vilberg*. Dept. Psych., Bowling Green State University, Bowling Green, OH 43403

Early childhood autism has several distinct behavioral characteristics which are amenable to being modelled in animals--a low incidence of crying during infancy, a failure to cling to parents, a low desire for social companionship, and a variety of learning abnormalities characterized by unusual strength or persistence. From the assumption that a primary disorder in autism is a deficit in the elaboration of social affect, and from our theoretical perspective that social affect in mammals may be controlled by brain opiates, we have hypothesized that autistic symptoms may be precipitated by excessive activity of endogenous brain opiate systems. Accordingly, we have attempted to generate the above-mentioned behavioral symptoms of autism in animals with injections of low doses of the opiate agonist morphine sulfate. We have found that morphine at doses of 0.25-1.0 mg/kg can markedly reduce acute isolation-induced crying in young puppies, guinea pigs, and chickens in dose-dependent fashion. For instance, in 8-16 day old guinea pigs, .25 and 1.0 mg/kg morphine reduced crying to 65% and 25% of placebo levels respectively. At the same dose levels, morphine reliably reduced clinging behavior of 12-18 day old rat pups, and the desire for social companionship (as measured by proximity maintenance time) in adult rats and adolescent guinea pigs. Finally, in a T-maze learning situation motivated by either the reward of food or return to home, morphine (1 mg/kg) led to marked persistence of behavior during extinction in 20-40 day old rat pups. Control animals extinguished within 3-6 daily sessions, while morphine treated animals continued to run without diminished speed for up to two weeks of testing. These results suggest that morphine has a very powerful capacity to modulate social affect, and are consistent with the possibility that autistic symptoms in human children may be due to endogenous overactivity of brain opiate systems. If so, a rational pharmacologic adjunct to psycho- or behavior-therapy may be the administration of relatively pure opiate antagonists such as naloxone or naltrexone.

Supported by Research Scientist Development Award MH-0086 to JP.

1602 STIMULANT DRUGS ENHANCE EFFECTS OF PUNISHMENT IN NORMAL AND CHLORDIAZEPOXIDE TREATED RATS. B.A. Pappas, R.A. Vogel, G.D. Frye, G.R. Breese and R.A. Mueller. Biol. Sci. Res. Ctr., UNC Sch. Med., Chapel Hill, NC 27514.

Animal models of minimal brain dysfunction (MBD) have focused upon reproducing the hyperkinetic aspects of this syndrome. There has been scant attention paid towards models for the attenuated impulse control which is a major symptom (Wender, Life Sci. 14, 1974). To this end we assessed the effects of stimulant drugs in a conflict procedure in which water-deprived rats, previously trained to drink in the apparatus, were briefly exposed to electric shock delivered through the water spout. The number of shocks received can be argued to reflect the capacity of the rat for inhibitory control of the drinking impulse when the latter leads to punishment. As previously reported (Vogel et al., Psychopharmacol. 21:1, 1971) prior injection of the anxiolytic chlordiazepoxide (8 mg/kg) more than doubled the number of shocks that the rats received. This disinhibition of punished behavior was counteracted by the stimulants d-amphetamine (0.5-4.0 mg/kg) and methylphenidate (0.75-6.0 mg/kg) in a dose related manner. Imipramine (4.0-32 mg/kg) and buproprion (2.5-40 mg/kg) were similarly effective. Imipramine and buproprion have not previously been reported to affect punished responding in other paradigms, such as the operant Geller conflict. These drugs, except methylphenidate, also reduced the number of punishments received by saline control rats in a dose related fashion.

The disinhibition caused by chlordiazepoxide in this procedure may be analagous to the weak impulse control displayed by children diagnosed as minimal brain damaged. Future research should further assess the possibility that antagonism of this disinhibition by drugs reflects their clinical efficacy for symptomatic treatment of MBD. (Supported by HD-10570, AA-02334 and MH-00013.)

1603 AMITRIPTYLINE INDUCED ALTERATIONS IN CEREBRAL CAPILLARY PERMEABILITY. S.H. Preskorn*, H.B. Clark*, B.K. Hartman, and M. Raichle (SPON: D.R. Justesen). Depts. of Psychiatry and Neurology, Washington University School of Medicine, St. Louis, MO 63110.

Intraperitoneal (i.p.) administration of amitriptyline (AMT) has been shown to profoundly alter cerebral capillary permeability to water (P_W). This has been studied using a modication of the indicator-dilution technique in rats (Oldendorf, et al., Am. J. Physiol. 229:1110, 1975) and a single injection, external registration technique in monkeys employing $H_2{}^{15}O$ as the tracer (Raichle, et al., Am. Physiol. 220:543, 1976). AMT at blood levels under 800ng/ml in rats induced an increase in extraction fraction of ^{3}H-water (E_W) from control values of 0.67 to 0.90 ($p < .001$) within five minutes of administration. Ethanol which normally has an extraction fraction (E_e) of 0.80 in rats becomes freely permeable (i.e. $E_e = 1.00$) under this treatment. Changes in E_W are a function of cerebral blood flow (CBF) as well as P_W. However, AMT at blood levels between 150-200ng/ml in monkeys caused a significant increase in the permeability of ^{15}O-water without altering CBF. Thus, AMT appears to specifically alter brain P_W in both rats and monkeys. The AMT effect was not blocked by phenoxybenzamine administered i.p. 24 hours before AMT treatment. Lidocaine administered i.p. at the same dose produced a comparable increase in water permeability. The relative potency of tricyclic antidepressants in producing this permeability alteration is: amitriptyline $\geq$ nortriptyline > imipramine > protriptyline. This is the same order as their relative local anesthetic effects. The AMT effect is thus compatible with the potent local anesthetic properties of AMT. The presumed psychopharmacology of tricyclic antidepressants has not previously considered such microculatory effects.

1604 PERSISTENCE OF CHOLERA TOXIN-INDUCED STIMULATION OF ADENYLATE CYCLASE AND BEHAVIORAL CHANGES. Linda F. Quenzer* and Linda G. Dusek*, (SPON: R.L. Volle). Dept. Pharmacol., U. Conn. Health Ctr., Farmington, CT 06032.

We have previously shown that stereotaxic injection of cholera enterotoxin (1 µg in 1 µl) unilaterally into the substantia nigra produces a 2-fold increase in basal adenylate cyclase activity in the ipsilateral caudate nucleus 24 hours after the injection (Quenzer et al., Sci. 195: 78, 1977). We now find that adenylate cyclase activation, beginning at 6 hrs after toxin treatment, occurs in homogenates of both substantia nigra and the ipsilateral caudate after a single cholera toxin injection into the substantia nigra. Basal activity in the caudate on the toxin side increases significantly ($p<.005$) from 42.7 pmol cAMP formed/mg wet weight/3 min to 110.1 pmol/mg tissue/3 min at 24 hrs and is maintained at that level for the duration of time tested (3 wks). Basal adenylate cyclase activity in substantia nigra (23.4 pmol cAMP/mg tissue/3 min) is much lower than that in caudate but it too is increased (46.9 pmol/mg tissue/3 min) 24 hrs after intranigral cholera toxin. Although the basal adenylate cyclase activity is increased 2-3 fold, the ability of 100 µM dopamine to further stimulate the enzyme is apparently unchanged. No change occurs in the activity of cAMP phosphodiesterase when measured using 1 µM and 100 µM substrate concentrations. The prolonged increase in adenylate cyclase activity in combination with unchanged phosphodiesterase metabolism of cAMP suggests that endogenous levels of cAMP are elevated on the toxin treated side of the brain for at least 3 weeks.

A 3.5-fold increase in motor activity also appears within 24 hrs after an intranigral injection of cholera toxin. The increased activity is characterized by spontaneous rotation contralateral to the toxin-treated side. Spontaneous rotation persists in most animals for at least 3 days but is entirely lost within 1 week of the toxin injection. At that time no direction bias is evident even in animals stimulated with tail pinch. Increased motor activity also diminishes by one week but is more persistent than the rotation. The difference in time course of neurochemical changes and behavioral changes following cholera toxin injection suggests that the toxin-induced increase in cAMP formation is unrelated to the increase in activity or spontaneous rotation.

1605 ONTOGENY OF AMPHETAMINE ANOREXIA: EFFECTS OF AMPHETAMINE ON NIPPLE ATTACHMENT. Lisa A. Raskin* and Mark J. Dollinger* (SPON: B. A. Campbell). Princeton University, Princeton, N.J. 08540

The psychomotor stimulant properties of amphetamine are evident in the rat as early as one day of age, but amphetamine anorexia is not observed until approximately 15 days of age. Prior to that age amphetamine appears to potentiate feeding (Lytle, Moorcroft & Campbell, JCPP, 1971, 77, 388-393). The following experiments examined this transition in the effects of amphetamine from facilitation to disruption of feeding in the developing rat. This was accomplished by using three tests of nipple attachment.

In the first test pups were placed near the ventrum of an anesthetized dam which made it necessary for them to move about in order to attach to the nipple. Consistant with the effects on feeding, amphetamine produced an increase in attachment in 5-day-olds but decreased it in 15-day-olds. In the second test 5- and 15-day-old pups were held at the nipple of an anesthetized lactator and nipple attachment was observed. In this test pups did not nave to locomote to the nipple before attaching. Amphetamine neither disrupted nor facilitated nipple attachment in 5-day-olds but markedly disrupted it at 15 days of age. Our third test was designed to dissociate the psychomotor stimulant properties of amphetamine from its direct effect on attachment. Latency to approach an anesthetized lactator was measured in 2-, 5-, 10-, and 15-day-old pups using a runway apparatus with the anesthetized lactator at one end. After the pup had reached or was placed at the ventrum, latency to attach was also recorded. Amphetamine facilitated approach to the mother at all ages but slowed attachment in 15-day-old pups. As in the second test, amphetamine did not effect speed of attachment in the younger pups.

These results demonstrate that amphetamine facilitates feeding in the young pup by enhancing the locomotor activity required to approach the nipple. In the older pup amphetamine disrupts feeding due to its anorexigenic properties.

1606 SMALL DOSES OF MORPHINE SULFATE AND PRESSING FOR HYPOTHALAMIC INTRACRANIAL STIMULATION (ICS) IN RATS. Larry D. Reid, Marcia D. Lind*, Michael A. Bozarth, Vicki J. Merriman*, and June M. Stapleton*. Dept. of Psychol., Rensselaer Polytechnic Institute, Troy, NY, USA, 12181.

There has been considerable exploration of the effects of larger doses of morphine (e.g., 10 mg/kg) on pressing for hypothalamic ICS. These doses are larger than those a rat might self-administer. We tested, therefore, for the effects of smaller doses on pressing for ICS (.125, .25, .5, 1.0, and 2.0 mg/kg, ip).

Rats were each fixed with a chronically indwelling bipolar electrode. The lateral hypothalamic sites of ICS were confirmed histologically after behavioral testing. After recovery from surgery, rats were trained to press a lever for a single train of ICS (60 Hz sine waves of .3 sec). Intensities of ICS differed for each rat, being selected during initial testing to produce stable, sustained pressing, and ranged from 15 to 40 μA. Doses of morphine or placebo were then given immediately before each 1-hr test session.

Doses of .125 and .25 mg/kg occasionally led to increased pressing for ICS, but overall increments in pressing were not reliably different from those following placebos. Doses of .5, 1.0, and 2.0 mg/kg did produce reliable increments in pressing.

A second study tested whether the doses of 0, .5, and 10 mg/kg of morphine produced a conditioned preference for the place where a rat had experienced morphine's effects. The testing procedure was nearly identical to the one used by Rossi and Reid (1976, Physiol. Psychol., 4, 269-274). Results indicated that the doses of .5 and 10 mg/kg lead rats to spend more time in the place of their morphine-experience compared to results from placebo-injected controls.

Small doses of morphine, therefore, (a) lead to increments in pressing for hypothalamic ICS and (b) induce a state that leads rats to go to the place where they experienced that state (i.e., the morphine produced a positive affective state). These observations (as well as others with larger doses) are concordant with the idea that morphine is self-administered for its positive affective effects. Such positivity might be due to morphine's ability to increase activity in the tissue of intracranial self-stimulation.

1607 NICOTINE AS A DISCRIMINATIVE CUE IN RATS: ISOMERIC SPECIFICITY. Carmelo Romano* and Avram Goldstein. Dept. Pharmacology, Stanford Univ. Sch. Med., and Addiction Research Foundation, Palo Alto, CA 94305

Rats were trained to discriminate (-)-3-nicotine (0.4 or 1.0 mg/kg, base) from saline, utilizing a T-maze-shock escape paradigm similar to Overton's (Psychopharm. 10:6, 1966). Training sessions (5 days per week) lasted a period of months so that consistent and reliable performance was maintained (91% mean correct responding for 0.4 mg/kg group, N = 3; 97% for 1.0 mg/kg group, N = 7; for most animals, this represents performance maintained for most than 90 sessions). A 10-session performance test preceded each test drug session to allow statistical analysis.

We report here responses of the animals to (+)-3-nicotine, (±)-2-nicotine, (±)-4-nicotine, and lobeline. Also included are the pooled data from tests with (-)-3-nicotine and saline administered blind. The fraction shows the number of animals giving a nicotine response (i.e., turning in their nicotine direction) and the total number of animals tested.

Drug and dose (mg/kg base)	Training dose 0.4	1.0
(-)-3-nicotine training dose	6/6*	11/11*
saline	2/6*	0/9*
(+)-3-nicotine 1.0	0/3	0/7
10.0		0/3
(±)-2-nicotine 1.0	0/3	1/4
10.0	0/3	0/4
(±)-4-nicotine 1.0	0/1	0/4
10.0	1/3	0/3
lobeline 10.0	0/3	0/4

*multiple tests on same animals.

We conclude that the receptor mediating the discriminative cueing property of nicotine possess a high degree of geometrical specificity.

(Supported by grants from the National Institute on Drug Abuse.)

1608 THE EFFECTS OF A LOW-TRYPTOPHAN DIET ON APOMORPHINE-INDUCED STEREOTYPED BEHAVIOR IN RATS. B. J. Sahakian, R. J. Wurtman, J. K. Barr*, and W. R. Millington. Lab. Neuroendocrine Regulation, Dept. Nutrition and Food Science, M.I.T., Cambridge, MA 02139.

Psychomotor stimulant drug-induced stereotyped behavior is thought to be mediated primarily by dopaminergic mechanisms. However, other neurotransmitter systems, such as the cholinergic, noradrenergic, and serotonergic (5-HT), are known to exert modulatory control over this behavior. Therefore, we examined the effects of decreased levels of brain 5-HT, produced by dietary manipulation, on apomorphine-induced stereotypy in rats. Rats on a low-tryptophan diet showed significantly shorter durations of stereotyped behavior as compared with rats on a control diet in response to most doses of apomorphine. In addition, low-tryptophan-diet rats showed significantly less intense stereotypy induced by 0.2 mg/kg apomorphine. The differences between the two groups in stereotypy were abolished by pretreatment with 50.0 mg/kg L-tryptophan. It was later confirmed that the rats on a low-tryptophan diet had decreased levels of brain 5-HT. In other experiments, it was found that pretreatment with MK-212, a novel 5-HT agonist, or with quipazine also was able to restore the apomorphine-induced stereotypy to control levels among low-tryptophan-diet rats. Results are discussed in terms of the role of the serotonergic system in the modulation of psychomotor stimulant drug-induced stereotypy, and the inverse relationship between amount of locomotor activity and the amount of stereotyped behavior.

Supported by grants from NIH (AM-14228) and NASA (NGR-22-009-627).

1609 EFFECTS OF MORPHINE ON ESCAPE FROM HYPOTHALAMIC STIMULATION IN THE RAT. Susan Schenk*, Terrence Williams* and Peter Shizgal. Cen. Res. Drug Depen., Dept. of Psychology, Concordia University, Montreal, Quebec, Canada, H3G 1M8.

The modulation by morphine of the aversive properties of electrical brain stimulation has been studied less extensively than the drug's interaction with rewarding effects of stimulation. The present study was undertaken in order to extend previous findings in several ways. First, we used a frequency threshold measure of stimulation-escape in an attempt to reduce the contribution of performance effects that often bias response rate measures. Second, we attempted to establish time- and dose-response relationships that could be compared with those describing the effects of morphine on brain stimulation reward. We also administered a given dose repeatedly to observe tolerance effects.

Rats were trained to press a lever in order to obtain a 1 second interruption of lateral hypothalamic stimulation. If the rat failed to respond, the train of 0.1 msec. monophasic, cathodal pulses was automatically terminated after 10 seconds and then reinstated 1 second later. The rectangular, constant current pulses were delivered through monopolar electrodes. Frequency thresholds were determined by decreasing the stimulation frequency in 0.1 $\log_{10}$ steps. Subjects were tested for 2.5 minutes at each step. Optimal current intensities were selected for each rat and then fixed for the duration of the experiment. After stabilization of baseline performance, a series of daily morphine HCl injections was begun. An initial dose of 20 mg/kg (i.p.) was incremented in 0.1 $\log_{10}$ steps until a dose of 320 mg/kg was achieved.

Morphine reliably produced a monophasic increase in threshold for stimulation escape. No consistent tolerance effects were observed; escape thresholds were unaltered during withdrawal. There were large individual differences in the magnitude of the drug's effect which may be related to the ratio of thresholds for self-stimulation and escape at each placement.

Experiments are now in progress to determine concurrent changes in self-stimulation and stimulation-escape from the same stimulating electrode. Initial findings suggest that changes in the aversive properties of stimulation are not due to the effects of the drug on the substrate underlying reward. This work was supported by Canadian NRC Grant A0308.

1610 CHRONIC ADMINISTRATION OF d-AMPHETAMINE TO SELECTED MEMBERS OF PRIMATE SOCIAL COLONIES AS AN ANIMAL MODEL OF PSYCHOSIS. R.F. Schlemmer, Jr., D.L. Garver, N. Narasimhachari, J.M. Davis. Illinois State Psychiatric Institute, Chicago, Illinois 60612

Chronic administration of large doses of amphetamine to human volunteers results in a syndrome (amphetamine psychosis) which is virtually indistinguishable from paranoid schizophrenia. Similar administration of amphetamine to selected members of primate social colonies may provide an animal model which can be used to study behavioral aberrations characteristic of psychosis. With this in mind, the following study was conducted. Following a baseline observation period, d-amphetamine (d-Amph), 1.6 mg/kg in time-release form, was administered n.g. every 12 hours for 12 consecutive days to 13 female Stumptail macaques selected from 6 stable, adult social colonies of 4-5 monkeys each. No more than 2 monkeys/group received drug treatment (Tx) during one time interval. Behavioral observation occurred once daily for at least 4 days prior to and for the 12 days during Tx. During this time, "blind" observers quantified and recorded the social and solitary behaviors of all members of each colony for 1 hr. using the focal-sampling technique. In general, d-Amph induced abnormal behavior and altered normal behavioral patterns in all Tx monkeys, however, there was considerable variation in response between animals, some of which could be correlated with social rank. All Tx monkeys developed stereotypy and hypervigilance as abnormal behaviors. The form of stereotypy varied between animals but higher ranking (HR) monkeys displayed more social stereotypy than lower ranking (LR) monkeys. No rank correlation could be detected for hypervigilance. d-Amph-induced changes in normal behavior primarily involved social withdrawal. Most Tx monkeys had decreased social grooming with increased self grooming. Most Tx monkeys became isolated from other monkeys as reflected by increased distancing scores. LR monkeys became isolated earlier in Tx than HR monkeys. HR monkeys had increases in submissive gesture scores during Tx. Several HR females displayed large increases in threats, but rarely followed with attacks. d-Amph-induced excessive scratching in 8 monkeys. Several of the behavioral changes induced in monkeys by d-Amph have been described in humans with amphetamine psychosis including: large individual variation in response, stereotypy, social withdrawal, hypervigilance, and excessive scratching. This study also demonstrates that social status may play a role in some behavioral responses seen with d-Amph. We conclude that chronic d-Amph treatment of selected members of primate social colonies provides a particularly relevant animal model for the study of the psychosociopharmacology of amphetamine and psychosis.

1611 MATERNAL BEHAVIOR AND OFFSPRING AMPHETAMINE RESPONSE. H. Schreiber; Dept. Psychiat., Washington Univ. Sch. Med., St. Louis, MO 63110; R. Bell, J. Palet*, K. Nau*, J. Eatwell*, B. Baker*, Dept. Psychol., Texas Tech Univ., Lubbock, TX 79409.

Recent studies in our laboratory have shown that when rat pups are handled in infancy and returned to a mother-present nest, they show, in adulthood, reduced amphetamine toxicity (Schreiber, et al., Psychopharm. 52, 173, 1977) and reduced stereotypy. Rat pups which are handled and returned to a mother-absent nest show no such diminished response in adulthood, indicating a crucial maternal component. The present study was undertaken to show that certain styles of behavior in rat mothers (elicited by stressing their pups) are sufficient to reduce amphetamine stereotypy--even when the offspring themselves are not directly stressed.

Young, primiparous (PRIMIP) and older, multiparous (MULTIP) mothers were exposed to (a) baskets of actively-signalling cold-stressed donor pups (STRZ), (b) baskets of anesthetized donor pups (ANES), or (c) baskets of ping-pong balls (BSKT) during the first week after parturition while the mothers' own offspring remained undisturbed. Maternal behavior was assessed before and during exposure to the baskets as well as during the week following exposure to the baskets (the second week after parturition). PRIMIP mothers responded to the distress calls of the donor pups by increasing their care-giving behavior. MULTIP mothers responded by decreasing their care-giving behavior. Both PRIMIP and MULTIP responded to ANES donor pups in a fashion intermediate to the STRZ and BSKT conditions, except that mothers exposed to ANES donor pups constructed better rated nests. Following weaning, these mothers' male offspring were subjected to six consecutive daily injections of saline, 2.5 mg/kg or 5.0 mg/kg d-amphetamine 30±5 minutes prior to a two-minute observation in a Y-maze. Following four days of an accelerating drug regimen, subjects were injected with saline. On the next day, subjects were tested in the Y-maze as before, except that all subjects were injected with 5.0 mg/kg d-amphetamine.

Offspring of PRIMIP mothers exposed to STRZ donor pups showed less stereotypy in comparison with the offspring of PRIMIP mothers exposed to ping-pong-ball-filled baskets. Offspring of MULTIP mothers exposed to STRZ donor pups showed more stereotypy in comparison with offspring of MULTIP mothers exposed to baskets. Although offspring of mothers exposed to ANES donor pups showed evidence of greater activity and lower body weight, stereotypy was largely influenced. These results indicate that the rat mother's behavior is sufficient to influence the offspring's later response to amphetamine.

1612 BIPHASIC EFFECT OF ACUTE ETHANOL ON AUDIOGENIC SEIZURES. Robert A. Schreiber, Dept. Biochemistry, UTCHS, Memphis, TN, 38163.

The metabolism of an acute dose of ethanol should lead to a transiently increased amount of ethanol-derived hydrogens in brain NADH, as well as an abundance of acetyl CoA (AcCoA). This should transiently lead to an abundant supply of ATP and should in turn transiently reduce the rate of production of glycolysis-derived and of TCA cycle-derived hydrogens in NADH, and of glycolysis-derived AcCoA. Brain glucose levels would be expected to (and do) transiently rise after acute ethanol, followed by a deep depression in brain glucose hours later, as ethanol is cleared from the body (Arch int Pharmacodyn, 154, 108, 1965). Glycogen levels in brain transiently fall, indicating a greater rate of glycogen to glucose-6-phosphate (G-6-P) than of high-ATP-suppressed glucose to G-6-P.

It has been proposed that decreased total immediately mobilizable energy reserves in brain may underly susceptiblity to audiogenic seizures (SAGS). (Res. Comm. Psychol., Psychiat., Behav., in press; Pharm. Bioch. Behav., 8(5), in press, 1978). Should this model hold, then one would predict an early protection from SAGS by ethanol, followed by a transient heightened SAGS as the ethanol-derived energy in brain (either directly in brain NADH, or indirectly via plasma ketone bodies) was cleared, and glycolytic-based energy reserve levels in the brain remained transiently at a low level prior to recovery.

C57BL/6J mice were subjected to audiogenic priming at 16 days of age (127 ± 2dB for 60 sec.) and tested at 21 days of age. Mice were injected with 1.96 mg/kg ethanol (0.1 ml of 25% per 10 gm mouse) ip; behavioral effects at this dose (staggering, and in some cases, mild sedation) dissipated by 30 min. post-injection.

Mice were tested for SAGS at 30-min or 60-min intervals up to 7 hr. post-injection. Data are shown below: (mice completely protected at 60" and 120" as well)

	control	+30"	+150"	180	210	240	270	300	360	420
N_1	12	4	4	7	7	7	6	6	7	7
$\bar{x}^1$	3.08	0	0	1.00	2.71	2.85	3.50	4.83	2.85	2.57
SEM	0.51	0	0	0.69	0.68	0.80	0.80	0.16	0.80	0.71
t vs C^2		5.73	5.73	2.27	0.39	0.22	0.40	3.08	0.22	0.54

1. Mice were assigned a "seizure severity score" based on the most severe level of seizure attained. Wild run=1, clonic-flexion=2, clonic-extension=3, tonic=4, death=5.
2. Student's t; statistically significant differences ($p<.025$) are underlined.

1613 BEHAVIORAL EVIDENCE FOR ANATOMICAL SPECIFICITY OF ACTION BY CLOZAPINE. Thomas F. Seeger* and Eliot L. Gardner, Depts. of Pharmacology, Psychiatry, and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

Biochemical evidence suggests that clozapine has a dopamine (DA) blocking activity specific to the mesolimbic system, while "classical" neuroleptics such as haloperidol have equal potency in both the nigro-striatal and mesolimbic DA systems. However, behavioral evidence for this specificity is lacking, perhaps due to the need for a good behavioral assay for mesolimbic DA function.

We have tested the effect of chronic clozapine or chronic haloperidol treatment on intracranial self-stimulation (ICSS) in rats with electrodes implanted in the A10 cell body area of the ventral tegmentum (origin of the mesolimbic pathway). Following three-week treatment with either 20 mg/kg/day of clozapine, or 1 mg/kg/day of haloperidol, the rats showed an average 35% increase in ICSS rate over pre-drug control levels (N=10 in each group). This increase was long-lasting in both groups, persisting for three weeks before beginning a week-long decline to pre-drug control rates.

In order to compare this apparent induction of supersensitivity in the mesolimbic system with behaviors that have been localized to the nigro-striatal system, we also quantified stereotypic behaviors (chewing, rearing, sniffing, head bobbing, and grooming) after injection of 1 mg/kg of apomorphine. The rats were then given the same chronic clozapine or haloperidol treatment that the ICSS rats received. After drug treatment, all animals showed a significant increase in locomotor activity (a behavior believed to have both striatal and mesolimbic components). However, the haloperidol group showed significant increases in chewing and sniffing (believed to be localized to the striatum), while the clozapine group showed no significant changes in these behaviors (N=6 in each group).

Taken together, we believe that these results show a relative specificity of action of clozapine for behaviors that are mediated by the mesolimbic DA system. In addition, they suggest a possible role for the self-stimulation assay in testing the anti-psychotic potency of atypical neuroleptics such as clozapine.

— (Supported, in part, by USPHS Grants NS-09649 and GM-07260)

1614 LONG-TERM EFFECTS OF HALOPERIDOL ADMINISTRATION DURING DEVELOPMENT. Ismail A. Shalaby*, Linda Patia Spear* and John Brick* (SPON: C. VanHartesveldt). Dept. Psychol. SUNY at Binghamton, Binghamton, N.Y. 13901.

Chronic haloperidol treatment during development was found to induce long-term behavioral and psychopharmacological effects. Pregnant Sprague-Dawley rats were injected subcutaneously twice daily with 0.25mg/kg haloperidol or saline, or were noninjected but handled twice a day, from day 1 of gestation until weaning of the offspring at postnatal day 21. The offspring of such treatments were tested on either postnatal days (P) 23-30, 35-42 or 47-54. At least seven animals were tested under each drug condition at each age for each of the treatment conditions (as there were no differences in the response of offspring of saline treated and noninjected control mothers, these two groups were combined into a single control group). Mothers from the experiment were also tested using the same procedures on P23-30 of their offspring.

After termination of a chronic treatment regime of haloperidol to adult rats, animals are typically hyperactive, have an enhanced response to drugs that stimulate the dopamine system (such as apomorphine and amphetamine), and have a decreased response to drugs that block dopamine receptors (such as haloperidol). However, the effects of chronic haloperidol treatment during development did not parallel that of adult chronic treatment, and varied with age at the time of testing. Offspring given haloperidol chronically during development were spontaneously hyperactive in the open field, showed an attenuated response to amphetamine and an accentuated response to haloperidol when tested at the early (P23-30) and late (47-54) testing intervals. Drug treated offspring did not differ from controls on any of these measures at the intermediate (P35-42) testing interval. Thus behavioral and pharmacological effects of chronic haloperidol treatment during development may vary with age at the time of testing. In addition, although both chronic neuroleptic treatment during development and in adulthood may produce behavioral hyperactivity, the later pharmacological responsiveness of such treated animals may vary: adult treated animals show an accentuated response to amphetamine and attenuated response to haloperidol; the opposite is found in developmentally treated animals when tested at early (P23-30) or late (P47-54) testing intervals.

The results of neurochemical studies on the effects of chronic haloperidol treatment during development will be presented. These results may help elucidate the synaptic basis for the paradoxical pharmacological responses observed in this study.

1615 LONG LASTING BEHAVIORAL SEIZURES IN THE YOUNG CHICKEN INDUCED BY PHENCYCLIDINE. Clifford J. Cherry and P. Scott Hunter*. Biology Dept. Texas A & M Univ. College Station, Tx. 77843

The abuse of the hallucinogen, phencyclidine (PCP) on the "street" is increasing at an alarming rate. Unfortunately, the mechanism and site of action of PCP is not clearly known, but the use of PCP is frequently associated with convulsive movements (Johnstone, M. Anesthesia 1959, 31, 433-) and status epilepticus (Kessler, G. F. et al., New Eng. J. Med. 1974, 291, 479-) in humans and seizures in animals (Chen, G. et al., J. Pharmac. Exp. Ther. 1959, 127, 241-) in animals. However, these papers do not give detailed quantitative descriptions of the seizures. In addition, little is known about the effect of PCP on developing organisms.

Male White Leghorn chickens were obtained at on day of age and housed under standard conditions. Seperate groups of chicks were exposed to 10 mg/kg of PCP, intraperitoneally, at 2, 3, 8, 9, 11, and 18 days of age, where the day of injection was choosen randomly. Seperate groups of chicks were exposed to 1, 5, 10, 20, 40, 60, or 80 mg/kg PCP at 2 days of age to determine the lethal dose 50% (LD50) and the effective dose 50% (ED50). The latency to the development and duration of two classes of behavioral seizures were noted: clonic seizures (i.e. the chick would lose posture and the legs and toes would move rapidly in a running motion, with eyes open) and tonic seizure (i.e. the chick would lose posture and the legs and toes would be vigorously extended, with eyes closed and frequently the wings extended up over the back.

The LD50 for PCP is the 2 day old chick is 43.58 mg/kg. The ED50 for seizure development in the 2 day old chick is 7.6 mg/kg. When compared to the 2 day old chick, older chicks tended, on the average, to have more clonic seizures and the average time between clonic seizures and the average length of clonic seizures decreased. In older chicks, the average lenght of tonic seizures tended to increase, while the average time between tonic seizures decreased. When the dose level of PCP was increased to 15 mg/kg in the 2 day old chick, it tended to increase the inter-clonic seizure interval, but during this time a tonic seizure developed which lasted for the entire inter-clonic interval. The higher dose level was also associated with an increase in the average length of clonic seizures and an increase in the average length of tonic seizures, as well as the frequency of tonic seizures.

1616 BEHAVIORAL EFFECTS OF ACUTE AND CHRONIC PHENCYCLIDINE IN THE RAT. R.C. Smith, C.A. Biggs*, D.E. Leelavathi, H.L. Altshuler. Texas Res. Inst. of Mental Sciences, Houston, Texas 77030.

Phencyclidine (PCP) is an anestetic drug with psychotomimetic properties, which has been shown to effect brain dopaminergic, noradrenergic, cholinergic, and serotontic systems. Recent experiments in our laboratory now indicate that pre-treatment with low to moderate doses of naloxone, intensifies some of the behavioral effects of PCP in the rat, and therefore suggests that endorphins and opiate receptors may also be involved in the mechanism of action of PCP. In other experiments rats were chronically administered PCP (10 mg/kg) for 30 days. We found that after 30 days of chronic treatment with PCP, chronic PCP rats exhibited greater behavioral effects of PCP on ataxia, stereotypied sniffing, and rearing, but not on circlinig behavior (during the first 60-90 minutes after i.p. injection of 10 or 15 mg/kg PCP) as compared to control rats treated chronically with saline and then given these acute doses. These results suggest that behavioral supersensitivity may develop to some of the effects of PCP during or after chronic treatment with the drug.

1617 INCREASES IN URINATION AND DEFECATION IN RATS FOLLOWING P-CHLOROAMPHETAMINE. J.M. Stein, K.M. Kantak, C.C. Loullis, M.J. Wayner, R.C. Cook* and J.A. Cudworth*. Regional Primate Research Center, Univ. of Washington, Seattle, WA 98195 and Brain Research Laboratory, Syracuse University, Syracuse, NY 13210.

p-Chloroamphetamine (PCA) produces long term and short term changes in brain amines. The long term effects of PCA include decreases in brain serotonin (5-HT), tryptophan hydroxylase and 5-hydroxyindoleacetic acid concentrations. Short term effects of PCA include increased extraneuronal 5-HT and various effects on central catecholamines. During the initial period, rats injected with PCA display a stereotyped behavioral syndrome which includes hyperactivity, arching of the back, piloerection, reciprocal forepaw treading, and hind limb abduction. It is also during this initial period that large decreases in food and water intakes and body weights have been reported. In a previous study, (Stein, et al., 1978), it was shown that 300 gm rats administered PCA, 5.0 mg/kg, lost up to 10 gm of body weight during the first hr post-injection, and up to 18 gm during the first 3 hr post-injection. Pretreatment with fluoxetine 10.0 mg/kg, a specific 5-HT uptake blocker, did not affect these changes. This weight loss was apparently due to increased urination and/or defecation because: 1) the decreases in weight occurred whether animals were injected during high or low probability eating periods; and 2) the decreases were significantly greater compared to a food and water-deprived control group.

In the present study, the body weight losses, urination and defecation following various doses of PCA were quantified. Rats were adapted to metabolism cages and injected, ip, 1.0 ml/kg of saline containing 0.0, 0.5, 1.0, 2.0, 5.0 or 10.0 mg/kg of PCA. A dose-dependent body weight loss was observed during the 3 hr post-injection period. Weight loss ranged from 4.8 gm in the saline group to 16.3 gm in the 5.0 and 10.0 mg/kg groups. Significant increases in defecation occurred during the 0-1 hr post-injection period in the 2.0, 5.0 and 10.0 mg/kg groups. Significant increases in urination occurred during this same period in the 0.5 - 5.0 mg/kg groups. In the 10.0 mg/kg group, the fur of the rats became saturated with urine, and measurable diuresis was masked. The role of these effects on PCA-induced hypophagia, hypodipsia and subsequent hyperdipsia (Stein, et al., 1978) will be discussed, as will potential central and/or peripheral nervous system involvement in these effects.

1618 THE INTERACTION OF PSYCHOTROPIC DRUGS WITH N,N-DIMETHYLTRYPTAMINE (DMT). David M. Stoff, J. Christian Gillin, Dilip V. Jeste,* Egidio A. Moja* and Richard Jed Wyatt. Lab. of Clinical Psychopharmacology, SMR, IRP, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

The hypothesis that schizophrenia results from the endogenous synthesis of methylated hallucinogenic agents was proposed about 25 years ago; among the possible compounds as an abnormally methylated agent N,N-dimethyltryptamine (DMT) has been the most vigorously pursued.

We have already reported dose response disruptive effects of DMT on rat shuttlebox avoidance and suggested that this paradigm may serve as an animal model for the hallucinogenic activity of DMT (Stoff et al., Biological Psychiatry, 1977, 12, 339-346). Here, we present evidence that chronic neuroleptic pretreatment protects against this effect of DMT.

A series of experiments was conducted to study the interaction of acute and chronic pretreatment of various psychotropic drugs with DMT. Fischer 344/mai rats were trained to a high stable conditioned avoidance response (CAR) baseline rate (>80% CARs), pretreated once with neuroleptics: chlorpromazine (1 mg/kg, n=10), haloperidol (.05 mg/kg, n=8), pimozide (.025 mg/kg, n=10), clozapine (2.5 mg/kg, n=9), methiothepin (.0675 mg/kg, n=8); pargyline (MAO inhibitor) (75 mg/kg, n=10); AMPT (inhibitor of CA synthesis) (100 mg/kg, n=10); 1,2,5,6-tetrahydropyridine-3-(N,N-diethylcarboxamide) (THPC) (postulated to occupy the DMT receptor) (20 mg/kg, n=15) or chronically with the same dose daily of chlorpromazine (21 days, n=10), haloperidol (14 days, n=9) or pimozide (14 days, n=10) followed by a challenge dose of DMT 4 mg/kg (n=81).

The following results were obtained: (i) none of the pretreatment schedules, by themselves, influenced the CAR; (ii) DMT caused about a 40% decrease in the CAR rate,; (iii) acutely, none of the drugs influenced DMT disruption; (iv) chronic chlorpromazine, haloperidol and pimozide attenuated DMT disruption with the degree of protection directly related to the length of neuroleptic pretreatment.

The findings that chronic, but not acute, neuroleptic pretreatment is effective in preventing disruptive effects of DMT may be related to the time dependent effects of neuroleptics on dopamine turnover and the therapeutic latency of these agents.

1619 ANTI-CONFLICT PROPERTIES OF THYROTROPIN RELEASING HORMONE (TRH), ALONE OR IN COMBINATION WITH ETHANOL (ETOH) OR CHLORDIAZEPOXIDE (CDZ). R.A. Vogel, B.A. Pappas, J. Wilson*, G.D. Frye, R.A. Mueller and G.R. Breese. Biol. Sci. Res. Ctr., UNC Sch. Med., Chapel Hill, NC 27514.

Anti-conflict activity was measured in water deprived rats using a modification of the procedure of Vogel et al. (Psychopharmacol. 21:1, 1971). Rats deprived of water for 24 hours were allowed 200 unpunished licks in the test apparatus to decrease intersubject variability in the subsequent drug test. After 48 hours of deprivation, the drug-treated rats were allowed to drink for 3 min., but after every 20 licks, licking was punished for 2 secs. by electric shock through the water spout. ETOH (0.5-1.5 g/kg, 10% w/v, ip) increased punished drinking, although not as strongly as CDZ (4-27 mg/kg, ip). Punished drinking was unchanged by 2 g/kg and decreased by 2.5 g/kg ETOH. In surprising contrast to the antagonism of acute depressant actions of ETOH observed after TRH, TRH (20 mg/kg, ip) enhanced the anti-conflict action of ETOH (1 or 2 g/kg). TRH (20 mg/kg) also increased the anti-conflict effect of a marginally effective dose of CDZ (4 mg/kg). TRH alone produced dose related increases in punished responding over a range of doses from 5 to 80 mg/kg (ip). This effect was maximal 15 to 60 min. post injection but could also be observed 2 or 20 hours after TRH administration. Intracisternal injection of TRH (100 µg/rat) also increased punished responding, suggesting that the locus of this action of TRH may be within the CNS. d-Amphetamine (2 mg/kg, ip) antagonized the anti-conflict effect of TRH (40 mg/kg). This observation provides further evidence for the view that TRH does not share a common mechanism of action with d-amphetamine. In summary, the present results indicate that TRH has anti-conflict activity in a paradigm sensitive to the anxiolytics CDZ and ETOH, and can enhance the anti-conflict activity of these agents. Thus, these findings with TRH may be consonant with human subjective reports of "relaxation" following TRH administration (Wilson et al., Archs. Gen. Psychiat. 29:15, 1973). (Supported by HD-10570, AA-02334, MH-00013, MH-05636 and AA-05047.)

1620 PENTOBARBITAL-INDUCED CROSS-TOLERANCE TO ETHANOL IS MEDIATED BY LEARNING AND NOT BY DRUG-EXPOSURE PER SE. John R. Wenger*, Pegi M. McEvoy* and Stephen C. Woods* (SPON: R.H. Lovely). Dept. of Psychol., Univ. Washington, Seattle, WA 98195

Cross-tolerance is the phenomenon wherein an organism has a decreased response to a drug as a consequence of having previously been given another drug. Cross-tolerance is a poorly understood phenomenon because the drugs used have often been believed to operate via different mechanisms. A learning interpretation of cross-tolerance can explain the phenomenon by positing compensatory skill learning in the altered physiological/psychological "state" as the common mechanism underlying cross-tolerance. A 3-group design (intoxicated practice vs. drug exposure vs. saline controls) was used to partition pentobarbital-induced cross-tolerance to ethanol into components attributable to learning and to drug exposure per se. Rats were given a 9-day series of i.p. injections using an escalating series of pentobarbital doses (ending with 18 mg/kg) or an equivolemic series of drug vehicle injections. All groups were then tested for tolerance to a 2.8 g/kg test dose of ethanol. The data indicated that all of the cross-tolerance was due to the effects of intoxicated practice (i.e., to learning). There was no support for the concept of drug-exposure-induced cross-tolerance. Moreover, subsequent testing showed that the slope of the ethanol dose-effect curve was less for the intoxicated practice group than for the other two groups (which were identical). This suggests that behavioral practice while pentobarbital-intoxicated causes a change of the receptor population acted upon by the ethanol. This is consistent with the idea that learned cross-tolerance acts via a fundamentally different mechanism than other types of tolerance. (Tolerance was operationally defined by means of the moving-belt task. Gibbins, et al. J. Pharm. Exp. Ther. 159: 236-242.1968)

Supported by funds from the Alcohol and Drug Abuse Institute and the Graduate School Research Fund both of the University of Washington.

1621 CAFFEINE-AMPHETAMINE INTERACTION: MULTIPLE CAFFEINE TREATMENTS ATTENUATE SUBSEQUENT AMPHETAMINE-INDUCED ACTIVITY IN RATS. Brent C. White, Karen L. Haswell*, Don Harkins, Jr.*, Cathy D. Kassab*, and Paula M. Crumbie*. Psychobiology Program, Centre College of Kentucky, Danville, KY. 40422.

Similarities and interactions between amphetamine and caffeine were studied through the locomotor activity effects of these drugs in a cross-tolerance design. Five days of twice daily injections of caffeine (30mg/Kg) significantly reduced subsequent amphetamine (1.5mg/Kg) induced activity; whereas, prior amphetamine treatment had no effect on caffeine induced activity.

The role of hepatic enzyme induction was assessed by giving amphetamine and caffeine by subcutaneous and intraperitoneal routes in a 2 x 2 x 2 factorial design (Route of injection x Caffeine x Amphetamine). Regardless of route of injection, caffeine treatment attenuated the activity level during the following 7 days of amphetamine treatment. However, route of injection was an important determinant of amphetamine activity levels across all treatment conditions, with subcutaneous injections producing more activity than intraperitoneal injections.

An additional experiment investigated the effect of prior caffeine treatment on the amphetamine dose/response curve. A general flattening of the dose/response curve would suggest a reduced sensitivity of the caffeine treated rat to amphetamine. A shift of the dose/response curve to the left would indicate an increased sensitivity to amphetamine, and a shift to the right a competitive inhibition. In this experiment, the amphetamine dose/response curve was depressed at each dose by prior caffeine treatment, indicating reduced sensitivity to amphetamine.

This series of studies has implications for several possible neurochemical systems which may account for the caffeine-amphetamine interaction. These include catecholamine metabolism, cyclic nucleotide involvement in neurotransmission, and induction of hepatic microsomal enzymes.

1622 EEG SPECTRAL CHANGES DURING 1-ALPHA-ACETYLMETHADOL (LAAM) SELF-ADMINISTRATION IN DEPENDENT RATS COMPARED TO THOSE DURING MORPHINE AND METHADONE SELF-ADMINISTRATION. Gerald A. Young, George F. Steinfels and Naim Khazan. Dept. of Pharmacol. and Toxicol., Univ. of Maryland Sch. of Pharm., Baltimore, MD 21201.

Five adult female Sprague-Dawley rats were implanted with chronic cortical and temporalis muscle electrodes and i.v. cannulae. Tolerance and physical dependence were induced by automatic hourly i.v. injections of increasing doses of morphine. The rats were then allowed to lever press on a fixed ratio 20 schedule of reinforcement in order to receive LAAM (1,2, or 4 mg/kg) to maintain dependence. Each rat had the opportunity to self-administer each LAAM dose for 6 or 7 successive days. The number of self-injections per 24 hrs were 5.8 ± 0.6, 4.3 ± 0.7 and 2.8 ± 0.5 for the 1,2, and 4 mg/kg doses, respectively. EEG samples of successive REM sleep episodes during the interinjection intervals were subjected to power spectral analyses with a Nicolet MED-80 system. Power spectra derived from REM sleep EEG samples have been shown to be associated with a predominant peak of spectral power in the theta wave range (Young et al, Pharmac. Biochem. Behav. 8: 89, 1978). During LAAM self-administration the first one or two REM sleep episodes occurring in the inter-injection interval were associated with the fastest peak EEG frequency that was observed. There was a general slowing of the predominant EEG frequency during the successive REM sleep episodes, reaching the lowest value in the last one or two episodes. However, a temporary increase in peak EEG frequency during REM sleep episodes was seen towards the middle of the interval. This non-linear trend of shifts in peak EEG frequency with LAAM is in contrast to that previously seen with morphine and methadone self-administration during which a linear slowing of the predominate EEG frequency occurred in successive REM sleep episodes (Young et al, Pharmacologist 19: 140, 1977; Steinfels et al, Fed. Proc. 37: 310, 1978). Each LAAM self-injection reinstated the faster peak EEG frequency. The majority of the lever pressing activity for all three narcotics occurred after the last REM sleep episode. These results suggest that the slowing of the peak EEG frequency may reflect declines in plasma levels of the respective narcotic or changes in the state of the CNS which precede "drug-seeking" behavior. The observed increase in the peak EEG frequency in the middle of the interval during LAAM self-administration may be related to the appearance of its active metabolites. This assumption is currently being investigated. (Supported by NIDA Grant DA 01050).

1623 URINARY MHPG AS A PREDICTOR OF TRICYCLIC-INDUCED MANIA. A.P. Zis,* R.W. Cowdry,* T. Wehr,* and F.K. Goodwin.* (SPON: Robert M. Post). Clinical Psychobiology Branch, NIMH, Bethesda, Maryland 20014 U.S.A.

Recent studies suggest that levels of urinary MHPG may predict the response of unipolar depressed patients to tricyclic antidepressants (TCA's). Specifically, low urinary MHPG is associated with responses to imipramine (IMI) and desmethylimipramine (DMI), which have predominant effects on norepinephrine reuptake; in contrast, high urinary MHPG is associated with response to the more "serotonergic" tricyclic antidepressants, chlorimipramine and amitriptyline.

This study explores the potential usefulness of urinary MHPG in predicting the response of bipolar depressed patients to TCA's. In bipolar patients, the occurence of hypomania or mania provides a particularly unambiguous marker of response. Two issues were addressed: 1) Does urinary MHPG predict the latency of clinical response? and 2) Do low and high MHPG excretors respond differentially to TCA's?

Twenty-three bipolar depressed patients were studied on an affective disorders unit at the National Institute of Mental Health. Twenty-four hour excretion of MHPG was determined while the patients were on placebo medication. Tricyclic response was assessed in a double-blind design.

There was a strongly positive correlation between urinary MHPG and latency of a hypomania or manic response (measured from the time of initiation of TCA treatment) ($r = .87$, $n = 14$, $p < .001$).

Furthermore, patients who developed severe and prolonged episodes of mania in response to IMI ($n = 5$) or AMI ($n = 1$) had low MHPG excretion. In contrast, patients with high MHPG excretion had either no response, a transient, mild hypomania, or an unequivocal therapeutic antidepressant response. Finally, high MHPG patients treated with AMI fared much better than high MHPG patients treated with IMI, a finding consistent with data from unipolar patients.

The theoretical implications of these findings regarding the postulated noradrenergic involvement in the switch process from depression into mania will be discussed.

RECEPTORS

1624 MULTIPLE HIGH-AFFINITY BINDING SITES FOR BUTYROPHENONES IN RAT STRIATUM. Anne C. Andorn* and Michael E. Maguire, Depts. of Psychiatry and Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

^{3}H-Spiroperidol(3H-SP) was used to assay butyrophenone binding sites in crude membrane preparations of rat striatum. The final assay volume of 100 µl contained 3H-SP, test drugs, and approximately 0.15 mg protein in buffer. Bound 3H-SP was isolated by adsorption to glass fiber filters. Specific binding determined as the difference in the amount bound in the absence and presence of 10 µM (+)-butaclamol represented 80% of total binding. (-)-Butaclamol was without effect on 3H-SP binding at 0.1 mM. Specific binding could be competed by physiologically relevant concentrations of putative dopaminergic agonists and antagonists, serotonergic agonists, and α-adrenergic agonists and antagonists. β-Adrenergic agents, γ-aminobutyric acid, and muscarinic agents had no effect on 3H-SP binding at concentrations of 1 nM to 1 mM. Because Scatchard analysis of (+)-butaclamol competition against concentrations of 3H-SP from 0.05 nM to 50 nM was curvilinear, indicating two or more binding sites, and because a multiplicity of agents could compete 3H-SP binding, we attempted to define single binding sites by generating Scatchard plots against representatives of all pharmacologic classes that were effective competitors of 3H-SP binding. A lower affinity site (K_D > 100 nM) was not effectively competed by any of these agents. Dopamine, phentolamine, and serotonin all competed effectively for a single site (K_D = 1.5-2 nM, n = 300-400 fmol/mg) at concentrations above 10 µM. However, even at 3 mM these agents do not compete for the highest affinity 3H-SP binding site. This site is competed by spiroperidol, (+)-butaclamol, and apomorphine at 10 µM ($K_D \sim 0.1$ nM, $n \sim 75$ fmol/mg).

The ability of the above agents to inhibit 3H-SP binding to the intermediate affinity site suggests that this site may behave as a pharmacologic α-receptor, whether or not it is a physiologic dopamine receptor. The inability of dopamine to compete for the very high affinity 3H-SP site suggests that this site is not a pharmacologic dopamine receptor and that actions of butyrophenones may be mediated through a system(s) other than dopaminergic or α-adrenergic.

1625 EFFECT OF ELECTROCONVULSIVE SHOCK TREATMENT ON MONOAMINERGIC RECEPTOR BINDING SITES IN RAT BRAIN. D.A. Bergstrom*, P. Iadarola*, K.J. Kellar. Dept. of Pharmacology, Georgetown University, Schools of Medicine and Dentistry, Washington, D. C. 20007.

Chronic administration of tricyclic antidepressants and monoamine oxidase inhibitors have been shown to decrease the sensitivity of the norepinephrine-stimulated adenylate cyclase in rat brain. In addition, chronic treatment of rats with desipramine for one week or longer decreases the apparent density of β-adrenergic binding sites in rat cerebral cortex without affecting α-adrenergic or serotonergic binding sites.

Sulser and his associates have reported that daily administration of electroconvulsive shock treatment (ECT) to rats for 8 days reduces the responsiveness of the norepinephrine-sensitive cyclic AMP generating system in brain. We have investigated the effect of ECT on rat brain monoaminergic receptor binding sites.

Electroconvulsive shock (150 mA, 200 msec) was administered to rats through saline-moistened corneal electrodes. Control rats were handled in the same manner except no current was passed. Shocks were administered daily for 1, 2, and 7 day durations. All rats experienced tonic forelimb extensions, with some rats experiencing tonic hindlimb extensions. Rats were decapitated 24 hours after their last shock.

The kinetic properties of the β-adrenergic receptor binding sites were measured with (^{3}H)-dihydroalprenolol, the α-adrenergic sites with (^{3}H)-dihydroergocryptine, and the serotonergic sites with (^{3}H)-serotonin. The data were analyzed with Scatchard plots.

We found no difference in the kinetic binding properties of the β-adrenergic, α-adrenergic, or serotonergic binding sites in rat brain cortex after a 1 or 2 day electroconvulsive shock regimen. However, after 7 consecutive days of shock we found a significant decrease, approximately 30%, in the number of cortical β-adrenergic binding sites. There was no apparent change in the affinity of the binding sites for the ligand.

Antidepressant drugs and ECT produce a rapid increase in the availability of norepinephrine to post-synaptic receptors. The present findings with ECT and previous findings with chronic antidepressant drug administration suggest that the delayed onset of these antidepressant treatments may be related to an adaptation of the β-adrenergic receptor binding sites to an increased availability of norepinephrine.

(Supported US PHS NS12566 and NASA NCA2OR258701)

1626 DISPERSAL AND REFORMATION OF ACETYLCHOLINE RECEPTOR CLUSTERS IN CULTURED RAT MYOTUBES. Robert J. Bloch. Dept. of Neurobiology, The Salk Institute, San Diego, CA 92112.

Acetylcholine receptor (AChR) clusters in the plasmolemma of rat myotubes are lost upon treatment of cultures with inhibitors of energy metabolism. This loss was not caused by a variety of other drugs, and was dependent both on inhibitor concentration and time of treatment. It was not due to loss of cells, as cell number and overall AChR titer per culture were only slightly (10-20%) reduced. Furthermore, AChR clusters on identified, living cells were seen to disperse in the absence of any gross morphological changes. Dispersal appeared to be by diffusion of small AChR aggregates away from the cluster. Upon removal of energy poisons, AChR clusters reformed. Reformation proceeded by 1) appearance of small foci of AChR aggregation in a limited region of the sarcolemma; 2) aggregation of AChR in the areas around the foci, to yield clusters or cluster pairs; 3) continued aggregation around cluster pairs to give larger single clusters. Metabolic energy appears to be required to "fix" AChR to the foci and then to surrounding regions; energy metabolism inhibitors weaken this interaction, presumably by blocking ATP biosynthesis.

1627 OPIATE BINDING TO MEMBRANE PREPARATIONS OF NEUROBLASTOMA X GLIOMA HYBRID CELLS NG108-15: EFFECTS OF IONS AND NUCLEOTIDES. A.J. Blume, G. Boone* and D. Lichtshtein* (SPON: J.H. Tarver). Department of Physiological Chemistry and Pharmacology, Roche Institute of Molecular Biology, Nutley, NJ 07110.

The binding of peptide and alkaloid opiate agonists to NG108-15 cell membranes is selectively decreased by mono-valent cations. Dihydromorphine (DHM), leu-Enkephalin (leu-E) and the stable met-enkephalin analogue D-ala^2-Met^5-amide (DAMA) binding under steady-state conditions are reduced about 50% by 135 mM Na^+ and similar inhibition is seen with Li^+, K^+ and choline, but with decreasing affinities respectively. Na^+ also increases the dissociation of leu-E and DAMA from the receptor. In contrast, none of the above cations significantly alters the binding of naloxone or naltrexone, two opiate antagonists. Certain nucleotides will also decrease DHM, leu-E and DAMA binding. The affinity of these nucleotides appears to be increased by Na^+. High nucleotide concentrations completely eliminate specific binding when Na^+ is present. In the presence of Na^+, the relative order of potency is GMP-P(NH)P≥GTP≥GDP≥ITP≥IMP-P(NH)P>>ATP = AMP-P(NH)P>GMP and half-maximal inhibition by GMP-P(NH)P occurs at 5 µM. These same nucleotides have also been found to increase the dissociation of leu-E and DAMA from the receptor. GMP-P(NH)P at 100 µM, with or without Na^+, causes only a 10% and 20% decrease in naloxone and naltrexone steady-state binding respectively. Dissociation of the opiate agonist leu-E is complex, being monophasic and relatively slow in the absence of Na^+ and GMP-P(NH)P; biphasic in the presence of either Na^+ or GMP-P(NH)P, with a portion of the ligand now dissociating rapidly; and finally, monophasic, yet rapid in the presence of both Na^+ and GMP-P(NH)P. The selective effects of Na^+ and nucleotides on opiate agonist binding are not due to the fact that opiate agonists bind to a different opiate receptor than do opiate antagonists. The affinity of DHM, naloxone, naltrexone and morphine as assessed in saturation binding experiments is found to be the same as its affinity determined from the inhibition curves of the binding of any of the other ligands, regardless of whether they are agonists or antagonists or alkaloids or peptides. Opiates, through their plasma membrane receptors, have been shown to inhibit NG108-15 adenylate cyclase. Therefore, nucleotides apparently regulate (within a single cell) receptors which inhibit and receptors which activate (i.e. PGE_1 and adenosine receptors) adenylate cyclase.

1628 NICOTINE CAUSES AN INCREASE IN SENSITIVITY TO MECHANICAL STIMULATION IN A CONTRACTILE PROTOZOAN. Peter A. Boxer* and David C. Wood. Psychobiology Program, Dept. of Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

A suprathreshold mechanical or electrical stimulus triggers an action potential in the ciliated protozoan, Stentor coeruleus, which in turn elicits a contraction of the animal. The animals response to mechanical stimulation is mediated by a receptor potential. Wood (Comp. Biochem. Physiol., 56C, 151, 1977) has shown that the organism's sensitivity to mechanical stimuli can be markedly reduced by addition of 3-60μM d-tubocurarine chloride (d-TC) and several other nicotinic cholinergic antagonists. Since the effect of d-TC is specific to mechanical stimulation d-TC must bind to the site involved in mechanical stimulus transduction. Based on this specificity a [^{14}C]-d-TC binding assay for mechanical stimulus transduction sites has been developed and validated.

To complement the work on cholinergic antagonists we studied the behavioral and biochemical effects of cholinergic agonists. Upon addition of nicotine (0.5 mM) to the medium there is an initial (5-30 min) decrease in the probability of contraction to mechanical stimuli followed in 1-3 hr by a significant increase in response probability. In response to electrical stimulation stentor exposed to nicotine show a similar initial decrease in excitability, but within an hour the response probability returned to pretreatment levels and remained there. Therefore, nicotine's initial inhibitory effect can be attributed to a non-specific debilitation of the contractile response, while the increase in sensitivity is specific to mechanical stimulation.

Nicotine also appears to compete with d-TC at the mechanical transduction site. Incubation in nicotine (0.5 mM) for 1 to 3 hrs. caused a 50% inhibition in the amount of bound [^{14}C]-d-TC, while shorter incubations had little effect. Therefore there is a correlation between the ability of nicotine to inhibit the binding of [^{14}C]·d-TC and its ability to increase the sensitivity of the animal to mechanical stimuli.

Other cholinergic agonists (1.0mM Dimethylphenylpiperazinium (DMPP), 10mM Carbachol (Carb), and 10mM Tetramethylammonium (TMA)) also caused the initial drop in response probability to mechanical and electrical stimulation. Carb and DMPP also produced a small increase in probability of contraction after 1 hr. These drugs produced smaller effects on the binding of [^{14}C]-d-TC than nicotine. The finding that nicotine and other cholinergic agonists affect mechanical stimulus transduction by acting on a curariform binding site implies that this site in stentor has molecular properties somewhat analogous to those found in higher organisms and may serve as a useful, simple model system.

1629 PHARMACOKINETICS OF NEUROLEPTIC DRUGS: IN VITRO INHIBITION OF ^{3}H-SPIROPERIDOL BINDING IN SHEEP CAUDATE BY NEUROLEPTICS AND THEIR METABOLITES. David B. Bylund and Jorge Perez-Cruet*, Dept. of Pharmacology and Missouri Institute of Psychiatry, Sch. Med., University of Missouri, Columbia, MO 65212.

Many of the clinically useful neuroleptic drugs are metabolized to give derivatives which may have clinical efficacy. In order to understand the mechanisms of action of these drugs, it is important not only to have an estimate of the plasma (or even better, CNS) concentrations of the various metabolites, but also an estimate of their potency at the receptor binding sites of various neurotransmitters. Since there is pharmacological evidence to suggest that dopaminergic receptors are involved in the action of neuroleptic drugs, we have evaluated the potency of various metabolites using an in vitro binding assay.

^{3}H-Spiroperidol recently has been shown to label the dopamine receptor binding site in human, rat and calf caudate nucleus. We find that ^{3}H-spiroperidol also appears to label dopamine receptors in the sheep caudate. At 23°, ^{3}H-spiroperidol specific binding to a crude particulate fraction of sheep caudate has an apparent dissociation constant of 0.3 nM and a binding capacity of about 35 pmol/g wet weight tissue. The binding reaches equilibrium in less than 30 min and then remains constant for at least one hour. The rate constant of association is 0.1/nM/min. The concentrations of various compounds which half maximally inhibit specific ^{3}H-spiroperidol binding are given below.

Compound	IC_{50}, nM
Fluphenazine	3
Chlorpromazine	8
Thioridazine	20
7-OH-Chlorpromazine	30
Mesoridazine	30
7-OH-Fluphenazine	60
Fluphenazine Sulfoxide	200
Apomorphine	300
Methoxychlorpromazine	400
Thioridazine-S-Sulfoxide	700
Sulforidazine	3000
Northioridazine	7000
Thioridazine-R-Sulfoxide	8000

All metabolites are less active than the respective parent compounds in this in vitro assay system using dopamine receptors from sheep caudate. Since there is a good correlation between the affinity of neuroleptics for dopamine receptor binding sites and their clinical potency, these results should be helpful in determining the involvement of these metabolites in the actions of neuroleptic drugs.

1630 RATES OF METABOLISM AND SITE OF INSERTION OF A PLASMA MEMBRANE PROTEIN IN RAPIDLY GROWING NEURONS. S.T. Carbonetto* and D.M. Fambrough, (SPON: K.J. Muller). Carnegie Institution of Washington, Baltimore, Md. 21210.

We have previously demonstrated that α-bungarotoxin binds selectively to chick sympathetic neurons, that this binding is saturable (10nM) and is completely blocked by d-tubocurarine (100μM). Chick sympathetic neurons depolarize in response to iontophoretically applied acetylcholine but α-bungarotoxin does not block this response (PNAS 75, 1015, 1978) and therefore may be binding to some membrane protein other than the acetylcholine receptor. The α-bungarotoxin receptor is not extracted from the membrane by 1M NaCl or 1mM EDTA but is readily extracted by non-ionic detergents (Triton X-100, Emulphogene BC720). α-Bungarotoxin-receptor complexes normally dissociate with a half-time of 3.5 hrs at 23° but in solution they can be cross-linked by glutaraldehyde (0.1%) to form complexes that are stable for several days. Both before and after cross-linking, α-bungarotoxin-receptor complexes sediment in sucrose gradients as a single peak with a sedimentation constant of approximately 11S (compared with 10S for skeletal muscle acetylcholine receptors).

Neurons grown in medium containing "heavy" ^{2}H, ^{13}C, ^{15}N-substituted amino acids incorporate these heavy amino acids into α-bungarotoxin receptors during protein synthesis, and the resulting heavy receptors can be separated from light receptors by velocity centrifugation in 25-40% sucrose-deuterium oxide gradients. We have used this technique to study the biosynthesis and degradation of the α-bungarotoxin receptor to obtain information about the kinetics and the localization of membrane synthesis in growing neurons.

The main features of the kinetic data are that new receptor incorporation into the surface begins after a lag of 2 hrs and the half-time for directly labeling the surface population with heavy molecules is about 7 hrs. The degradation rate, obtained by monitoring the decrease in a population of previously synthesized heavy receptors is an exponential decay with a half-time of 10 hrs. The difference in the rate of synthesis and degradation is reflected in a net increase in the number of receptors during the first 3-4 days in culture.

After culturing ganglia whole, rather than as dissociated sympathetic neurons, a "halo" of axons grows from each ganglion that can be simply dissected to yield a preparation of pure axons. Initial experiments to localize receptor incorporation within this preparation indicate that up to 10 times more α-bungarotoxin receptors are incorporated into the ganglion than the axons and that the rate of incorporation of receptors, expressed as per cent of the surface population incorporated per hr, is about the same in both regions. This suggests that receptors are incorporated at several sites in the neuron and not exclusively at the growth cone.

1631 LOCALIZATION OF GAMMA-AMINOBUTYRIC ACID RECEPTORS BY [3H]-MUSCIMOL BINDING: LIGHT AND ELECTRON MICROSCOPE AUTORADIOGRAPHY IN MAMMALIAN CENTRAL NERVOUS TISSUES. Victoria Chan-Palay and Sanford L. Palay. Depts. Neurobiology and Anatomy, Harvard Medical School, Boston, MASS 02115.

Previous reports indicate that muscimol (M) is a potent gamma-aminobutyric acid (GABA) agonist. GABA receptors are visualized by autoradiographic techniques after [3H]-muscimol ([3H]-M) administration into incubated tissue slices, by in vivo injections, and to intraocular transplants in rats. [3H]-M is bound and after fixation subsequent autoradiograhic procedures allow visualization of the [3H]-M binding sites which are equivalent by all three modes of administration. The cerebellar cortex and nuclei, hippocampus, caudate nucleus and cerebralcortex have been studied. Control experiments demonstrate that [3H]-M binding is sensitive to pretreatment with guvacine and -nipecotic acid, two known GABA uptake and transport system inhibitors. Whereas cold M passes the blood brain barrier, [3H]-M does not produce visible autoradiographic label after intravenous administration. Quantitative analyses of [3H]-M autoradiographic label in the cerebellar cortex show highest amounts in the basket area around Purkinje cell somata , primary and secondary dendritic shafts. Intracortical interneurons are also labeled. Electron microscopy reveals label over the axosomatic and axodendritic synaptic membranes at these locations and over axonal membranes in the basket area where few synaptic junctions are found. In the hippocampus highest[3H]-M binding occurs in the area dentata neuropil between granule cells and in the molecular layers of the hippocampus. Cells of varying size between 20 to 40 micronmeters in diameter, non granular in morphology, possibly basket cells are also labeled. It is suggested that [3H]-M demonstrates one of a possible group of GABA receptors.

1632 GLIAL LOCALIZATION OF BENZODIAZEPINE RECEPTORS IN THE MAMMALIAN BRAIN. Raymond S.L. Chang, Vinh T. Tran, Shirley E. Poduslo and Solomon H. Snyder. Dept. Pharmacol., Johns Hopkins Sch. Med., Baltimore, MD 21205.

We have attempted to determine the localization of benzodiazepine receptors (BZR) in the brain by evaluating the influence of specific lesions and by comparing the density in the purified neuronal and glial fractions from rat brain. Lesions destroying most of the neuronal populations of corpus striatum include intrastriatal kainic acid injection, cerebral cortex ablation, hemisection rostral to substantia nigra and nigral injection of 6-hydroxydopamine. None of these lesions reduce BZR binding in the corpus striatum. In the cerebellum, the elimination of almost all the neuronal types except granule cells by intracerebellar kainic acid administration, mutation leading to loss of granule cells (Weaver mice), as well as destruction of climbing fibers with 3-acetylpyridine also fail to reduce BZR binding. The failure of lesions destroying almost all neurons in the striatum and cerebellum to decrease BZR binding suggest that BZR may be associated with glia. Direct assays of ^{3}H-flunitrazepam binding in the purified astrocytic and neuronal fractions show that both fractions display saturable binding with K_D (4 nM) similar to those found in the brain membranes. The number of binding sites is almost twice as great in astrocytic as in neuronal fractions. The drug specificity in the two fractions is similar, with IC_{50}'s of 3, 3, 20 and 1500 nM for clonazepam, flunitrazepam, diazepam and chlordiazepoxide respectively. Oligodendroglia show negligible binding. We also evaluated binding of ^{3}H-flunitrazepam in membranes prepared from C6 glioma (an astrocytoma) culture. The binding is saturable with a maximal number of binding sites of 2 pmole/mg protein, which is comparable to that found in brain membranes and with a K_D of 30 nM, which is about 10 times of that found in the rat brain membranes. The drug specificity of binding sites in the C6 glioma also differs from that of rat brain membranes to a certain extent. The IC_{50}'s of clonazepam, flunitrazepam, diazepam and chlordiazepoxide in these membranes are 5,000, 32, 28 and 22,000 nM respectively.

1633 α-BUNGAROTOXIN BLOCKS NICOTINIC TRANSMISSION IN THE AVIAN CILIARY GANGLION. V.A. Chiappinelli and R.E. Zigmond. Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115

Specific, high affinity binding sites for α-bungarotoxin have recently been found in the avian ciliary ganglion (V. Chiappinelli and E. Giacobini, Neurochem. Res., in press). In order to determine whether α-bungarotoxin has an effect on chemical transmission through the ciliary ganglion, isolated ganglia were exposed to the toxin, and the response of the ganglion neurons to preganglionic stimulation was monitored by recording compound action potentials from their postganglionic nerves.

α-Bungarotoxin (10^{-6}M) completely blocked nicotinic transmission between preganglionic neurons and both the ciliary and choroid neurons of chick and pigeon ciliary ganglia. The time required for complete blockade of chemical transmission was 30-40 min for the ciliary neurons and 60-90 min for the choroid neurons. The blockade was partially reversed by prolonged washing (2-8 h).

Incubation of ganglia with d-tubocurarine (10^{-4}M) prior to the addition of α-bungarotoxin significantly reduced the duration of the washout period necessary to restore transmission. These results suggest that d-tubocurarine and α-bungarotoxin are interacting with the same receptor.

Under similar conditions, α-bungarotoxin did not block nicotinic transmission in the rat superior cervical ganglion, a finding which is in agreement with previously published reports.

The avian ciliary ganglion is the only vertebrate autonomic ganglion in which both α-bungarotoxin binding and α-bungarotoxin blockade of transmission have been shown to occur. The ganglion, therefore, provides a model system for using α-bungarotoxin to study neuronal nicotinic receptors. (Supported by NIH grant NS 12561 and training grant NS 07009).

1634 NUCLEOTIDE EFFECTS ON OPIATE RECEPTOR BINDING. Steven R. Childers and Solomon H. Snyder. Depts. of Pharmacol. and Psychiat., Johns Hopkins Sch. Med., Baltimore, MD 21205.

Guanine nucleotides play an important role in β-adrenergic systems by coupling receptors and adenylate cyclase, and by decreasing affinity of β-adrenergic agonists for receptors to produce desensitization. Blume (Proc. Natl. Acad. Sci., April, 1978) has shown that guanine nucleotides decrease receptor affinity for both opiate agonists and antagonists in brain opiate receptor binding studies. In our laboratory, guanine nucleotides have specific effects on rat brain opiate receptor binding which distinguish between opiate agonists and antagonists depending on the presence of ions in the assay medium.

In the absence of sodium, GTP is potent in decreasing agonist binding and is much less effective in decreasing antagonist binding. At 50 μM concentration, GTP decreases ^{3}H-dihydromorphine binding by 40-50%, ^{3}H-met-enkephalin by 60-70%, but ^{3}H-naloxone binding by only 15-20%. Scatchard analysis of saturation binding data reveals that the principal effect of GTP on ^{3}H-dihydromorphine binding is to reduce the number of high affinity agonist binding sites, while the effect of GTP on ^{3}H-naloxone binding is minimal, with a slight decrease in affinity detected in high-affinity antagonist binding sites. Experiments with different guanine nucleotides showed that GTP, GDP, and GMP-P(NH)P are effective in decreasing agonist binding, but GMP has no effect.

In the presence of sodium, GTP is even more effective in distinguishing opiate agonist and antagonist binding. GTP has no detectable effect on ^{3}H-naloxone binding, but decreases ^{3}H-dihydromorphine binding by 70-80% and ^{3}H-met-enkephalin binding by 90-95%. The order of potency of the various guanine nucleotides parallels that seen in the absence of sodium. These results suggest that guanine nucleotides may play an important physiological role in regulating the actions of opiate agonists. (Supported by USPHS grant MH-18501 and by USPHS postdoctoral grant MH-7329 to SRC).

1635 THE INTEGRATION OF NEURAL AND ANABOLIC STEROID INFLUENCES ON SKELETAL MUSCLE. Harold Chin*, Joseph Tropea*, Joan Warrenski* and Richard R. Almon . Division of Cell and Molecular Biology, SUNY at Buffalo, Buffalo, New York 14260.

Cell populations are presented with a vast array of small molecules that interact with receptor structures and consequently influence the behavior of this tissue. In the case of skeletal muscle, some of the most important influences come from anabolic steroids and surface acting neurotransmitters. In particular, interactions with the acetylcholine receptor population are the determinants of contraction; interactions with the Beta-adrenergic receptor population influence the strength and duration of contraction; and anabolic steroids have a substantial influence on both the character and quantity of protein synthesis in skeletal muscle. The present studies examine the characteristics of the Beta-adrenergic and cholinergic receptor populations in membranes and androgen receptor in cytosol from three unique muscles in the male rat. The first is the predominantly fast fiber, extensor digitorum longus; the second is the predominantly slow fiber, soleus; and the third is the highly androgen sensitive levator ani. The dissociation constant of ^{125}I-alpha burgarotoxin is in the order of $1x10^{-7}$ molar for all three muscles. This is in distinct contrast to the previously reported values with detergent-extracted AcCH receptor (Almon et al., Biochemistry 1974). This result supports our previous observation that the hydrophobic environment of the receptor macromolecule can significantly influence receptor binding affinity. The number of AcCH receptor sites varies amongst the three muscles from $5x10^{-14}$ moles/mg membrane protein to $2x10^{-13}$ moles/mg membrane protein. The Beta adrenergic receptor population was characterized using the ligand ^{125}I-hydroxybenzylpindolol. The dissociation constant of this ligand from the population of characterized Beta-adrenergic sites is $3x10^{-11}$ molar. The number of Beta-adrenergic receptor sites varies amongst the muscles from $1.4x10^{-M}$ moles/mg membrane protein to $5x10^{-14}$ moles/mg membrane protein. The androgen cytosol receptor population was characterized using ^{3}H-dihydrotestosterone. The results clearly demonstrate that muscle cytosol contains a population of androgen receptors which have a binding affinity and specificity comparable to the population previously characterized in prostate cytosol. The dissociation constant for ^{3}H-Dht is in the order of 10^{-9} M. Depending on the conditions, the number of sites varies amongst the three muscles from $7x10^{-15}$ moles/mg cytosol protein to $2x10^{-14}$ moles/mg cytosol protein. In addition, castration has significant and selective effects on the receptor populations in the three muscles. (This work was supported by a grant from the Muscular Dystrophy Association).

1636 BINDING OF OPIATE AGONISTS, ANTAGONISTS AND A PEPTIDE TO C57BL/6J MOUSE BRAIN HOMOGENATES. E.E. Codd and W.L. Byrne, Dept. of Biochem., UTCHS, Memphis, TN 38163.

The "opiate" receptor was first identified using a radiolabeled opiate antagonist ligand (Sci. 179: 1011-1014, 1973). Subsequently, radioreceptor assays for the opiate receptor employed as ligands opiate agonists and antagonists and peptides, with the implied assumption that they all bound to the same receptor. More recently, experiments have begun to discriminate differences between ligands in ligand-receptor interactions. [Eur. J. Pharm. 40: 241-248, 1976; Nat. 267: 495-499, 1977; Eur. J. Pharm. 41: 247-248, 1977]. The present study involves the binding of radiolabeled opiate agonists and antagonists and peptide ligands to opiate receptor(s) from the brains of an inbred strain of mice, C57BL/6J. The binding assay was performed as described previously (Prog. Neuro-Psychopharmac. 1: 259-264, 1977). The tritiated ligands used were: agonists morphine, dihydromorphine; antagonists: naltrexone and naloxone; peptide: D-ala-enkephalin-amide. Saturable binding was defined as the difference between binding in the presence and absence of 10^{-7}M of the unlabeled form of each ligand used. Scatchard plots of saturable binding were linear and indicate fewer sites for dihydromorphine than for any other ligands tested. All other ligands investigated have similar numbers of sites. Since the lower number of sites is obtained for dihydromorphine but not morphine, the difference is not common to agonists as we had previously thought (Neurosci. Abs. vol III, #1447, 1977), but appears to be a property of dihydromorphine itself. However, in a brain slice system, others have reported fewer numbers of sites for morphine than naloxone (PNAS 74: 5764-5766, 1977). Although they did not investigate other agonists or antagonists, they generalize their results with morphine and naloxone to the classes of drugs they represent. Most investigators report ED50's for the displacement of one radiolabeled drug by many other unlabeled drugs. No reports of the independent determination of the number of binding sites for several agonists and antagonists under the same conditions have been found. From the present work it would appear that there are differences in sites labeled even by drugs in the same class.

1637 β-ADRENERGIC RECEPTORS IN THE RAT HIPPOCAMPAL AND DENTATE GYRI. Keith A. Crutcher and James N. Davis. VA Hospital, Duke University Medical Center, Durham, NC 27705.

[^{3}H] Dihydroalprenolol (DHA) binds to β-adrenergic membrane receptors in the rat cerebral cortex. Recent reports that fluorescent analogues of propranolol localize to the stratum pyramidalis of the rat hippocampal gyrus prompted us to study the distribution of [^{3}H] DHA binding in the hippocampal and dentate gyri. Fresh hippocampal formations from decapitated male Sprague-Dawley rats were separated into dentate and hippocampal gyri using the hippocampal fissure as a landmark. The accuracy of this separation was confirmed by histological examination. Some of the hippocampal formations were blocked and frozen on cryostat chucks. Beginning at the alvear surface, 30 μ-thick sections were cut in parallel to the long axis of the hippocampus. Sections from stratum pyramidalis and stratum radiatum were collected using occasional sections for histological examination. Membranes were prepared from these sections and from the separated gyri for binding studies. Nonspecific binding was determined in the presence of 1 μM (±) propranolol. As in whole cerebral cortex, specific binding was 40 to 60% of total binding. [^{3}H] DHA bound to sites on membranes from both dentate and hippocampal gyri with an affinity (K_D) of 9 nM. The density of binding sites was roughly equivalent in both gyri (0.17 pmol/mg in the dentate and 0.19 pmol/mg in the hippocampus) and the ability of catecholamines to compete for [^{3}H] DHA binding was as expected for β-adrenergic receptors. [^{3}H] DHA binding in the strata pyramidalis and radiatum was also similar (28 and 23 fmol/mg respectively at 3 nM [^{3}H] DHA). These data indicate that 1) [^{3}H] DHA binding is roughly homogeneous throughout the hippocampal formation, 2) the distribution of [^{3}H] DHA binding sites does not correlate with the catecholamine innervation of the hippocampal formation, and 3) the distribution of binding sites does not suggest a concentration of β-adrenergic membrane receptors in stratum pyramidalis. (Supported by VA 1680, NIH NS13101, NS06233, AG00029).

1638 ASCORBIC ACID INDUCED DESTRUCTION OF OPIOID STEREOSPECIFIC BINDING SITES IN GUINEA PIG BRAIN HOMOGENATE. Claud E. Dunlap III* and B.M. Cox.* (SFON: D.B. Goldstein).
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L-ascorbic acid was found to reduce stereospecific binding of ^{3}H-etorphine, ^{3}H-leucine-enkephalin, ^{3}H-naloxone, and ^{3}H-naltrexone to guinea pig brain homogenate. Incubation with 1mM ascorbate destroyed 50% of ^{3}H-etorphine stereospecific binding in 50 min at 22°C, complete destruction occurring within 180 min. Stereospecific binding was not recovered following repeated washing of the ascorbate treated membranes. Dose-response curves in which tritiated ligands were added to homogenates incubated with ascorbate for one hour displayed a dose related inhibition of stereospecific binding by increasing concentrations of ascorbate up to 1mM, above which a decrease in the amount of inhibition produced by ascorbate was observed.

Inhibition of opioid stereospecific binding by ascorbate was observed to be pH dependent, with maximal inhibition occurring between pH 7.4 and 7.8. One millimolar concentrations of ascorbic acid analogs such as dehydroascorbic acid and L-ascorbic acid-2-sulfate did not affect opioid stereospecific binding. D-isoascorbic acid, however, produced dose-response curves similar to those for L-ascorbic acid. Reducing agents, including glutathione, dithiothreitol, and sodium borohydride produced little or no effect on opioid binding at concentrations of 1mM. The mechanism of ascorbate destruction of opioid stereospecific binding capacity is currently under investigation.

(This work was supported by National Institute on Drug Abuse grant #1199).

1639 A METHOD FOR THE OBSERVATION OF AND RECORDING FROM SINGLE CELLS OF LIVING ORGANS: A REFINEMENT OF TISSUE SECTIONING TECHNIQUES. C. Eyzaguirre, R. Gallego*, Y. Hayashida* and L. Monti-Bloch*.
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Nodose ganglia excised from cats were placed on the removable stage of a Sorvall TC-2 Tissue Sectioner and covered with 4% agar-physiological saline at 38°C. Cat carotid bodies were vascularly isolated, the vessels flushed with physiological saline and then injected with 2% agar-saline. Afterward they were removed and treated like the ganglia. The stage and either preparation were then placed in a refrigerator (about 4°C) until the agar hardened (about 10 mins). The stage and preparation were then positioned in the sectioner and sliced by the vertical strokes of a razor blade mounted on the cutting arm of the instrument. Thus, sections of 25-150 μ were obtained, removed with a thin camel hair brush and placed in a chamber through which physiological saline at 36-37°C was allowed to flow. The chamber consisted of a thin plastic frame whose bottom was a microscope cover glass glued to it. The sections were covered with an EM grid kept in place by a platinum loop, operated by a micromanipulator, exerting pressure on the grid to keep the section flat against the bottom glass. The chamber and preparation were mounted on the stage of an inverted microscope equipped with Nomarski optics. The cells were visualized and impaled with conventional microelectrodes which were slid between the condenser (working distance 6 mm) and the bath. The trajectory of the electrode was seen after closing the condenser diaphragm. Once the cell was impaled and resting potential obtained, the diaphragm was opened for proper Normaski optics and photography. Most cells in the sections survived well. For instance, ganglion cells showed normal resting and action potentials. In addition, neuron visualization and photography (at 640 X) permitted measurements of membrane constants R_m ($\Omega \cdot cm^2$) and C_m ($\mu F/cm^2$) without intracellular staining which has the disadvantage that cells may shrink during histological processing giving spurious values for specific constants. Carotid body (type I) cells could be easily seen and distinguished from other cellular elements in spite of their small size (about 10 μ). For identification of type I cells magnifications of 1600 X were employed, by using oil immersion objectives. The cells had normal resting potentials (20-50 mV) and input resistances (about 40 MΩ). They responded to temperature changes and chemical stimulation in normal fashion. At times, active nerve fibers were impaled although electrodes could not be kept inside these small fibers for long. The glomus cell-nerve ending junctions could be seen and this gives hope of studying these junctions with electrophysiological methods under visual observation. Also, this technique may be useful to others in studying different neurons or other cells. Supported by NIH grants NS 05666 and NS 07938.

1640 ^{3}H-SPIROPERIDOL BINDS TO TWO RECEPTOR SITES IN BOTH RAT FRONTAL CORTEX AND CORPUS STRIATUM. J.Z.FIELDS, N.W.PEDIGO*, T.D.REISINE, AND H.I.YAMAMURA. Dept. of Pharmacology, Univ. of Arizona Health Sciences Center, Tucson, Arizona 85724.

Spiroperidol is both a potent antipsychotic drug and a potent dopamine antagonist. ^{3}H-Spiroperidol (^{3}H-Sp)(NEN, 26.4 Ci/mmol) binds to receptor sites in the frontal cortex (FC) and corpus striatum (CS) of rat brain. In the CS, these ^{3}H-Sp binding sites have been called dopaminergic because pharmacologically active dopamine agonists and antagonists are the most potent inhibitors of ^{3}H-Sp binding. The dissociation constant (K_D) in this area, determined by kinetic experiments using the ratio of the rate constants ($^{K_{-1}}/_{K_{+1}}$) is between 10 and 20 pM. Determination of the K_D by Scatchard analyses yields an apparent K_D (K_D app) of 20 to 40 pM at a tissue receptor concentration ($[R]_t$) of 4 to 8 pM. Since the K_D app increases linearly as a function of the $[R]_t$, extrapolation ofthe K_D app to an infinitely small receptor concentration gives the "true" K_D. In rat striatal tissue the extrapolated "true" K_D is approximately 10 pM, which is in good agreement with the data from our kinetic experiments.

^{3}H-Spiroperidol binding in rat FC is complicated by the existence of two distinct receptor sites. The high-affinity ^{3}H-Sp binding site in the FC has a K_D app similar to the K_D observed in the CS (≈20 pM). However, the density of high affinity sites in the FC (≈2.5 pmol/g tissue) is 16 times less then in the CS (≈40 pmols/g tissue). The high-affinity binding site in FC is revealed only at the lower concentrations of ^{3}H-Sp used in saturation studies (less than 15 pM). Scatchard analyses of ^{3}H-Sp binding at ligand concentrations of 20 pM to 5000 pM indicate a second, low-affinity binding site with a K_D app of approximately 340 pM at an $[R]_t$ of 5 to 9 pM. The density of low-affinity binding sites in the rat FC (≈18 pmoles/g tissue) is approximately 7 times greater than the density of high-affinity sites.

In light of these data, saturation studies were done in rat CS using high concentrations of ^{3}H-Sp (up to 5000 pM). These experiments indicated that a low-affinity binding site is present in CS but at a relatively low density (≈14 pmoles/g tissue). The K_D app for this low-affinity binding site in CS is the same as the low-affinity K_D observed in the FC (approximately 330 pM at an $[R]_t$ ≈3 pM). Thus, ^{3}H-Sp appears to bind to two sites in both rat FC and CS. These binding sites have similar affinities in the two brain areas (20 pM and 340 pM), but the high-affinity site predominates in the CS while the low-affinity site is predominant in the FC. These ^{3}H-Sp binding sites may represent two types of dopamine receptors in rat brain. Alternatively, either of these binding sites may be non-dopaminergic in nature, as recently suggested by Leysen et al. (Nature 272: 163, 1978). Supported by USPHS grant 27257, by the C.C.H.D., by an RSDA(MH-00095) to H.I.Y. & USPHS (MH-05248) to J.F.

1641 A COMPARATIVE STUDY OF THE EFFECTS OF HYPOXIA ON TYROSINE HYDROXYLASE ACTIVITY IN THE CAROTID BODY OF RAT, RABBIT AND CAT. Constancio Gonzalez*, Yan Kwok*, James W. Gibb* and Salvatore J. Fidone. Dept. Physiol. and Dept. Biopharm. Sci., Univ. Utah, Salt Lake City, UT 84108.

The Type I cells of the carotid body are rich in catecholamines, particularly dopamine. However, the possible role of dopamine as neurotransmitter or modulator at the Type I cell synapse is complicated by the apparent finding that exogenous dopamine is excitatory to chemosensory activity in some species (rabbit, dog, and perhaps rat), but inhibitory in others (cat).

The activity of tyrosine hydroxylase (TH) is known to be stimulus-dependent, and a recent study by Hanbauer, Lovenberg and Costa (1977) demonstrated an induction of TH in rat carotid body at 1 and 2 days following exposure of the animals for 1 hr. to 5% O_2 (two 30 min exposures, 20 min apart). In the present study, we repeat the experiments of Hanbauer et al. in the rat, and extend them to include the carotid bodies of rabbit and cat as well.

Carotid bodies were removed from adult cats, New Zealand rabbits and Sprague-Dawley or Lewis rats. The organs were cleaned of surrounding connective tissue and homogenized in Triton X-100 (0.2%). TH activity was assayed according to the method of Nagatsu et al. (1964). The basal levels of TH activity varied widely between the different animal species. Expressed in pmols (hydroxylated tyrosine)/mg carotid body/hr., control TH values were: rat, 5247±461; rabbit, 1291±87; cat, 840±98. When the animals were exposed in a chamber to 5% O_2 for two 30 min periods, 20 min apart, and the enzyme activities assayed 48 hr. later, there was an increase in TH activity of 37% in the rat, 24% in the rabbit, and no change in the cat. In the rat, continuous exposure to 10% O_2 for 3 hr. again resulted in an increase in TH activity of 32% when assayed 48 hr. following the hypoxic episode. However, the enzyme activities were unchanged in rabbit and cat.

The delayed increase in TH activity following hypoxia agrees with the observed induction of TH reported by Hanbauer et al. However, the basal TH level which we find for rat carotid body is more than 100 times larger than the value which they reported. It is difficult to reconcile such a large discrepancy to differences in the assay method (Nagatsu vs. modified Waymire), since the TH levels which we find for rat superior cervical ganglion are similar to theirs, i.e., 6391±188 vs. approximately 4500, respectively. The absense of increased TH activity in cat carotid body following hypoxia is an interesting finding, and may be related to the apparent differences in dopamine action in the cat compared with the rabbit and perhaps rat. (Supported by USPHS Grants NS-12636, NS-07938 and DA-00869, and by a grant from the Utah Heart Association.

1642 ^{3}H-CLONIDINE AND ^{3}H-WB-4101 BINDING TO TWO α-NORADRENERGIC RECEPTOR POPULATIONS IN PERIPHERAL TISSUES. David A. Greenberg, David C. U'Prichard and Solomon H. Snyder. Dept. Pharmacol., Johns Hopkins Sch. Med., Baltimore, MD 21205.

^{3}H-WB-4101 (25 Ci/mmole) and ^{3}H-clonidine (CLO, 26.7 Ci/mmole) bind to two different postsynaptic α-receptors in CNS (U'Prichard et al., Mol. Pharmacol., 13:454, 1977). These α-receptors may be analogous to α_1- and α_2-receptors respectively (Berthelsen and Pettinger, Life Sci., 21:595, 1977). The WB site is predominant in rat kidney, and exclusive in rat ventricle and vas deferens, where there is little or no CLO binding. WB binding in these tissues represents CNS WB binding. The antagonist ligand ^{3}H-dihydroergokryptine (DHE) binds with high affinity to both WB and CLO sites in CNS (Greenberg and Snyder, Mol. Pharmacol., 14:38, 1978). In rat kidney, heart and vas deferens, the DHE binding site appears identical to the WB binding site. Conversely, in rat salivary gland, there is CLO binding, which resembles CNS CLO binding, but no WB binding. The DHE binding site in rat salivary gland is identical to the CLO binding site. These data suggest that two populations of α-receptors, which have been found in the CNS, also exist throughout the body, and are differentially located in various tissues. (Supported by USPHS grant MH-18501 and by grants from the McKnight and Hartford Foundations).

1643 ISOELECTRIC FOCUSING VARIANTS OF THE NICOTINIC ACETYLCHOLINE RECEPTOR FROM DROSOPHILA MELANOGASTER. Linda M. Hall, Thomas H. Hudson*, Reid W. von Borstel*, Barbara C. Osmond*, and Sydney D. Hoeltzli*. Biology Department 16-711, M.I.T., Cambridge, MA. 02139.

Our laboratory is using genetic approaches to study specific molecular components of the nervous system. We are particularly interested in isolating mutants affecting the acetylcholine receptor because of the key role that this component plays in synaptic function. Previous work from our laboratory (J. Neurochem. 29, 1013, 1977) has shown that the central nervous system of the fruit fly Drosophila melanogaster is a rich source of an α-bungarotoxin binding component which has the properties expected of a nicotinic acetylcholine receptor. In the experiments to be described, we will present the techniques we are using to identify genes involved in the production of acetylcholine receptors in Drosophila. Using heads from adults of the Canton-S wild-type strain as a source of binding component, we have developed a procedure for the solubilization and isoelectric focusing of this component. We show that a complex of this component and ^{125}I-α-bungarotoxin focuses as a single peak with an apparent pI of 6.6. This is substantially more basic than the toxin-receptor complex prepared from purified acetylcholine receptors from Torpedo electroplax since this complex focuses in our system at a pH of 5.2. This isoelectric focusing procedure is being used to screen for Drosophila strains with alterations in their acetylcholine receptors. A strain (designated HR) has been identified in which the isoelectric point is shifted from 6.6 to 6.7. When extracts from the wild-type and HR stocks are mixed, two peaks are clearly distinguishable on the isoelectric focusing gels. Feeding experiments have shown that the HR strain is more resistant to nicotine than is the Canton-S wild-type strain. The alteration in pI and the nicotine-resistance phenotype could both be due to a mutation affecting the acetylcholine receptor. Genetic experiments in progress will allow us to determine if the two alterations are due to a change in the same gene product. These experiments open the way for molecular genetic analysis of the acetylcholine receptor. (Supported by Council for Tobacco Research grant No. 1126 and NSF grant No. BNS 75-22581. LMH is a McKnight Scholar in Neuroscience.)

1644 POSSIBLE EXPLANATION FOR α-TOXIN FAILURE IN BLOCKING CNS ACETYLCHOLINE RECEPTORS INFERRED FROM α-TOXIN BINDING STUDIES.
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Certain snake venom α-type (post-synaptic) toxins, notably α-bungarotoxin (α-Bgt), fail to block transmission at central nicotinic synapses in spite of their demonstrated potency on neuromuscular junctions. Thus, is the saturable, high-affinity rat brain binding site for radiolabeled α-Bgt related to an authentic nicotinic acetylcholine receptor (nAChR)? To directly address this issue, we have studied the binding of an [^{125}I]-labeled derivative of Dendroaspis viridis long toxin 4.7.3 (Ddt), which exhibits antagonism towards CNS nicotinic synapses, to rat brain and Torpedo californica electric tissue and compared it to that of [^{3}H]- α-Bgt. There are two [^{125}I]-Ddt sites for every [^{3}H]-α-Bgt site in both intact and solubilized rat brain membranes, but the toxin binding is 1-to-1 in intact and solublized Torpedo nAChR-rich vesicles. Unlabeled α-Bgt and Ddt quantitatively displace both the homologous and heterologous radiolabeled toxin in both tissues. Membrane-bound [^{125}I]-Ddt and [^{3}H]/[^{125}I]-α-Bgt sites from rat brain migrate together in a sucrose gradient, co-enrich on solubilization, and have a similar nicotinic pharmacology as judged from cholinergic ligand displacement potencies. Furthermore, differences in the ability of cholinergic agonists to displace [^{125}I]-Ddt and [^{3}H]-α-Bgt emerged when competition effectiveness was tested on nAChR in experimentally-stabilized high- and low-affinity states; arguing against physical overlap of α -Bgt and Ddt sites. These observations indicate: 1) α-Bgt and Ddt bind to the same nAChR in each tissue, 2) Torpedo and rat brain nAChR are significantly different on the basis of their binding sites for closely-related protein toxins, 3) the binding to additional sites in brain must be related to Ddt's physiological blocking-activity in CNS, and 4) while α-Bgt recognizes rat brain nAChR and can apparently prevent all Ddt binding (including presumptive agonist-activation sites), it fails to block agonist triggering of the receptor.

(Supported by the Division of Biomedical and Environmental Research, U.S.Dept. of Energy. RJL is a postdoctoral fellow of the National Institute of Neurological and Communicative Disorders and Stroke.)

1645 THE HORMONE RECEPTOR/ADENYLATE CYCLASE SYSTEM OF HUMAN ASTROCYTOMA CELLS: DIFFERENTIAL EXPRESSION OF COMPONENTS DURING CELL GROWTH. T.K. Harden, S.J. Foster*, and J.P. Perkins*, Dept. of Pharmacology, Univ. of N.C. Med. Ctr., Chapel Hill, N.C. 27514

Cultured human astrocytoma cells (132 1N1) respond to isoproterenol (ISO) and prostaglandin E_1 (PGE_1), with marked increases in intracellular cAMP levels. The amount of cAMP that accumulates in intact cells in response to maximally effective concentrations of ISO (10 μM) and PGE_1 (10 μM) varies as a function of time after subculture of cells. ISO-stimulated cAMP accumulation increases 3-4 fold during the first 2 days after subculture, then subsequently declines to the initial (6 hr) responsiveness after 8 days of culture. In contrast, PGE_1 responsiveness gradually increases by approximately two-fold over an 8 day culture period. An analysis of the individual components of the hormone receptor/adenylate cyclase system was carried out to determine the mechanism(s) responsible for this differential expression of astrocytoma cell responsiveness to hormones. The changes in intact cell responsiveness were reflected by similar alterations in hormone stimulated adenylate cyclase activity in membrane preparations. ISO-stimulated enzyme activity increased 2-4 fold during the first 48 hr after subculture and decreased during the next 6 days to a level that was 15-25% of the peak activity. PGE_1-stimulated adenylate cyclase activity gradually increased by 1.5-3.0 fold during 8 days of culture. A small increase (1.2-1.5 fold) was observed in basal and NaF stimulated enzyme activity during the first 48 hr after subculture. The role of β-adrenergic receptors in the observed changes in ISO responsiveness was examined using ^{125}I-hydroxybenzylpindolol (^{125}IHYP) as a specific receptor ligand. The affinity (≈0.15 nM) of ^{125}IHYP for β-adrenergic receptors did not change as a function of days in culture. In contrast, the density (moles/mg protein or moles/cell) of receptors increased markedly during the first two days in culture, reaching a level that was 3-5 times greater than the receptor density observed after 6 hr of culture. During the following 6 days β-receptor density decreased, reaching a level that was 20% of the 48 hr receptor density. When cells were plated at high density (> 2×10^5 cells/cm^2), no increase in ISO-responsiveness and β-adrenergic receptor density occurred during the period immediately following subculture. Taken together these results suggest that the individual components of the hormone receptor/adenylate cyclase systems of astrocytoma cells are regulated differentially. The role of cell growth and density in this regulation of the components of the cAMP system are under further investigation. Supported by HL 22490 and GM 25163.

1646 7S NERVE GROWTH FACTOR DOES NOT BIND TO NGF RECEPTORS ON EMBRYONIC CHICK SENSORY NEURONS. RONALD M. HARRIS-WARRICK AND ERIC M. SHOOTER. Department of Neurobiology, School of Medicine, Stanford University, Stanford, California, 94305

When Nerve Growth Factor (NGF) is isolated from mouse submaxillary gland, the active protein, βNGF, is found in a complex with two other proteins, α and γ,with a sedimentation coefficient of 7S. The binding of βNGF and the 7S NGF complex to specific receptors on sensory neurons from 8-day embryonic chick dorsal root ganglia was compared to determine which molecular species of NGF interacts with the cells to elicit neurite outgrowth. When ^{125}I-βNGF is stabilized in the 7S complex by addition of a large excess of α and γ subunits (4×10^{-7}M) and zinc ion (25μM), specific binding of radioactive NGF is completely abolished. There is no binding to either the high affinity receptor, S_I (which mediates neurite outgrowth) or to the lower affinity receptor, S_{II} (with unknown function) over a wide concentration range of ^{125}I-βNGF when it is stabilized as a 7S complex. The kinetics of association of the subunits into the 7S complex were measured using CM52 columns to rapidly separate free ^{125}I-βNGF from that bound in the complex (Bothwell and Shooter, in press). The rate of complex formation is the same as the rate of reduction of binding of radioactive NGF to neuronal receptors under the same conditions. Carboxypeptidase B cleaves the carboxy-terminal arginyl residues from βNGF; the resulting bis-desarg-NGF has been previously shown to be unable to form a 7S complex with α and γ subunits. However, bis-desarg-NGF has normal biological activity and binds specifically to S_I and S_{II}. Mixture of ^{125}I-bis-desarg-NGF with excess α and γ subunits and zinc ion does not inhibit specific binding to either S_I or S_{II}. Incubation of ^{125}I-βNGF with zinc alone, or with α subunit (with or without zinc) does not inhibit binding. Incubation with γ subunit (with or without zinc) selectively inhibits binding of radioactive NGF to S_I, with no effect on binding to S_{II}. The γ subunit, an arginine esteropeptidase, does not degrade βNGF or NGF receptors on sensory neurons. The inhibition of binding to S_I is due to formation of a β-γ or β-γ_2 complex; such complexes have been shown to form under these experimental conditions (Bothwell and Shooter, in press). These results suggest that the biological role of 7S NGF is at a stage prior to interaction with responsive neurons, and that dissociation of the 7S NGF complex is required for the activation of NGF. (Supported by grants from the NIH and NINCDS.)

1647 BIOCHEMICAL AND PHARMACOLOGICAL STUDIES OF PUTATIVE ACETYLCHOLINE RECEPTORS OF INVERTEBRATES. Stephen W. Jones*, Puppala Sudershan* Katumi Sumikawa* and Richard D. O'Brien. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

Although some invertebrate tissues have long been known to be sensitive to acetylcholine and other cholinergic drugs, it is not clear whether this is due to nicotinic and/or muscarinic receptors (as in vertebrates) or to novel acetylcholine receptors (AChRs). We report here a more extensive pharmacological comparison of sites in invertebrates that bind cholinergic drugs with the classical vertebrate AChRs.

House fly heads have an α-bungarotoxin (α-BGT) binding site [FEBS Lett. 76:211 (1977)]. It is similar to that of vertebrate brain and autonomic ganglia: α-BGT binds reversibly, with nanomolar affinity and an off time of hours. The potent nicotinic agents decamethonium, hexamethonium, and succinylcholine are rather inactive, and atropine is mildly effective in blocking α-BGT binding, but the pharmacology is otherwise nicotinic.

House fly heads [J. Neurochem. 17: 1287 (1970)] and horseshoe crab axons [J. Neurochem. 29:803 (1977)] contain sites that bind both nicotinic and muscarinic agents. Both sites are present in concentrations greater (per gram tissue) than is the AChR of Torpedo electroplax. The house fly site binds decamethonium, dimethylphenylpiperazinium, benzoquinonium, hexamethonium, methacholine, and dexetimide well (>80% inhibition of 0.1 μM decamethonium binding by 10 μM drug), but also binds the anticholinesterase BW284 and hemicholinium-3. Among other effective agents are the anthelminthic drugs hycanthone (57%), pyrantel (75%), and levamisole (63%), which are thought to act by binding to the AChRs of helminths. The site in horseshoe crab axons also has a "mixed" pharmacology, but it differs in several ways from the house fly site. In particular, it binds both α-BGT and the "specific" muscarinic agent quinuclidinyl benzilate (QNB) with micromolar affinity (compared to nanomolar or better for their classical targets).

The absence of physiological evidence [but see J. Neurobio. 2:247 (1971)] makes it impossible to conclusively identify these sites as AChRs. The location of the horseshoe crab site (in axons) argues against its functioning as an AChR. The similarity of the α-BGT site to that in autonomic ganglia of vertebrates, which is not an AChR, casts suspicion on its physiological role.

1648 ALPHA-ADRENERGIC RECEPTORS IN THE RAT SUPERIOR CERVICAL GANGLION. Marian S. Kafka and Nguyen B. Thoa*. Biological Psychiatry Branch and Laboratory of Clinical Science, NIMH, Bethesda, MD 20014.

A radioactive α-adrenergic antagonist, [^{3}H]dihydroergocryptine (^{3}H-DHE) binds specifically to membranes from Sprague-Dawley rat superior cervical ganglia. The binding is saturable and reversible and the binding sites have a high affinity for ^{3}H-DHE. The binding is stereospecific as l-epinephrine (l-E) and l-norepinephrine (l-NE) inhibit binding more potently than d-norephinephrine. The binding sites are α-adrenergic as the α-adrenergic agonists l-E and l-NE and the α-adrenergic antagonist phentolamine inhibit binding potently, whereas the β-adrenergic agonist l-isoproterenol and the β-adrenergic antagonist dl-propranolol inhibit weakly. Decentralization of the superior cervical ganglia by severing the preganglionic cholinergic nerve supply resulted in a decrease in ^{3}H-DHE binding. The data suggest that there are membrane α-adrenergic receptors in the rat superior cervical ganglion and that some of the receptors are present on pre-ganglionic sympathetic nerve endings where they may participate in the regulation of neurotransmission through the ganglion.

1649 WHITE-NOISE ANALYSIS OF ELECTRORECEPTOR DYNAMICS: A CONCEPTUAL BASIS FOR THE FREQUENCY SELECTIVITY OF THE AFFERENT RESPONSE.

Howard I. Krausz and Terry A. Viancour, UTMB, Galveston, Texas and UCSD, San Diego, California.

The electric fish Eigenmannia produces a continuous, pseudo sinusoidal Electric Organ Discharge (EOD) at frequencies of 250 to 500 per second, depending on the individual. An individual has a 'signature' EOD frequency. The EOD is used for communication, and distortions of the EOD field produced by objects can be detected by the fish. Electroreceptor organs contain modified acousticolateralis receptor cells which respond to local changes in electric field intensity and regulate the spike activity of primary afferent neurons. The afferent discharge is selectively sensitive to the frequency of exogenously applied sinusoidal electric fields -- the maximum frequency sensitivity correlates with an individual's EOD frequency. The afferent discharge occurs in phase with single EOD cycles: some afferents fire a spike for each EOD cycle, others discharge for less than 60% of the EOD cycles. The afferent discharge pattern and its frequency selectivity suggests that the system may act as a linear filter followed by a spike generator with a fixed threshold.

To test this hypothesis further, we presented white-noise modulated electric fields and averaged the stimulus waveforms that preceded each spike in the primary afferent nerve. If the hypothesis is correct, this trigger correlation or first kernel will be the impulse response of the linear filter that precedes the threshold device. When the noise stimulus was filtered by the measured kernel, a continuous signal resulted that crossed a set threshold at times nearly identical to the actual times of spike discharges. Therefore, we conclude that the first kernel represents the impulse response of the receptive processes prior to spike generation and furthermore, the close resemblence between kernel waveform and EOD waveform suggests that receptors are matched or tuned to the fishes' own EOD waveform, i.e. it is tuned to the entire waveform and not just to the repetition frequency. The frequency tuning of electroreceptors has commonly been measured with sinusoidal inputs at various frequencies, and we now report that such measurements are closely predicted by the Fourier transform of the first kernel.

1650 STATE TRANSITIONS OF A CENTRAL NICOTINIC ACETYLCHOLINE RECEPTOR: INVOLVEMENT OF THIOL GROUPS AND DIVALENT CATION SPECIFICITY. Ronald J. Lukasiewicz*, Hiromi Morimoto* and Edward L. Bennett. Lab. Chem. Biodynamics, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720.

The ability of cholinergic agonists to displace [^{3}H]α-bungarotoxin (α-Bgt) binding to nicotinic acetylcholine receptors (nAChR) from rat brain when agonist is preincubated with nAChR prior to the addition of toxin (preincubation) is increased relative to the case where toxin and agonist are added to nAChR simultaneously (coincubation). In order to elucidate the molecular mechanisms by which cholinergic agonist induces transformation of nAChR to a high-affinity form toward agonist, the role of receptor thiol groups and some effects of solvent composition in manifestation of this phenomena were examined. Reduction of nAChR thiol groups with dithiothreitol (DTT), followed by alkylation of reduced nAChR sulfhydryls with N-ethylmaleimide (NEM) prevents agonist-induced transformation of nAChR to a high-affinity state; for NEM-treated nAChR, the concentration of acetylcholine (ACh) at which half specific α-Bgt (10 nM) binding is displaced (IC-50) is 30 μM, whether or not nAChR is preincubated with agonist prior to exposure to toxin. This IC-50 value is similar to that for the transient low-affinity form of native nAChR under the coincubation condition. Oxidation of DTT-reduced nAChR thiol groups by treatment with 5,5'-dithiobis-2-nitrobenzoate (DTNB), which presumably promotes formation of nAChR-nAChR disulfide linkages, leaves nAChR in a high-affinity state for both pre- and coincubation; IC-50 is 3 μM for DTNB-treated nAChR, which is identical to that for native nAChR pretreated with ACh. The apparent affinity of ACh for DTT-treated nAChR is markedly altered, IC-50 values being ∿2 mM for coincubation. However, DTT-reduced nAChR is responsive to pretreatment with ACh, as IC-50 values are ∿50 μM for the preincubation case. Furthermore, a low-affinity state of nAChR, with IC-50 of 30 μM, is preserved in Ca^{++}-free medium. These affinity state observations hold for all cholinergic agonists tested, including ACh. In contrast, the IC-50 for d-tubocurarine competition toward 10 nM α-Bgt is identical for NEM- and DTNB-treated or native nAChR, in the presence or absence of Ca^{++}, for either pre- or coincubation paradigms. None of these treatments significantly alter the quantity of α-Bgt sites on nAChR or the affinity of toxin for receptor. These results suggest that the redox state of nAChR thiol groups and/or some primary or secondary effect(s) of Ca^{++} may mediate activation of NAChR and/or receptor desensitization, the presumed physiological correlates of these agonist-specific alterations in receptor state.

Supported in part by the Division of Biomedical and Environmental Research of DOE and by an NINCDS postdoctoral fellowship (RJL).

1651 SEROTONIN RECEPTORS COUPLED TO ADENYLATE CYCLASE IN A NEUROBLASTOMA X BRAIN HYBRID CELL LINE. John MacDermot* and Marshall Nirenberg. Laboratory of Biochemical Genetics, NHLBI, NIH, Bethesda, MD 20014.

Serotonin stimulates adenylate cyclase in neuroblastoma (N18TG2) x fetal Chinese hamster brain hybrid cells (NCB-20). The concentration of serotonin required for half-maximal stimulation (Ka) is 0.5 μM, and an increase of 75-115% in adenylate cyclase activity is produced by a saturating concentration (10 μM) of serotonin. Tryptamine, bufotenine, 5-methoxytryptamine and N,N'-dimethyl-5-methoxytryptamine also stimulate adenylate cyclase (Ka = 0.5-0.7 μM). Hill and Eadie-Scatchard analyses suggest a simple bimolecular interaction between serotonin and its receptor. An increase in adenylate cyclase activity is also produced by D-lysergic acid diethylamide, D-LSD, (Ka = 12 nM) and its analogues metergoline (Ka = 250 nM) and methysergide (Ka = 620 nM). The increase is less than half that produced by serotonin. Serotonin-stimulated adenylate cyclase is inhibited by the antagonists mianserine (Ki= 43 nM) and cyproheptadine (Ki = 95 nM). Similar Ki values are obtained for the inhibition of D-LSD-stimulated adenylate cyclase. Phentolamine and propranolol have little or no effect on the stimulation of adenylate cyclase by serotonin (Ki > 10 μM). However, the dopamine antagonists fluphenazine (Ki = 47 nM) and pimozide (Ki = 250 nM) both inhibit serotonin-stimulated adenylate cyclase. Basal and PGE_1-stimulated adenylate cyclase are partially inhibited by histamine, dopamine, norepinephrine and carbamylcholine; no effect is observed with GABA. Serotonin has little or no effect on adenylate cyclase activity of NG108-15 neuroblastoma x glioma hybrid cells. This confirms the observation that intracellular levels of cAMP are not affected by serotonin (Matsuzawa, H. and Nirenberg, M., in preparation). Serotonin depolarizes NG108-15 cells, but D-LSD neither mimics nor antagonizes this effect (Christian, C. N., et al., in press). Serotonin receptors of NCB-20 hybrid cells also mediate cell depolarization (Higashida, H., unpublished results) and in addition the activation of adenylate cyclase. These results indicate that there are two serotonin receptor functions and that they can be independently inherited in a clonal fashion.

1652 DENDROAXONIC NEUROTRANSMISSION II: EVIDENCE OF NERVE ENDING RECEPTOR SITES FOR DENDRITICALLY RELEASED TRANSMITTER.
P.L. McGeer, E.G. McGeer and V. Innanen*. Kinsmen Lab. Neurol. Sci., Dept. Psych., UBC, Vancouver, B.C. Canada V6T 1W5.

Local injections of kainic acid (KA) into the striatum of rats destroy neurons in the striatum without injuring afferent dopaminergic (DA) nerve endings. Among the neurons destroyed are GABAergic and substance P systems that project to the substantia nigra (SN). Intraventricular injections of 6-OHDA can be used to destroy DA systems selectively. Animals with these lesions were used to study the localization of muscarinic and nicotinic receptors in the striatum and DA receptors in the SN by standard binding assays with ^{3}H-QNB, ^{125}I - α-bungarotoxin (α-Btx) and ^{3}H-spiroperidol: unlabeled excess oxotremorine, D-tubocurarine and dopamine were used in the blanks. Rats were given unilateral injections of KA into the striatum followed one week later in some animals by intraventricular injections of 6-OHDA. Tyrosine hydroxylase (TH), choline acetyltransferase (CAT), glutamic acid decarboxylase (GAD), and protein, as well as QNB and α-Btx binding were measured on the individual striata. Following the lesions, ^{3}H-QNB binding correlated with both residual GAD and CAT activity in the striatum. There was no correlation of QNB binding with TH activity, although this correlated with α-Btx binding. These results suggest that muscarinic receptors in the striatum are on neuronal systems destroyed by KA-injections[1] while nicotinic receptors may exist on dopaminergic nerve endings[2]. Spiroperidol binding in the SN correlated more closely with striatal GAD than with striatal TH. The localization of DA receptors in the SN on afferent nerve endings partly destroyed by striatal injections of KA is in accord with prior reports on DA-sensitive adenylate cyclase in the SN being located on such striatal afferents[3]. Since dopaminergic nerve endings connect with cholinergic dendrites in the striatum, and GABAergic nerve endings connect with dopaminergic dendrites in the SN, these data suggest that dopamine and acetylcholine may be released from dendrites to act on receptors on axonal endings. They support morphological studies (Hattori, McGeer and McGeer, Dendroaxonic Neurotransmission I) indicating that structures suitable for synthesizing, storing and releasing these neurotransmitters are located in dendrites in the striatum and SN

[1]cf. R. Schwarcz and J.T. Coyle, Brain Res. 127, 235-249 (1977).
[2]cf. M.F. Giorguieff et al., Brain Res. 106, 117 (1976).
[3]K. Gale et al., Science 195, 503 (1977); P.F. Spano et al., Science 196, 1343 (1977).

1653 EFFECT OF PHOSPHOLIPASE A_2 ON ACETYLCHOLINE RECEPTOR FUNCTION IN TORPEDO CALIFORNICA MEMBRANES.
Mark G. McNamee* and Terrence Andreasen* (SPON: L. Chalupa). Dept. Biochem. & Biophys., Univ. of California, Davis, CA 95616.

A protein isolated from Naja naja siamensis venom on the basis of its phospholipase A activity inhibits acetylcholine receptor function in postsynaptic membrane vesicles from Torpedo californica. Specifically, the phospholipase A prevents the large increase in sodium efflux that can normally be induced by carbamylcholine, a receptor agonist. The phospholipase A inhibition shows the following properties: 1) it occurs at concentrations 50 times lower than the concentrations required for inhibition by α-neurotoxins; 2) the phospholipase A has no effect on the binding properties of the receptor; 3) the inhibition is abolished by removal of calcium ions; and 4) some phospholipid hydrolysis accompanies inhibition. Introduction of free fatty acids or lysophosphatidylcholine into the membranes also results in inhibition of receptor activation, although much higher concentrations than those generated in situ are required. Lysophosphatidylethanolamine does not inhibit receptor function.

The inhibition by phospholipase A_2 or by fatty acids and lysophosphatidylcholine can be completely reversed by treatment of the membranes with bovine serum albumin. Inhibition and its reversal can be directly correlated with the uptake and removal of fatty acids, using both radioactive and spin-labeled fatty acids.

It is suggested that phospholipase A_2 acts enzymatically to produce fatty acids and some lyso-derivatives that effectively uncouple ligand binding from ion permeability in the receptor containing membrane vesicles.

1654 **CHARACTERIZATION OF STRIATAL ^{3}H-APOMORPHINE AND ^{3}H-SPIROPERIDOL BINDING AND DOPAMINE SENSITIVE ADENYLATE CYCLASE ACTIVITY UTILIZING PROTOBERBERINE ALKALOIDS.**
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Tetrahydroprotoberberine alkaloids contain a nitrogen atom in a fixed position (cis;gauche) to the catechol nucleus. These compounds inherently possess two isoquinoline moieties within their molecular geometry. The recent availability of several positional and optical isomers in various states of phenolic methylation and nitrogen quaternarization made it possible to further characterize the geometric, stereospecific and topographic requirements for agonist and antagonist radioligand binding to the dopamine receptor. The optical isomers of 2,3,9,10- and 2,3,10,11-tetrahydroxyberbine (THPB) and selective mono-O-methylated 2,3,10,11-THPBs were tested for their ability to displace 5nM [^{3}H] apomorphine and 0.2nM [^{3}H] spiroperidol and to inhibit adenylate cyclase activity in the presence of 100 μM dopamine. The results of these studies appear in the table below:

COMPOUND	Specific Binding EC_{50} (nM)		DA-Cyclase IC_{50} (nM)
	^{3}H-APO	^{3}H-SPIRO	
(-)-2,3,9,10-THPB	60	500	3000
(+)-2,3,9,10-THPB	500	28000	—
(-)-2,3,10,11-THPB	65	4500	1000
(+)-2,3,10,11-THPB	600	>100000	50000
(±)-10-OMe-THPB	150	4500	59000
(±)-3-OMe-THPB	150	3000	500
(±)-2-OMe-THPB	2000	1200	15000

These data indicate that the S-(-)-isomer of both THPBs was more potent than the R-(+)-isomer in displacing labelled apomorphine and spiroperidol from their respective binding sites. Selective mono-O-methylation altered the potency of these compounds to displace antagonist or agonist labelled recognition sites. It is noteworthy that these compounds were generally as potent inhibitors of dopamine sensitive adenylate cyclase activity as they were antagonists of [^{3}H] spiroperidol binding. However, these same alkaloids were considerably more potent displacing agents of [^{3}H] apomorphine. Results of additional studies employing various quaternary protoberberine and quaternary dehydroprotoberberine salts to further characterize the recognition sites of dopaminergic receptor agonist and antagonist binding will be discussed.
(Supported in part by grants from the Veterans Administration, USPHS AA 00226 and Pharmaceutical Manufacturer's Association.)

1655 FIBER SIZE CONTENT AND ORIGIN IN THE RAT CAROTID SINUS NERVE.
Jasleen Mishra and Arthur Hess. Dept. Anat., Rutgers Med. Sch., CMDNJ, Piscataway, N.J. 08854.

Before performing experiments involving physiological recording from the carotid sinus nerve of the rat, studies were made of the fiber size distribution and the source of these fibers. Normal nerves were studied, as well as after ganglionectomy (removal of the superior cervical ganglion for 10-12 days), severance of the ninth nerve (for 10-12 days), and section of the sympathetic chain below the superior cervical ganglion (degeneration of preganglionic fibers for 10-18 days). The normal nerve contains about 50-60 myelinated fibers in some rats, up to 150 myelinated fibers in others, probably depending on size and age. The myelinated fibers are scattered throughout the nerve. Most are fairly uniform in diameter and are about 2mμ. The 5-10 larger fibers are about 4-5mμ in diameter and appear more or less segregated in a peripheral area of the nerve. There are about 500-550 non-myelinated fibers. Most of these occur alone in their own Schwann cells and are about 1-1.5mμ in diameter. Smaller non-myelinated fibers (0.3-0.5mμ in diameter) occur in bundles of 3-8 and share a Schwann cell. There are about 20 of these bundles scattered throughout the nerve. It is suggested that the smaller non-myelinated fibers sharing a Schwann cell are sympathetic in origin and are postganglionic fibers arising from cell bodies in the superior cervical ganglion. The larger non-myelinated fibers appearing individually in a Schwann cell are the sensory distal processes of petrosal ganglion cells. Preliminary studies indicate thus far that the bundles of non-myelinated fibers degenerate after ganglionectomy, while the individually ensheathed non-mylinated fibers degenerate after section of the ninth nerve. A combination of operations (ganglionectomy and section of the ninth nerve) results in degeneration of almost all fibers. However, consistently thus far, about 1/3 of the population of myelinated fibers 2mμ in diameter are still present, scattered throughout the nerve and apparently surviving the surgical procedures; the origin of these fibers remains obscure. Section of preganglionic fibers to the superior cervical ganglion results in no degeneration of myelinated or non-myelinated fibers; hence, no preganglionic fibers arising from the spinal cord or fibers having their origin in sympathetic ganglia below the superior cervical ganglion are present in the carotid sinus nerve.

(Supported by NIH RR05576.)

1656 DEVELOPMENTAL STUDIES OF α-BUNGAROTOXIN BINDING SITES IN MAMMALIAN BRAIN. Barbara J. Morley and George Kemp*, Neurosciences Program, University of Alabama in Birmingham, Birmingham, Alabama, 35294.

α-bungarotoxin, (BTX), a specific marker for the nicotinic acetylcholine receptor (nAChR) in skeletal muscle, has been employed to investigate the possibility of these receptors in mammalian brain. Approximately 2-3 pM toxin sites/g wet weight is found in adult brain, with large variation among several brain areas (e.g., Morley et al, Brain Res. 134: 161-166, 1977). Although these binding sites are associated with other cholinergic markers, their function(s) in nervous tissue have not been demonstrated.

Little is known concerning their rate of appearance during development in mammalian brain. One report (Salvaterra & Moore, BBRC 55: 1311-1318, 1973) has suggested that these sites are present in newborns, increases gradually in the cortical/forebrain areas, and reaches adult levels by about 10 days.

We now report a thorough study of BTX binding sites in rat brain during development utilizing a biochemical assay for I^{125} BTX. In addition to large regions (cortex, forebrain, brainstem, cerebellum), selected areas throughout the brain (e.g., olfactory bulbs, inferior colliculus, superior colliculus, raphe, reticular formation) were also investigated from days 1-60 postnatal. In the newborn rat the whole brain levels of BTX binding sites was high (1.5 pM BTX sites/g wt.; .04 pM/mg pro.). The concentration found in cortex and forebrain were similar (approximately 1.2 pM/g wet wt.; 0.36 pM/mg pro.). The concentration in brainstem is very high (1.8 pM/g wt.; .06 pM/mg pro.).

In general the development of BTX sites follows a caudal-rostral pattern and appears to increase gradually during postnatal development in all nuclei. Both rostral and caudal areas contain adult levels of BTX binding by 10 days. Pharmacological studies have not revealed any differences in the inhibition of toxin binding by cholinergic ligands throughout development, suggesting that there is no change in the conformation of receptors postnatally.

Data on the prenatal appearance of BTX binding sites in rat, rhesus monkey, and human will also be presented.

It is hoped that the relationship of toxin binding sites to the development of synapses and other cholinergic markers will shed light on its possible role in the formation and/or maintenance of synapses.

1657 BETA-ADRENERGIC RECEPTOR IN MAMMALIAN CHOROID PLEXUS. James A. Nathanson. Dept. Neurology, Yale Medical School, New Haven, Ct. 06510.

The choroid plexus plays an important role in cerebrospinal fluid (CSF) production but, as yet, control mechanisms for CSF secretion by the choroid are poorly understood. This organ receives adrenergic innervation, and it has been reported that intraventricular cholera toxin, a known activator of adenylate cyclase, increases the rate of CSF production. These facts raise the possibility for an involvement of cyclic AMP in the control of CSF production.

The present study reports the identification of a beta-adrenergic-sensitive adenylate cyclase in broken cell preparations of choroid plexus from several mammalian species. This enzyme is markedly stimulated by low concentrations of isoproterenol (K_a= 2 X 10^{-7}M) and norepinephrine (K_a= 2 X 10^{-5}M) but is only poorly activated by phenylephrine, an alpha-adrenergic agonist. Maximal stimulation by isoproterenol in cat choroid plexus is as much as 800% of basal activity, and the enzyme shows a regional distribution within the choroid. Isoproterenol activation is inhibited by propranolol but not by phentolamine or fluphenazine. This, plus other data, distinguish beta-adrenergic-sensitive adenylate cyclase from dopamine- and histamine-sensitive adenylate cyclases, also present in broken cell preparations of choroid plexus.

Because of the known intimate association of adenylate cyclase with certain hormone receptors, these data support the existence of specific β-adrenergic receptors in mammalian choroid plexus. The cellular localization and possible significance of these receptors in CSF secretion will be discussed.

1658 ACETYLCHOLINE (AcCh) AND LOCAL ANESTHETIC BINDING PROPERTIES OF TORPEDO CAL. NICOTINIC POST-SYNAPTIC MEMBRANES AFTER REMOVAL OF NON-RECEPTOR PEPTIDES. Richard R. Neubig*, Elizabeth K. Krodel*, Jonathan B. Cohen. Dept. Pharmacol., Harvard Med. Sch., Boston, MA 02115.

Comparison of the polypeptide composition of post-synaptic membranes isolated from Torpedo electric tissue with that of the nicotinic cholinergic receptor (AcChR) isolated in detergent solution from that tissue has revealed that the major difference resides in a 43,000 dalton peptide (43K) present in the membranes, but absent in the isolated AcChR (Sobel et al., Eur. J. Biochem. 80:215 (1977)). The 43K protein is distinct from the 40,000 dalton peptide known to contain the site of binding of cholinergic ligands and α-neurotoxin. In order to analyze the possible functional role of the 43K protein in the post-synaptic membrane, we developed a procedure to extract quantitatively the 43K protein and other minor non-receptor peptides while preserving the fundamental structural integrity of the membrane.

Analysis by dodecylsulfate/polyacrylamide gel electrophoresis of the peptide composition of the 43K-depleted membrane established peptide components of 40, 50, and 66,000 daltons, peptides characteristic of isolated AcChR. The 43K-depleted membranes contained 40% of the protein and 80-90% of the initial [^{3}H]-AcCh binding sites. Functional integrity of the 43K depleted membranes was measured by: (1) an analysis of the kinetics of binding of [^{3}H]-AcCh; (2) the effect of local anesthetics on that binding; (3) a determination of the number and affinity of specific binding sites for the local anesthetic [^{14}C]-trimethisoquin. By these three criteria, the ligand binding properties of the 43K depleted membranes were unaltered relative to the control membranes. For example, for both the control and 43K depleted membranes, the kinetics of binding of [^{3}H]-AcCh (35nM) with a suspension 50nM in binding sites was characterized by a biphasic reaction: 45% of the binding occurred with a half time of 45 sec. However, in the presence of 10μM dimethisoquin the AcCh binding attained equilibrium in less than 5 sec. Furthermore, for both 43K depleted and control membranes, the [^{14}C]-trimethisoquin was bound with a dissociation constant K_D=1μM when all AcCh binding sites were occupied by carbamylcholine, and the bound anesthetic was displaced by micromolar concentrations of histrionicotoxin (HTX).

It is concluded that the 43K protein is unrelated to the site of binding of local anesthetics or HTX, which must be associated with one of the remaining peptides. Flux studies will specify whether the cholinergic ionophore remains functional in these membranes.

Supported in part by USPHS grants NS 12408, GM-02220, and by MDAA fellowship to EKK.

1659 CHARACTERIZATION OF THE α-BUNGAROTOXIN BINDING COMPONENT OF GOLDFISH BRAIN. R.E. Oswald*, J.T. Schmidt and J.A. Freeman. Depts. of Biochem. & Anat., Vanderbilt Univ. Med. Sch., Nashville, TN.

Previous work has indicated that the nicotinic cholinergic receptor protein (AChR) might have a role in the maintenance of synaptic connections in the optic tectum (Freeman, Nature, 269, 218 (1977)). Schmidt and Freeman (in preparation) have demonstrated that visual responses in goldfish tectum are abolished by α-Btx. We have undertaken the biochemical characterization of the kinetic and molecular parameters of the goldfish brain nicotinic AChR using ^{125}I-α-Btx. The distribution of receptors was studied using light autoradiography. Equilibrium binding of α-Btx to goldfish brain synaptosomes revealed a single-class of toxin-binding sites with a dissociation constant of 0.92 ± 0.02 nM. Kinetic studies, however, revealed that the binding is more complex. Although a single association rate constant (3.46 ± 0.39 x 10^5 M^{-1} sec^{-1}) is observed, α-Btx exhibits a biphasic dissociation curve. The fast dissociation rate constant (6.6 x 10^{-4} sec^{-1}) is exhibited by approximately 20% of the sites; whereas, 80% of the sites have a slow dissociation rate constant of 4.3 x 10^{-6} sec^{-1}. Dissociation constants calculated from the kinetic constants are 1.9 nM for the low affinity site and 12.5 pM for the high affinity site. The binding of α-Btx exhibits nicotinic pharmacology as evidenced by the following K_I's for cholinergic ligands: d-tubocurarine=10.7 nM, nicotine=36.3 nM; carbamylcholine=1.3 uM; hexamethonium=11.2 uM; and atropine=0.10 mM. The toxin receptor complex is readily solubilized by treatment with 1% Triton X-100. Preparative isoelectric focusing of the toxin-receptor complex yields an isoelectric point of 5.01 ± 0.01. Gel filtration on Sepharose 4B gives a Stokes radius of 88.1 ± 1.2Å and a diffusion constant of 2.4 x 10^{-7} cm^2/sec for the toxin-receptor-detergent complex. Sedimentation velocity experiments in sucrose-H_2O and sucrose-H_2O gradients yield a sedimentation constant of 11.3 S and a partial specific volume of 0.76 cm^3/g. The molecular weight of the toxin-receptor-detergent complex was calculated to be 470,000 daltons, assuming a partial specific volume of 0.735 cm^3/g for the protein portion of the complex, 15% (w/w) or the complex is detergent and 85% is protein. Thus, the molecular weight of the toxin-receptor complex was 400,000 daltons. Using light autoradiography, the optic tectum was observed to contain the greatest concentration of toxin-binding sites. The receptors were distributed in specific lamina with the greatest concentration localized in the layers of optic projection. Thus, the goldfish brain contains an α-Btx binding protein similar in most respects to the nicotinic AChR of electroplax and muscle. A large proportion seems to be in the optic tectum associated with the retinotectal projection.

1660 MECHANICAL PROPERTIES OF THE INTRAFUSAL MUSCLE OF MAMMALIAN MUSCLE SPINDLES. R. E. Poppele, W. R. Kennedy, and D. C. Quick,* Laboratory of Neurophysiology and Department of Neurology, University of Minnesota, Minneapolis, Minnesota 55455

Properties of muscle spindle behavior including sensitivity and adaptation have been assumed to be explainable by the mechanical properties of intrafusal muscle. We have investigated the role of intrafusal muscle in determining the steady-state sensitivity of spindle receptors. Small stretches ($\leq$ 100 μm) were applied to isolated muscle spindles removed from cat tenuissimus muscle for various steady-state lengths of those spindles. The sensitivity of both the primary and secondary endings was greater in stretched spindles than in relaxed ones.

We measured the steady-state strain of intrafusal muscle in sensory and non-sensory regions of the spindles as the incremental length change per unit length induced by the applied stretch. We found that there was a proportional relationship between sensory strain and receptor sensitivity. By comparing intrafusal strain of the sensory and non-sensory areas with and without the spindle capsule, we conclude that the spindle capsule does not contribute to the observed non-linearity. Further measurements of steady-state tension indicated that there is a non-linear stiffness of the striated portions of the intrafusal muscle which can quantitatively account for the increased sensitivity in a stretched spindle. Therefore, we propose that the sensory endings act as proportional strain gauges in transforming steady-state stretches of the sensory endings into receptor potentials. (Supported by NSF grant #76-10791 and USPHS grant NS 109690-04.)

1661 DISSOCIATION BETWEEN THE PRESYNAPTIC DOPAMINE SENSITIVE ADENYLATE CYCLASE AND ^{3}H-SPIPERONE BINDING SITES IN RAT SUBSTANTIA NIGRA. Maryka Quik*, Piers C. Emson*, Eileen Joyce* and Leslie L. Iversen. MRC Neurochemical Pharmacology Unit and Dept. of Experimental Psychol., University of Cambridge, Cambridge, England.

Seven or 14 days after lesions which destroyed various components of the striato-nigral system, ^{3}H-spiperone binding and dopamine (DA)-sensitive adenylate cyclase were measured in rat substantia nigra. Hemisections, which resulted in an approximately 70% decrease in tyrosine hydroxylase (TH), cyclic nucleotide phosphodiesterase (PDE) and glutamate decarboxylase (GAD), led to a 50% decline in ^{3}H-spiperone binding and an almost complete disappearance of DA-sensitive adenylate cyclase. 6-Hydroxydopamine injection into the substantia nigra, which depleted TH in the substantia nigra by 85%, whilst leaving PDE and GAD unaffected, resulted in a 40% decrease in ^{3}H-spiperone binding but no change in DA-sensitive adenylate cyclase. Intrastriatal injections of kainic acid did not alter TH in the substantia nigra, but decreased both GAD (54%) and PDE (68%); ^{3}H-spiperone binding was unaffected by this lesion, while the DA-sensitive adenylate cyclase was greatly reduced (50 to 75%). These results suggest that within the substantia nigra the DA receptor sites defined by using ^{3}H-spiperone are located mainly post-synaptically, while the DA-sensitive adenylate cyclase is bcated pre-synaptically.

1662 PRE- AND POSTSYNAPTIC LOCALIZATION OF NEUROTRANSMITTER RECEPTORS IN THE RAT CORPUS STRIATUM. T.D.Reisine, J.I.Nagy, H.C.Fibiger and H.I.Yamamura. Univ. of Arizona, Col. of Med., Tucson, AZ 85724 and Division of Neurosci., Univ. of Brit. Col., Vancouver B.C. Canada

Presynaptic neurotransmitter receptors have been hypothesized to be localized on dopaminergic (DA) nigral-striatal pathways (NSP). Neurotransmitter receptor binding assays in rats injected with 6-hydroxydopamine (6-OHDA)(into the lateral hypothalamus which destroys the NSP)may help to elucidate the existence of these presynaptic receptors. Dopaminergic (^{3}H-spiroperidol), muscarinic cholinergic (^{3}H-quinuclidinyl benzilate,QNB) and beta-adrenergic (^{3}H-dihydroalprenolol, DHA) receptor binding assays were done on the corpus striatum(CS) and substantia nigra (SN) of 30 day 6-OHDA treated rats. Tyrosine hydroxylase activity and dopamine levels in the CS were dramatically reduced in these animals (>90%). In the CS of these treated rats, we found a significant 20-30% increase in the Bmax of ^{3}H-spiroperidol binding with no alterations in ^{3}H-QNB binding. There was,however, a significant 30% decrease in the Bmax of ^{3}H-DHA binding within the CS of these treated rats. There was also a significant 40% decrease in ^{3}H-spiroperidol binding within the SN of 6-OHDA treated rats. These data indicate, a denervation supersensitivity of postsynaptic DA receptors within the CS, a lack of presynaptic muscarinic cholinergic receptors on the NSP and the possible existence of beta-adrenergic receptors on the NSP. The decrease in ^{3}H-spiroperidol binding within the SN of 6-OHDA treated rats indicates the existence of DA receptors on DA perikarya or dendrites. Supported by USPHS grants and RCDA.

1663 RECEPTORS APOUND VERTEBRAE IN THE CAT NECK. F.J.R. Richmond, D.A. Lakanen and V.C. Abrahams. Department of Physiology, Queen's University, Kingston, Ontario, Canada. K7L 3N6.

A variety of neck reflexes and clinical disorders have been thought to involve "joint" receptors of upper cervical vertebrae, but the morphology and distribution of receptors which might sense vertebral position have not been described. To provide this information, serial sections were made through whole decalcified vertebrae whose muscle and connective tissue attachments were preserved. The muscles, interspinalis,transversospinalis and intertransversarii which have both their origins and insertions on vertebral processes were reconstructed and their receptor contents were mapped. Spindles were particularly numerous in perivertebral muscles, where they commonly occurred in large chain-like complexes of several spindles extending for almost the whole muscle length. In these complexes up to 5 spindles may be seen in juxtaposition at a single level of section. Occasional spindles formed parallel conjunctions or included an extrafusal fibre within their capsules.

Golgi tendon organs were common at musculotendinous junctions, where they were often associated with spindles and/or small pacinform corpuscles. No Ruffini corpuscles were seen but this negative result may relate to the difficulty in distinguishing this receptor in transverse sections. Numerous small nerves coursed within loose connective tissue particularly near blood vessels. However nerves were not observed in highly structured tendon or synovial linings. The obvious receptors of the neck were thus not joint receptors but muscle receptors in perivertebral muscles around vertebral articulations.

Supported by M.R.C. of Canada

1664 BENZODIAZEPINE RECEPTOR BINDING IN 'EMOTIONAL' AND 'NON-EMOTIONAL' RATS: A BIOLOGICAL BASIS FOR ANXIETY?
Harold A. Robertson*, John C. Candy* and Ian L. Martin*
Univ. Lab. of Physiol., Oxford and MRC Unit of Neuropharmacology, Med. Sch., Birmingham, England. (SPON: R.A. Leslie)

The presence of brain-specific benzodiazepine receptors is now established and there is a significant correlation between the affinities of various benzodiazepines for the receptor in rat brain and the anxiolytic efficacy of these benzodiazepines. We have examined benzodiazepine receptor binding in 9 regions of the CNS of 2 inbred strains of rats, the Maudsley Reactive (MR) and Maudsley Non-Reactive (MNR) strains. These strains have been selectively bred for high (MR) and low (MNR) fearfulness and there is much support for the contention that they represent extremes of emotional behaviour. Measurement of specific ^{3}H-diazepam binding (at 2nM ^{3}H-diazepam concentration) revealed a differential regional distribution of binding sites with highest binding in the cortex and lowest in the thoracic spinal cord.

In every brain region examined, the NMR rats showed higher specific benzodiazepine binding than the emotional MR animals although this difference was only statistically significant in the hippocampus, hypothalamus, mid-brain, medulla-pons and thoracic spinal cord. There was no significant difference in benzodiazepine binding between the 2 strains in cortex, entorhinal cortex, striatum or cerebellum. Scatchard analysis of binding activity in hippocampus of the 2 strains indicates that the increased binding seen in the MNR rats is due to an increase in the number of binding sites rather than a change in the affinity of the receptor for the ligand.

There would now appear to be reasonable evidence that the anxiety-lessening effects of the benzodiazepines are due to the interaction of benzodiazepines with the benzodiazepine receptor. Our finding of a difference in benzodiazepine receptor binding between 2 strains of rats representing extremes of emotionality may provide a biological basis for an understanding of emotional behaviour based on alterations in the benzodiazepine receptor and, possibly, in changes in an endogenous ligand for this receptor.

(supported by the MRC and The H.F. Guggenheim Foundation)

1665 PUTATIVE HEPATIC SODIUM RECEPTORS ACTIVATE NEURONS IN THE VENTRAL BASAL THALAMUS.
Richard C. Rogers*, Donald Novin, and Larry L. Butcher. Brain Research Institute, UCLA, Los Angeles, CA 90024.

Recently there have been several reports concerning the existence and function of hepatic sodium and/or osmoreceptors. Furthermore, it appears that the major afferent nerve carrying such information to the brain is the vagus, since its resection eliminates the physiological and behavioral effects of hepatic osmotic or ionic stimulation.

Although the central pathways carrying this information are presently unknown, Norgren and Leonard (J. comp. Neurol. 166: 17, 1976) hypothesized that taste pathways and visceral afferent pathways share the same anatomical structures, which include the n. tractus solitarius, parabrachial nucleus, and ventral basal thalamus.

Our most recent experiments provide information concerning both the afferent hepatic pathway and the transduction mechanism involved in hepatic-afferent, sodium ion sensitivity.

Both the portal vein and vena cava of rats were cannulated and small volumes, 0.1-0.2 ml, of either water or twice-isomotic NaCl, choline chloride, mannitol, or sucrose was infused simultaneously such that the ionic/osmotic challange was limited to the hepatic circulation. Cells in the anterior ventro-basal complex responded to hepatic infusions of NaCl or choline but not to mannitol or sucrose. Given that the electrogenic Na+-K+ pump is inhibited by both Na+ and choline, this finding lends support to the view that some chemoreceptors may involve the electrogenic cation pump.

Following the electrical recording experiments small amounts of horseradish peroxidase were iontophoresed through the glass recording electrode. With restricted injections of peroxidase (halo diameter < 100 μm) retrogradely labeled neurons appear only in the parabrachial nucleus, a finding that supports the hypothesis of Norgren and Leonard (above ref.). [This research supported by USPHS grants NS 7687 to DN and NS 10928 to LLB]

1666 HIGH AFFINITY DIMETHYLTRYPTAMINE BINDING TO RAT BRAIN MEMBRANES.
Helen Rosengarten* and Arnold J. Friedhoff. Millhauser Labs, Dept. Psychiatry, NYU Medical Center, New York, NY 10016.

Postsynaptic membranes of rat brain were found to bind dimethyltryptamine. Binding of ^{3}H-DMT to a Whittaker membrane preparation was relatively rapid, saturable, reversible, and did not chemically alter the ligand. Scatchard plots revealed both high and low affinity binding sites for DMT. In this report we describe detailed properties of the binding of ^{3}H-DMT to membranes of rat brain.

1667 CHARACTERIZATION OF BRAIN MEMBRANE BINDING SITES FOR [^{125}I]$_2$-α-BUNGAROTOXIN AND [^{3}H]-QUINUCLIDINYL BENZILATE.
Paul M. Salvaterra and Dee Ann Matthews. Div. Neurosci., City of Hope Nat'l. Med. Ctr., Duarte, CA 91010.

As a preliminary step in designing effective experimental protocols for isolation of purified synaptic membranes we have studied the effects of various physical, chemical and enzymatic treatments on the particulate binding sites for [^{125}I]$_2$-α-bungarotoxin (α-BuTX) and [^{3}H]-Quinuclidinyl benzilate (QNB).

Membranes were prepared from rat forebrains by a modification of a combined floatation-sedimentation sucrose density gradient procedure (Jones and Matus, Biochem. Biophys. Acta 356:276, 1974) using a fixed angle rotor. This procedure yields a synaptic plasma membrane enriched fraction as judged by electron microscopy and enzymatic activity (5-7 fold enrichment of Na^+ - K^+ATPase). The specific activity of α-BuTX and QNB binding was enriched 3-4 fold over a total particulate fraction with a 15-25% yield of the binding sites.

Treatment of the membranes with reducing agents such as β-mercaptoethanol and dithiothreitol (DTT) (10^{-2}-10^{-4}M) had little effect on QNB binding. β-mercaptoethanol had only a slight inhibitory effect on α-BuTX binding while DTT treatment showed a dramatic increase in toxin binding possibly due to intermolecular crosslinking of the toxin to membrane proteins. Chelating agents (EDTA and EGTA) show only a slight inhibitory effect on binding at concentrations up to 10^{-2}M. The alkylating agent N-ethylmaleimide shows no effect when used alone but results in a complete loss of binding when used after prior reduction of the membranes with β-mercaptoethanol. Both binding sites are resistant to treatment of the membranes with trypsin, bacterial protease, and DNAse I. Treatment of the membranes with certain phospholipases results in a marked reduction in detectable binding sites. The effects of one phospholipase, β-bungarotoxin were studied in more detail. The decrease in binding due to this protein may not be explained entirely by its enzymatic activity since similar inhibitory effects were seen when the membranes were treated under conditions where it should be enzymatically inactive (ie: no Ca^{+2}, replacement of Ca^{+2} with Sr^{+2}, addition of EGTA to the incubation mixture). Membranes could be prepared at room temperature or 4° with little effect on the binding. Nearly identical results were also obtained when membranes were subjected to several freeze thaw cycles or sheared in a polytron rather than handled with more gentle homogenization techniques. (Supported by NIH 1 RO1 NS13813-01).

1668 THE USE OF FLUOXETINE IN THE BINDING ASSAY FOR SEROTONIN RECEPTORS. Daniel D. Savage*, Alan Frazer* and Joe Mendels* (SPON: B. Weiss). Dept. Pharmacol., Univ. of Penna. and Veterans Administration Hospital, Phila., Pa. 19104.

Because of the possibility of abnormalities in central serotonergic activity in affective disorders, it was of interest to study the effects of antidepressant treatment on 5-hydroxytryptamine (serotonin) receptors using the receptor binding assay developed by Bennett and Snyder, (Mol. Pharm. 12:373, 1976). It was noted that when crude mitochondrial fractions prepared from rat cerebral cortex were incubated for 15 min. at 30° with concentrations of ^{3}H-serotonin (^{3}H-5HT) up to 40nM, saturation of specific binding did not occur. Analysis of these data by the method of Scatchard indicated two populations of binding sites. The apparent binding constant (K_D) and maximum specific binding capacity (V_{max}) of the higher affinity site were 7.0 ± 0.25nM and 178 ± 14fmole/mg protein respectively, (N=4). For the lower affinity site, the K_D was 38 ± 4.2nM and the V_{max} was 453 ± 25fmoles/mg protein, (N=4).

The effect of fluoxetine, a specific serotonin uptake inhibitor, on ^{3}H-5HT binding was examined to investigate whether the lower affinity site was some form of a binding site for serotonin uptake. Fluoxetine in concentrations up to 0.5µM, produced no inhibition of binding of 5nM ^{3}H-5HT. However, when this concentration of fluoxetine was used in studies measuring the binding of ^{3}H-5HT at concentrations up to 40nM, only a single population of binding sites was obtained. The K_D and V_{max} of this binding site were 7.7 ± 0.35nM and 170 ± 8fmole/mg protein, values not significantly different from those of the higher affinity site measured in the absence of fluoxetine. When binding analyses were performed at <1°, only a single population of higher affinity sites were present, whether measured in the absence or presence of fluoxetine.

These data indicate that fluoxetine prevents binding of ^{3}H-5HT to a lower affinity population of binding sites, the characteristics of which are under investigation. The binding sites for ^{3}H-5HT measured in the presence of fluoxetine exhibit the selectivity characteristic of serotonin receptors as evidenced by the order of potency of the following drugs in displacing ^{3}H-5HT: serotonin>d-LSD>5-methoxytryptamine>>tryptamine>methysergide> cyproheptadine>>5-hydroxytryptophan, phentolamine, haloperidol, dopamine, norepinephrine, isoproterenol. (Supported by Research Funds from the Veterans Administration, NIMH Grant MH29094, and USPHSGM 07302).

1669 ELECTROPHYSIOLOGIC EVIDENCE THAT RETINOTECTAL TRANSMISSION IN THE GOLDFISH IS NICOTINIC CHOLINERGIC John T. Schmidt and Robert E. Oswald*, Depts. of Anatomy and Biochemistry, Vanderbilt Univ. School of Medicine, Nashville, TN 37232

Last year's report (Abst.#276) demonstrated three layers of optic input to the goldfish tectum using the anatomical technique of cobalt filling: the superficial gray and white layer (with superficial satellite band), the middle of the central gray layer, and the deep white layer. These correspond to three classes of optic fibers by conduction velocity: two faster ones in the dense superficial band and the slowest in the two sparse deeper bands. Postsynaptic components elicited by optic nerve stimulation were analyzed via the current source density technique: field potentials were recorded at depth intervals of 25µm, averaged on a PDP-12 computer, and their second differences were computed to localize the depths of the sources and sinks of synaptic current. Using this technique, the effects of various nicotinic agents on retinotectal transmission were studied. Alpha-bungarotoxin, when topically applied in Ringers, had no effect even at 10^{-5}M; but when injected into the tectal layers via a micropipette (low pressure, volumetric, with tip size <10µm), it quickly and irreversibly blocked all three components. Control injections of Ringers had very little or no effect on the waveforms or sources and sinks with low pressures (<180mm Hg). Since this system allowed local treatment and did not saturate the tissue, it was used with other nicotinic agents where a return to control was necessary. Acetylcholine (10^{-4}M) greatly diminished the size of all three components of the response. BW284C51 (10^{-4}M), an anticholinesterase, both diminished the size of all three components and prolonged their time course. Curare (10^{-3}M) greatly reduced or abolished the faster two components, but enhanced and greatly prolonged the time course of the slower deeper component. This may reflect either anticholinesterase activity or the elimination of an inhibitory intratectal circuit from the faster optic components. Carbamylcholine (10^{-3}M), an agonist, severly decremented all responses, moreso than acetylcholine but presumably via the same mechanism of receptor desensitization. Atropine (10^{-3}M), a muscarinic antagonist, had no apparent effect at all. The responses are being further tested for sensitivity to other nicotinic agents.

1670 STUDIES OF RECEPTOR BINDING AND CYCLIC AMP SYNTHESIS IN THE BRAINS OF YOUNG AND OLD HUMANS POSTMORTEM. M. J. Schmidt, B. Ghetti, A. Maggi*, and S. J. Enna. Eli Lilly and Company, Indiana Univ. Med. Cntr. and The Univ. of Texas Medical School at Houston.

These experiments were designed to assess the feasibility of using human brain tissue obtained at autopsy for neurochemical studies of drug-receptor interaction. The frontal pole of the cerebral cortex and the lateral pole of the cerebellum were removed from humans of various ages 6-16 hrs after death. Neurohormone-stimulated cAMP accumulation was measured in tissue slices. ^{3}H-Dihydroalprenolol binding, adenylate cyclase assays, and estimates of metabolic intermediates were run on tissue homogenates.

Norepinephrine increased cAMP levels in cortical slices from the brains of neonates 2 day - 2 year of age. Most tissues from older individuals were unresponsive to norepinephrine, histamine or adenosine. cAMP concentrations in the cerebellum were low and no hormone-induced rise in cAMP was detected in any sample at any age.

^{3}H-Dihydroalprenolol bound to membrane fragments from all samples of the cerebral cortex and cerebellum of all samples. There was no difference across age in the affinity constant or number of binding sites in the cerebral cortex, but a significant decline in binding was observed in cerebellar tissue from individuals over 60 yrs. of age. The age-related decline was due to a reduction in the number of binding sites (80 vs. 40 femtomoles/mg prot.) with no change in the affinity constant (1.5 nM vs. 1.4 nM).

Experiments with rats indicated that norepinephrine could stimulate cAMP synthesis as long as 16 hrs after death if the animals were cooled within 1 hr after death. However, elevated body temperatures (36° C) for 3 hrs postmortem, drastically reduced cAMP accumulation. Determination of brain temperature after death revealed that only in the case of the very young would rapid cooling of the brain take place in the clinical setting.

These experiments indicate that ligand binding studies can be conducted on human brain tissue obtained at autopsy. However, components of the cAMP generating system appear to be temperature sensitive postmortem. Studies in progress are examining what defects might account for the malfunction (e.g., adenylate cyclase activity, insufficient ATP concentrations, lowered metabolic conversion of ATP precursors). The data also show an age-related decline in beta adrenergic receptor density in the cerebellum but not cerebral cortex of humans.

1671 DIFFERENCES BETWEEN CURRENT AND VOLTAGE RESPONSES OF TOAD RODS. Julie L. Schnapf* and Robert N. McBurney. LNP, NINCDS, NIH, Bethesda, Md. 20014

Vertebrate photoreceptors respond to light with membrane hyperpolarization. A striking characteristic of these responses is that for bright flashes an initial hyperpolarization decays to a plateau level which is maintained for several seconds. It has been suggested that this transient reflects a transient in the kinetics of the phototransduction process in the outer segment. To test this hypothesis we have compared both outer segment current and intracellular voltage recorded from toad rods (Bufo marinus). A slice of isolated retina was placed in a superfusion chamber. A single rod outer segment was drawn into a glass pipette filled with toad Ringer. The pipette had a resistance of 1-3 MΩ which rose to as much as 10 MΩ with a rod in place. Current was recorded as the voltage output of a virtual-ground current-to-voltage converter connected between the inside of the electrode and the solution bathing the retina. Intracellular voltage changes were measured in an identical preparation except that a single rod was now impaled with a microelectrode (resistance 100-400 MΩ).

Membrane potential responses to bright stimuli in this preparation displayed the initial transient hyperpolarization and plateau phase typical of similar measurements by other investigators. Surprisingly, no such transient was seen in membrane current responses. However, current responses to bright stimuli did have a plateau phase that was maintained for several seconds. We also observed that the peak of the current response saturated at a lower intensity than did peak voltage.

We suggest that the peak component of the voltage response is probably a result not of the kinetics of the transduction process but a second process located proximally to the outer segment. Furthermore, a comparison of the time course of current and voltage responses to dim stimuli indicates that the nonlinear current-voltage characteristics of this second process operates on signals as small as the response to a single photon.

1672 SELECTIVE BLOCKADE OF HYPOTHALAMIC CHOLINERGIC PATHWAYS BY ANTIBODY TO G_{M1} GANGLIOSIDE. Nicole Schupf and Curtis A.Williams Dept.Psychology,Manhattanville Coll.and Div.Nat.Sci.SUNY College at Purchase,N.Y. 10577

Previous investigations of the functional activity of an anti-ganglioside antibody(aGS)have shown EEG abnormalities and inhibition of a passive avoidance task to be induced in rats following intracranial administration of aGS.(Karpiac et al,Science 194:735,1977).Karpiac et al(Neurosci.Abstr.739,1977)demonstrated that this neuroactivity was eliminated by absorption with G_{M1} ganglioside. We have found that focal intrahypothalamic injection of aGS(donated by Dr.M.Rapport)causes depression of drinking in 23-hour water deprived rats, identical to the effect we reported for an anti-rat brain microsomal membrane(aRB)antibody(Williams and Schupf,Science 196:328, 1977). A discriminating assay system, pharmacological induction of food and water intake, demonstrated that the activity of aRB appeared to be selective for inhibitory hypothalamic neurons mediating adrenergic-cholinergic antagonism. This test was employed to determine the functional activity of aGS.

Rats were prepared with cannulae in the perifornical hypothalamus, a region where direct chemical stimulation of sated animals with the alpha-adrenergic agonist norepinephrine(NE)will elicit excessive eating and stimulation with the cholinergic agonist carbamyl choline(CC)will elicit excessive drinking. Treatment with aGS(2ng Ig in 1 ul) 6 hours before drug stimulation depressed drinking in CC-stimulated rats but produced no change in appetitive behavior in either NE-stimulated or saline control rats. The activity of the antibody was eliminated by absorption with G_{M1} ganglioside. These results suggest that the neuroactivity of aGS in this brain region is best defined by blockade of cholinergic neurons. This discrete activity contrasts with that reported for aRB.

While G_{M1} ganglioside is distributed widely on cell membranes in the brain and other tissues, we conclude that it has a close functional relationship either to the ACh receptor itself or to some other gating structure on cholinergic neurones.

1673 PRE-SYNAPTIC DOPAMINE RECEPTORS LABELED BY ^{3}H-APOMORPHINE. P. Seeman and J. Tedesco, Department of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8

Previous work[1] has shown that ^{3}H-apomorphine (at 3 nM) prefers presynaptic dopamine receptors over post-synaptic dopamine receptors in the caudate, since 6-hydroxydopamine lesions of the nigrostriatal fibers caused a 56% fall in the binding of ^{3}H-apomorphine, but a 22% rise in the binding of ^{3}H-haloperidol. Intrastriatal injections of kainic acid, moreover, had no effect on the striatal binding of ^{3}H-apomorphine but did lower the binding of ^{3}H-spiperone, supporting the theory that ^{3}H-apomorphine favours attachment to pre-synaptic dopamine sites, while ^{3}H-spiperone prefers post-synaptic dopamine receptors[2].

To test further the hypothesis that ^{3}H-apomorphine (at 3 nM) prefers pre-synaptic dopamine receptors, we tested a number of dopamine-mimetic compounds for their ability to compete with ^{3}H-apomorphine binding; we then compared these competing concentrations with those which are known to act on peripheral pre-synaptic dopamine receptors[3-5]. The concentrations which 50% inhibited the specific binding of ^{3}H-apomorphine to calf caudate homogenates are shown in the Fig. in correlation to the concentrations which 50% inhibit the cardioacceleration responses[3-5].

The results are thus compatible with the theory that ^{3}H-apomorphine (at 3 nM) may bind to high affinity pre-synaptic sites for dopamine.

(Supported by MRC of Canada and the Ontario Mental Health Foundation).

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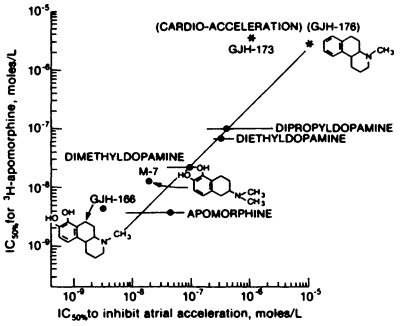

1674 IMMUNOCHEMICAL PROPERTIES OF SEROTONIN-BINDING PROTEINS. Jean C. Shih and Helen Young*. School of Pharmacy, University of Southern California, Los Angeles, California 90033.

By affinity column chromatography, two serotonin-binding proteins have been isolated from steer hypothalamus. One of them was eluted from the column by chlorimipramine (SBP-CIP), the other one was eluted by LSD (SBP-LSD). Recently, antibody has been developed against each protein. They are immunochemically identical.

When antiserum-anti-SBP-CIP (or antiserum anti-SBP-LSD) was reacted with rat brain synaptosmal membranes, one immunoprecipitin line was observed and it was immunochemically identical to the purified SBP from steer hypothalamus. Furthermore, both antibodies inhibited specific serotonin-binding to a similar degree.

1675 MUSCARINIC ACETYLCHOLINE RECEPTOR REGULATION IN CULTURES OF EMBRYONIC CHICK BRAIN. Robert G. Siman* and William L. Klein* (SPON: Virginia Carr). Dept. of Biological Sciences, Northwestern Univ., Evanston, Illinois, 60201.

Homogenates of chick embryo cerebral lobes specifically bind 3H-QNB to muscarinic ACh receptors. Pharmacological characteristics of the binding are similar to those reported elsewhere. Aggregate cell cultures of cerebral lobes develop muscarinic ACh receptors as monitored by 3H-QNB binding. Cultures prepared from 8 day old chick embryos and maintained for 7 days have about 100 fmol receptors/mg protein. These cultures can be used to study factors which regulate receptor density.

Muscarinic ACh receptor activation by addition of agonist to culture medium causes a subsequent reduction in muscarinic ACh receptor density. Cells activated for 24 hours with carbachol show a dose-dependent loss of muscarinic ACh receptors which closely resembles the receptor occupancy profile for carbachol. A saturating dose (10^{-4}M carbachol) lowers receptor density by 75%. A half-maximal reduction in receptor density is induced by 10^{-6}M carbachol. The regulation is muscarinic ACh receptor mediated and requires receptor activation and not simple occupancy. Thus 10^{-6}M oxotremorine induces a receptor loss which is blocked by 10^{-8}M atropine. Maximal receptor loss occurs after 9 hours exposure to agonist, while half the effect occurs by 1.5 hours. Receptor density returns to control level 48 hours after agonist withdrawal, indicating that receptor reduction is not due to loss of cholinoceptive cells. Our results suggest that regulation of muscarinic ACh receptors may be a mechanism for CNS synaptic plasticity.

Supported by the American Epilepsy Foundation.

1676 INHIBITORY EFFECTS OF SOME TRICYCLIC ANTIDEPRESSANTS AND IMIDAZOLINE DRUGS ON THE PINEAL α-ADRENERGIC RECEPTOR-MEDIATED STIMULATION OF PHOSPHOLIPID METABOLISM. Thomas L. Smith* and George Hauser, Ralph Lowell Labs., McLean Hospital, Belmont, MA 02178 and Harvard Medical School, Boston, MA 02115.

Norepinephrine (NE) *in vitro* selectively stimulates the incorporation of ^{32}P into phospholipids of the rat pineal gland. The most prominent stimulation occurs in phosphatidylinositol, while modest increases are seen in phosphatidic acid and phosphatidylglycerol. Phosphatidylcholine and phosphatidylethanolamine are not affected. This increased incorporation of ^{32}P by NE has been shown to be blocked by the highly specific α-adrenergic antagonist WB4101, as well as by classical antagonists such as phenoxybenzamine, phentolamine, and dihydroergotamine, whereas sotalol, a β-adrenergic antagonist, has no effect. A variety of α-agonists also enhance ^{32}P labeling of phospholipids producing a labeling pattern similar to that seen with NE. It thus appears that the increased rate of metabolism of certain phospholipids in the rat pineal gland is mediated through activation of α-adrenergic receptors. Pineals incubated with 10 μM NE plus 10 μM cocaine, an inhibitor of presynaptic NE reuptake, exhibit no greater stimulation than with NE alone. Thus, the NE stimulation of phospholipid metabolism in this system is not affected by presynaptic reuptake of NE under the conditions used.

One μM concentrations of the tricyclic antidepressants, amitriptyline, doxepin, imipramine, desipramine, and protriptyline inhibit completely the stimulation of ^{32}P incorporation elicited by 5 μM NE. Amitriptyline (1 μM) displaces the dose response curve for NE to the right, in a parallel manner, indicating a competitive type of inhibition with a K_D for amitriptyline of about 1 μM. Incubations of pineal glands with 1.5 μM tricyclic antidepressants plus 10 μM NE yield a rank order of inhibitory potency tertiary amines > secondary amines, similar to that seen in other α-adrenergic systems. Among the imidazolines, clonidine, oxymetazoline, naphazoline, xylometazoline, and tolazoline antagonized the stimulation caused by 10 μM NE. Clonidine also possesses partial agonist activity. Although many of the tricyclic and imidazoline drugs have strong local anesthetic properties which can affect labeling of membrane phospholipids in a characteristic manner, these effects were not observed at the concentrations tested. Since some of the imidazolines have been reported to act as pineal presynaptic α-agonists, their action inhibiting increased ^{32}P incorporation into phospholipids occurs probably at a postsynaptic α-receptor locus. (Supported by USPHS grant NS06399 from the National Institutes of Health.)

1677 HETEROLYTIC CLEAVAGE OF A CRITICAL DISULFIDE BOND ON THE CHOLINERGIC RECEPTOR. A. Steinacker. Rutgers Med. Sch., Piscataway, NJ 08854.

A modification of the cholinergic receptor has been produced by heterolytic cleavage of a critical disulfide bond on the receptor molecule. Strict reduction of this bond adjacent to the binding site for acetylcholine has been shown to reduce the post synaptic response to acetylcholine and specific alkylating and oxidizing agents have been used as affinity labels for the bond (Karlin, Fed. Proc. 32, 1973). Using sodium bisulfite and the frog neuromuscular junction and hatchetfish Mauthner fiber-giant fiber synapse, I have produced a heterolytic cleavage of a disulfide bond which, unlike the reduction, produces an increased response to acetylcholine. No change in time course or input impedance is produced. The affinity labels used following reducing agents are also active following heterolytic cleavage indicating that the same disulfide bond is involved. The heterolytic cleavage results in the addition of a strongly nucleophilic sulfonate to a site near the cholinergic binding site and increased current flow through subsynaptic ionic channels. This aspect will now be investigated at the single channel level.

There is also a presynaptic effect of this heterolytic cleavage which is manifested as an increase in the frequency of miniature end plate potentials (mepp's) and a decrease in the quantal content. The presynaptic response to modification of this disulfide bond has not been investigated previously sine the reducing agents used decreased the cholinergic response and mepps could not be seen. The most likely hypothesis for the increased frequency (up to 200/sec) and reduced quantal content is action on a presynaptic cholinergic receptor. There is no change in resting potential postsynaptically in either preparation and in the hatchetfish where it is possible to record presynaptically, no change in resting potential is seen. The response is not dependent on external calcium and does not appear to include mitochondrial involvement. The same affinity agents which act on the post synaptic receptor molecule also act presynaptically on the mepp frequency. Both these pre and post synaptic effects can be blocked by prior treatment with physostigmine or acetylcholine. The increased acetylcholine binds to the receptor preventing access to the disulfide bond by the sulfite ion. This data may provide evidence for an activation of a presynaptic cholinergic receptor mediating feedback control of neurotransmitter. (Supported by the General Research Support Grant of Rutgers Medical School.)

1678 VASOACTIVE INTESTINAL POLYPEPTIDE: SPECIFIC BINDING BY RAT BRAIN MEMBRANES. Duncan P. Taylor* and Candace B. Pert. Biological Psychiatry Branch, NIMH, Bethesda, MD 20014.

Immunohistochemical localization of vasoactive intestinal polypeptide (VIP) in central as well as peripheral nerve systems led us to search for specific binding of VIP by brain membranes. High specific activity [^{125}I]VIP was prepared and incubated with once-washed brain membranes in the presence or absence of 10^{-7} M unlabeled VIP. Bound VIP was separated from free by centrifugation of membranes through 10% sucrose. Membranes obtained from cerebral hemispheres, thalamus, and hypothalamus bound VIP specifically. Specific binding of VIP represented 40-50% of total binding. Specific VIP binding was reversible and saturable (B_{max} = 8 fmole/mg tissue). Brain membranes exhibited a high affinity for VIP (K_D = 3 nM) and a Hill plot indicated no cooperativity of binding. Binding was maximal at 37°C and 20 minutes. Millimolar concentrations of calcium, magnesium and manganese cations enhanced binding, while sodium ions decreased binding. Bacitracin and aprotinin preserved binding, presumably by competing with endogenous proteases, while soybean trypsin inhibitor was not effective. Binding of VIP paralleled its immunohistochemical localization, being enriched in cerebral cortex, hippocampus, hypothalmus, striatum and thalamus. Secretin and partial sequences of VIP competed for binding to brain membranes with an order of potency similar to that obtained in binding studies of the VIP receptor found in pancreatic acinar cells (Christophe et al., J. Biol. Chem., 251: 4629, 1976). These characteristics suggest that this binding is associated with a physiologically relevant VIP receptor. (D.P.T. is a NIDA NRSA Fellow.)

1679 PRE-SYNAPTIC DOPAMINE RECEPTOR BINDING: INVOLVEMENT OF THE NITROGEN LONE ELECTRON PAIR. J.L. Tedesco and P. Seeman. Pharmacology Department, University of Toronto, Toronto, Canada.

We have previously reported that ^{3}H-apomorphine receptors in calf striatum crude homogenate (Seeman et al., P.N.A.S. 73, 4354, 1976; Seeman et al., Fed. Proc. 37, 130, 1978) are identical in all respects to high affinity ^{3}H-dopamine receptors. In the current study we have determined that the lone electron pair of the tertiary amine nitrogen of apomorphine is necessary in ligand-receptor binding.

Dopamine itself has an IC_{50} of 2 nM when competing for binding to ^{3}H-apomorphine receptors. Secondary and tertiary amine analogues of dopamine retain high to moderate affinities even when N-propylated. Thus, N,N-dimethyl- ,N,N-diethyl- , N,N-dipropyl-, and N,N-dibutyl-dopamine have IC_{50} values of 10.5, 55, 74 and 625 nM, respectively. The secondary amine analogues N-methyl- , N-ethyl- , and N-propyl-dopamine have IC_{50} values respectively of 2.5, 25 and 215 nM. In contrast the quaternary amine analogue N,N,N-trimethyl-dopamine has a very low affinity (IC_{50} = 1200 nM). This compound lacks a nitrogen lone electron pair. Similarly, while R-(-)-apomorphine and R(-)-N-n-propyl-norapomorphine have high affinities, with respective IC_{50} values of 3.8 and 4 nM, the quaternary amine analogue N-methyl-apomorphine has an IC_{50} value of 710 nM.

We suggest that the position-vector for the amine lone pair is a more critical factor than the actual position of the nitrogen atom in determining potency. All previous studies have stressed the latter. It is however not a trivial problem to separate these two variables. We have tested an apomorphine analogue (Berney et al., Experientia 31, 1327, 1975) which is identical to R-(-)-apomorphine save that the nitrogen is part of a 7-membered ring instead of a six-membered ring. It is inactive in binding at 10,000 nM. However, crystallography indicates that the nitrogen atom and its lone pair have both altered in position. Again, S-(+)-apomorphine has a low affinity, with an IC_{50} of 325 nM for the ^{3}H-apomorphine receptor, but while its amine lone pair is pointing in a direction opposite to that in R-(-)-apomorphine, its nitrogen atom is also about 1.4 Å removed from its position in R-(-)-apomorphine relative to the plane of the catechol ring.

The involvement of the tertiary amine lone pair of opiates in binding to their receptor has been elegantly demonstrated (Belleau et al., J. Med. Chem. 17(8), 907, 1974). A similar demonstration for dopamine analogues would provide a fundamental insight into the nature of the dopamine binding-site. (Supported by the Ontario Mental Health Foundation and MRC of Canada).

1680 PRE- AND POST-SYNAPTIC CATECHOLAMINE RECEPTORS IN CALF CAUDATE. M. Titeler and P. Seeman, Department of Pharmacology, University of Toronto, Toronto, CANADA M5S 1A8.

This study was designed to improve the selective labelling of alpha-adrenoceptors by ^{3}H-dihydroergocryptine (^{3}H-DHEC) in calf caudate homogenates, since it is known that ^{3}H-DHEC can also bind to dopamine receptors[1]. In the presence of 5 nM spiperone, which served to block dopamine receptors, the specific binding of ^{3}H-DHEC was inhibited by 90 nM epinephrine (K_i values), 260 nM (-)-norepinephrine, and 1000 nM dopamine, indicating that ^{3}H-DHEC was selectively binding to brain alpha-adrenoceptors under these conditions. These numbers agree with those for ^{3}H-WB-4101, published by others[2].

An important problem, however, is that these inhibitory concentrations for ^{3}H-DHEC do not match those which inhibit the binding of ^{3}H-clonidine or ^{3}H-noradrenaline. A simple and straightforward resolution of this dilemma would be to propose that ^{3}H-DHEC and ^{3}H-WB-4101 label the post-synaptic alpha-receptors, while ^{3}H-clonidine and ^{3}H-noradrenaline label the pre-synaptic autoreceptors. This suggestion is compatible with the data shown below indicating that the stimulated release of ^{3}H-norepinephrine and the specific binding of ^{3}H-clonidine binding are both inhibited by almost identical concentrations (or K_i values) of the drugs listed.

	EC_{50}% to block overflow[3,4]	^{3}H-Clonidine[2] IC_{50} (25°) Rat Brain	^{3}H-DHEC[1] K_i (25°) Calf Caudate
clonidine	16 nM	10 nM	40 nM
(-)-norepinephrine	30 nM	29 nM	260 nM
phentolamine	50 nM	37 nM	6 nM
phenoxybenzamine	100 nM	102 nM	12 nM

This proposal is analogous to that for dopamine receptors, wherein we suggest that 1-2 nM ^{3}H-neuroleptic labels post-synaptic sites, while 2-4 nM ^{3}H-apomorphine or ^{3}H-dopamine primarily label dopamine autoreceptors (in press).
(Supported by the MRC of Canada, the Ontario Mental Health Foundation, and the W. Garfield Weston Foundation).

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1681 HISTAMINE H_1 RECEPTOR IDENTIFIED IN MAMMALIAN BRAIN WITH [^{3}H]-MEPYRAMINE. Vinh T. Tran, Raymond S.L. Chang and Solomon H. Snyder. Dept. Pharmacol., Johns Hopkins Sch. Med., Baltimore, MD 21205.

Histamine H_1 receptors have been labeled in mammalian brain with [^{3}H]mepyramine. Binding is saturable, with a dissociation constant of 4 nM and a maximal number of binding sites of 11 pmoles/g tissue. [^{3}H]Mepyramine binds reversibly to the receptor; binding occurs rapidly at 25° C with a $t_{1/2}$ (assoc.) = 2 min, and the bound ligand dissociates from the receptor with a half-life of 2.5 min. [^{3}H]Mepyramine binding is selectively blocked by classical H_1-antagonists with high affinity and stereoselectively, with d-chlorpheniramine being 100 times more potent than its l-isomer, while H_2 antagonists have no effect. The affinities of various H_1 antagonists at the histamine receptor correlate closely with their potencies in reversing histamine stimulated guinea pig ileal contraction. Tricyclic antidepressants block [^{3}H]mepyramine binding potently with K_i values for doxepin, amitriptyline, imipramine and nortriptyline of 0.7 nM, 3 nM, 20 nM and 20 nM respectively. Regional variations of binding do not parallel the distribution of endogenous histamine.

1682 TWO ^{3}H-CLONIDINE α-NORADRENERGIC SITES IN BRAIN WITH DIFFERENTIAL RESPONSE TO 6-HYDROXYDOPAMINE TREATMENT. David C. U'Prichard and Solomon H. Snyder. Dept. Pharmacol., Johns Hopkins Med. Sch., Baltimore, MD 21205.

^{3}H-Clonidine (CLO, 26.7 Ci/mmol) binds to α-noradrenergic receptors in rat and bovine cortex in a pharmacologically similar fashion to that previously reported for low S.A. CLO. Binding is 90-95% specific at low CLO concentrations. Saturation experiments indicate CLO binds to two populations of receptors, with K_D values of 0.4 nM (1-2 pmol/g cortex) and 2-4 nM (8-10 pmol/g cortex). These sites can be analyzed separately. Agonists have higher affinity at the high-affinity CLO site, with K_is of 0.5 and 1.7 nM for epinephrine (EPI) and norepinephrine (NE). α-Methyl-NE is two times less potent than NE at the high-affinity CLO site, but five times more potent than NE at the low-affinity CLO site. The rat CNS regional distributions of high- and low-affinity CLO binding differ. High-affinity CLO distribution is very marked, with highest binding in cortex, followed by anterior thalamus, colliculi and hippocampus, and insignificant binding in striatum, midbrain and cerebellum. Low-affinity CLO distribution is much more widespread, with more than 35% of cortical levels found in all areas. I.c.v. 6-OHDA increases the B_{max} of high-affinity CLO binding by 50-100% in cortex, compared to 50% increases for ^{3}H-EPI and ^{3}H-WB-4101 α-receptor binding. This increase is found in all brain areas except pons, where there is a 50-70% decrease in high-affinity CLO binding. I.c.v. 6-OHDA does not alter the B_{max} of low-affinity CLO binding, but increases CLO affinity two-fold at this site. At a single CLO concentration, a reduction in low-affinity CLO binding is most apparent in striatum, midbrain area ventral to the colliculi, and cerebellum. It is suggested that high-affinity CLO binding is to true neuronal postsynaptic α-receptors, and possibly α-receptors on locus coeruleus NE cell bodies, whereas low-affinity CLO binding is to glial receptors and possibly autoreceptors on NE terminals. (Supported by USPHS grant MH-18501 and by grants from the McKnight and Hartford Foundations.)

1683 REGULATION AND LIGAND SPECIFICITY OF DOPAMINE RECEPTORS. Ted Usdin*, Ian Creese and Solomon H. Snyder. Dept. Pharmacol., Johns Hopkins Sch. Med., Baltimore, MD 21205.

Striatal dopamine (DA) receptors activate an adenylate cyclase and have also been characterized by the binding of various radiolabeled ligands. A potent DA agonist, 2-amino-6, 7-hydroxyl-1,2, 3,4-tetrahydronapthalene (ADTN), has recently become available in a tritiated form (3 Ci/mmol). Rat striatal binding of ^{3}H-ADTN is saturable and occurs rapidly at 37°. Binding is stereospecifically displaced by butaclamol with the (+) enantiomer being 4000 times as potent as the (-) form. Among the agonists the potency order for displacing ^{3}H-ADTN is ADTN (IC_{50} = 13 nM) >apomorphine >dopamine (IC_{50} = 400 nM) >norepinephrine = epinephrine >5HT. Among DA antagonists (+)-butaclamol (IC_{50} = 4 nM) >fluphenazine >chlorpromazine >haloperidol >spiroperidol (IC_{50} = 250 nM) >promazine. The drug specificity of this binding site is similar to that of sites labeled by other DA agonists. The DA antagonist ^{3}H-spiroperidol (^{3}H-SPIRO) also labels sites in rat striatum with a drug specificity similar to that of sites labeled by ^{3}H-haloperidol. Although little cortical binding of ^{3}H-haloperidol is detectable, ^{3}H-SPIRO binding in the cortex is 20% of striatal levels. DA (IC_{50} = 2.2 μM) is 20 times more potent than 5HT in displacing striatal ^{3}H-SPIRO binding, however, 5HT (IC_{50} = 4.4 μM) is 50 fold more potent than DA in displacing cortical ^{3}H-SPIRO binding. That ^{3}H-SPIRO labels 5HT receptors is supported by ^{3}H-SPIRO binding in hippocampus which has a large 5HT but no DA innervation, and the high affinity of SPIRO for 5HT receptors labeled by ^{3}H-LSD (IC_{50} = 30 nM). Kainic acid microinjection into the striatum lesions intrinsic neurons removing almost all DA-sensitive adenylate cyclase activity but only 40% of ^{3}H-antagonist binding. However such lesions remove 70% of ^{3}H-apomorphine (^{3}H-APO) binding suggesting that ^{3}H-agonists may label receptors mediating DA stimulated cAMP production. Supporting this hypothesis guanyl nucleotides, which in other receptor-cyclase systems modulate agonist receptor affinity and cyclase activity, decrease ^{3}H-APO and ^{3}H-ADTN binding (GTP IC_{50} = 10 μM). Adenyl nucleotides are inactive. GTP does not alter absolute binding of ^{3}H-SPIRO. Specific DA receptor binding of ^{3}H-agonists in rat striatum is cation dependent with Mn^{++} active at μM, Ca^{++} and Mg^{++} at mM concentrations while monovalent cations Na^+, K^+ and Li^+ only decrease nonspecific binding at high concentrations. Specific DA receptor binding of ^{3}H-SPIRO does not require added cations, but is increased to the same maximal extent by both mono and divalent cations. These anatomical, pharmacological and biochemical differences between agonist and antagonist binding sites and the DA-sensitive adenylate cyclase indicate the existence of two distinct DA receptors.

1684 REDUCTION IN STEREOSPECIFIC BINDING OF ^{3}H-DIHYDROMORPHINE IN CNS REGIONS OF AGED RATS. Beatriz J. Vasquez, Vina R. Spiehler*, Rita B. Messing, Robert A. Jensen, Joe L. Martinez, Jr., and James L. McGaugh. Department of Psychobiology, School of Biological Sciences, University of California, Irvine, CA 92717, U.S.A.

There is extensive evidence that neuroanatomical and neurochemical changes occur in aged humans and infrahumans. Observed decreases in number of neurons and dendritic spines, which in turn means fewer synapses, suggest that aging may result in a deficit in the number of receptors for various neurotransmitters and neuromodulators. To investigate this we measured opiate receptor-ligand interactions in young and old animals.

The animals used were 10 young (5 month old) and 10 aged (24 month old) F344 female rats. They were killed by decapitation and the CNS dissected into 9 regions. Tissues were homogenized in ten volumes of 0.32M sucrose. Aliquots (0.2 ml) were then diluted in 1.8 ml of 0.05M tris buffer, pH 7.4, to determine stereospecific binding of ^{3}H-dihydromorphine (3 nM). Stereospecific binding was defined as the difference in binding in the presence of dextrorphan minus binding in the presence of levorphanol. Protein content was determined by the Lowry method and the binding reported as fmoles/mg protein. Differences in receptor binding with age are shown in the table.

	fmoles/mg protein	
Region	Young	Old
Cerebral hemispheres	61±2.23	51±3.27*
Hippocampus	19±1.43	15±1.11*
Striatum	58±3.74	45±2.33**
Amygdala	37±4.18	35±3.0
Hypothalamus	30±2.27	30±2.75
Diencephalon	14±1.68	15±2.09
Midbrain	24±2.99	22±2.0
Pons medulla	15±1.40	17±1.50
Spinal cord	7±0.38	7±0.86

* p<.05; ** p<.01, t-test. All values are mean±S.E.M.

These results indicate that there is a significant decrease in opiate receptor binding in cerebral hemispheres, hippocampus, and striatum, all forebrain regions. The decrease in opiate binding may be related to the decline in brain and cognitive functioning observed with age. [Supported by UPHS grant MH 12526 (J.L.McG.), AG 00469, MH 05358 (R.A.J.); BNS 76-17370; and the McKnight Foundation].

1685 DECREASED β-ADRENERGIC RESPONSES IN RAT CEREBRAL CORTEX FOLLOWING CHRONIC ESTROGEN TREATMENT. H. Ryan Wagner*, Keith A. Crutcher and James N. Davis. Duke University Medical Center and Durham VA Hospital, Durham, NC 27705.

Estrogen appears to be capable of binding to and altering the activity of noradrenergic neurons in the central nervous system. Since we previously demonstrated that increases in brain noradrenergic activity may be reflected by decreases (subsensitivity) in postsynaptic β-adrenergic receptor-coupled adenylate cyclase sensitivity, we began to study the effects of chronic estrogen exposure on this system. Six-week old female Sprague-Dawley rats were bilaterally ovariectomized. Following recovery (three weeks) all rats were implanted with a silastic pellet containing ethynylestradiol or nothing. Twelve to fourteen days after receiving the pellet, rats were killed and cerebral cortices from each group were removed and pooled. Approximately 1/2 of the tissue from each group was homogenized, made into membranes, and assayed for its ability to bind the β-adrenergic radioligand, [^{3}H] dihydroalprenolol (DHA). The remaining tissue was sliced (260 μM) and measured for its ability to accumulate cyclic 3', 5'-adenosine monophosphate (cAMP) in response to various concentrations of the β-adrenergic agonist, (-) isoproterenol (ISO). Maximum accumulations of cAMP in the presence of 10 μM ISO were reduced in estrogen-treated rats (31 pmoles/mg protein/12 min, control; 23 pmoles/mg protein/12 min, estrogen). The maximum density of [^{3}H] DHA membrane binding sites was also decreased in ovariectomized females exposed to estradiol (145 fmoles/mg protein, control; 119 fmoles/mg protein, estrogen; $p < .001$) with no change in the affinity of the ligand for the receptor (K_D = 3.7 nM, control; K_D = 3.5 nM, estrogen). Preliminary studies in other brain regions showed [^{3}H] DHA binding capacity was also significantly decreased in the striatum, hypothalamus, and olfactory bulb ($p < 0.05$) but not in the cerebellum, hippocampus, thalamus, midbrain, pons, or medulla. These data indicate 1) that β-adrenergically mediated cAMP responses are reduced by high in vivo estrogen levels, 2) that this subsensitivity is mediated at least in part by a decrease in β-adrenergic membrane receptors, and 3) that receptor changes reflect a decrease in maximum receptor number with no change in the affinity of the remaining receptors for the radioligand. Although these data are consistent with an estrogen-induced increase in noradrenergic neuronal activity, we presently cannot exclude direct effects of estrogen on postsynaptic β-adrenergic receptors or indirect effects on those same receptors resulting from estrogen-induced alterations in a second unspecified variable (e.g., weight loss or decreased body temperature) as alternative explanations. (Supported by VA 1680, NIH NS13101, NS06233, MH06058, and AG00029.

1686 BINDING OF JUNCTIONAL AND EXTRAJUNCTIONAL ACETYLCHOLINE RECEPTORS BY SERA FROM PATIENTS WITH MYASTHENIA GRAVIS. Crispin B. Weinberg* and Zach W. Hall. Dept. Neurobiol., Harvard Med. Sch., Boston, MA 02115 and Dept. Physiol., Univ. Calif. Sch. Med., San Francisco, CA 94143.

Sera from patients with myasthenia gravis have been reported to bind acetylcholine receptor in crude extracts of denervated rat muscle, but not to bind receptor from normally innervated muscle (Almon and Appel (1975) Biochim. Biophys. Acta 393, 66). We have investigated this difference using highly purified preparations of receptor from denervated and normal rat muscles which were shown by isoelectric focusing to consist largely of extrajunctional and junctional receptors, respectively. Binding was measured by incubation of human sera with a complex of ^{125}I-α-bungarotoxin and receptor, addition of a second antibody to human IgG and determination of precipitated radioactivity. In sera from ten patients, the ratio of the titer against extrajunctional receptor to that for junctional receptor ranged from 1.2 to 2.6. In control experiments antibodies raised in rabbits to purified rat muscle extrajunctional receptor or to receptor from eel electric organ gave equal titers to junctional and extrajunctional receptors. Thus the difference seen with myasthenia gravis sera was not due to the presence of inactive receptor or to an artifact of the binding assay. To compare the determinants present on the two receptor types, myasthenic sera were preincubated with unlabelled receptor prior to incubation with radioactive toxin-receptor complex. These competition experiments demonstrated that the sera contained two types of antibodies: 1) those directed against determinants common to junctional and extrajunctional receptors; and 2) those directed against determinants that are present only on extrajunctional receptors. No evidence for antibodies directed against unique determinants on junctional receptors was found.

1687 STRIATAL INTERDEPENDENCE AND PRE- AND POST-SYNAPTIC DOPAMINE RECEPTORS. P. Weinreich*, T. Lee & P. Seeman (SPON: K. Livingston) Pharmacology Department, University of Toronto, Toronto, Canada.

It has recently been found that 6-hydroxydopamine-induced lesions of the nigro-striatal neurones resulted in a 50% reduction in the number of striatal receptors for ^{3}H-apomorphine, supporting the theory that ^{3}H-apomorphine has a high affinity for pre-synaptic dopamine receptors[1]. To test further this hypothesis, we injected kainic acid into rat striatum and found that ^{3}H-apomorphine binding was unaffected while ^{3}H-spiperone binding decreased by 22% when compared to the contralateral unlesioned side. Since kainic acid destroys neurone cell bodies while sparing the nerve terminals[2,3], these results are compatible with the concept of ^{3}H-apomorphine labeling pre-synaptic receptors, while ^{3}H-spiperone prefers post-synaptic dopamine receptors.

We also found that either a pre- or post-synaptic chemical lesion of the rat striatum (with either 6-hydroxydopamine or kainic acid, respectively) elicited an approximate doubling of the ^{3}H-apomorphine and ^{3}H-spiperone specific binding in the contralateral unlesioned striatum when compared to unlesioned or intrastriatally saline injected animals (Fig.). This indicates that chemically-induced damage of either pre- or post-synaptic neurones in the striatum may cause a non-specific increase in the number of sites for ^{3}H-apomorphine or ^{3}H-spiperone in the contralateral side. These results thus suggest a bilateral communication between the two striata, as postulated by Nieoullon et al.[4].

(Supported by MRC and OMHF).

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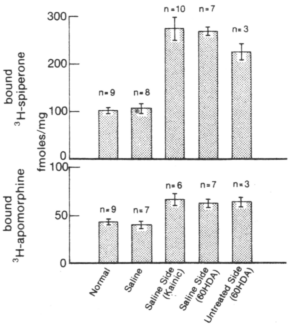


1688 IMMUNOFLUORESCENCE LOCALIZATION OF ANGIOTENSIN II (AII) AND AII RECEPTORS IN CULTURED RAT BRAIN CELLS. James A. Weyhenmeyer*, Mohan K. Raizada*, Detlev Ganten*†, M. Ian Phillips and Robert E. Fellows. Dept. of Physiology and Biophysics, University of Iowa, Iowa City, IA 52242 and †Dept. of Pharmacology, University of Heidelberg, West Germany.

In our earlier studies, we have found specific high affinity AII receptors in cultured cells from fetal rat brain. Here we report the localization of AII and AII receptors in these cultures by immunofluorescence.

Brain cells from rat fetuses of 20 day gestational age were prepared by trypsinization (Wilson et al.[1972], J. Biol. Chem. 247, 3159). $2.8x10^6$ cells in Dulbecco's modified Eagle's medium (DME) with 10% fetal calf serum were inoculated in Falcon culture dishes containing 11x22 mm coverslips and cultured at 37°C in a humidified CO_2 incubator. On days 3 and 5, the cultures were fed with DME containing 10% horse serum, 40 µg/ml fluorodeoxyuridine and 100 µg/ml uridine. By day 7 the morphological appearance of the brain cells on glass coverslips showed a background monolayer of large flat cells on the top of which were present individual and small clusters of "phase dark" cells. These "phase dark" cells showed numerous processes and resembled neuronal morphology. Four groups of duplicate dishes were tested. Group 1 was incubated with AII for 30 min, washed and fixed to test for receptors. Group 2 was similarly treated but incubated with PBS for 2 hr before fixation to test for nonspecific binding. Groups 3 and 4 were treated in the same way without AII to test for the presence of endogenous AII.

Groups 3 and 4 demonstrated significant intrinsic staining for AII on the cell body. This staining was not evident when the primary antibody was previously absorbed with AII. In group 1, which had been pre-treated with AII and fixed immediately, extensive staining in the processes and cell bodies was observed. Cells in group 2 demonstrated a loss of specific staining in the neural like cell process and perikaryon.

In conclusion, groups 3 and 4 indicated the presence of endogenous AII in fetal rat brain cells. A significant increase in fluorescence in cells from group 1 compared to group 2 indicates the specific AII receptors in these cultures. They are localized in the bodies and processes of the neuronal like cells. (Supported by NSF grant # BNS77-24415 to MIP; NIH grant # 1 RO1 HD11184-01 to REF. JAW is supported by a PMA Foundation Fellowship Award. MIP is recipient of RCDA from NIMH.)

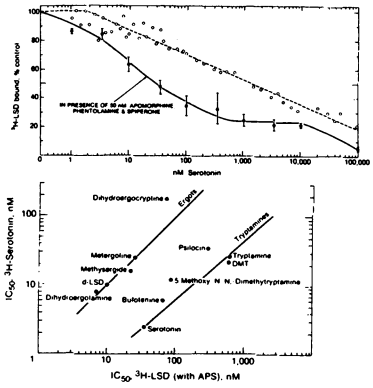
1689 SELECTIVE LABELING OF SEROTONIN RECEPTORS BY ^{3}H-LSD. P.M. Whitaker* and P. Seeman (SPON: P. Brawley). Department of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

Since it is known that LSD (d-lysergic acid diethylamide) acts on both catecholamine and indoleamine neurones, we sought conditions wherein ^{3}LSD would selectively label primarily serotonin receptors in vitro.

Using crude homogenate of calf caudate, which contains many types of neuroreceptors, we found that approximately 20% of the total binding of ^{3}H-LSD was inhibited by 50-1000 nM dopamine or apomorphine, 1000-10000 nM noradrenaline, or 10-100 nM phentolamine. These findings indicated that ^{3}H-LSD bound appreciably to alpha-adrenoceptors and dopamine receptors.

We then tested the effect of serotonin in competing against ^{3}H-LSD in the presence of a simultaneous combination of 50 nM apomorphine (to block dopamine autoreceptors), 50 nM phentolamine (to block alpha-adrenoceptors) and 50 nM spiperone (to block post-synaptic dopamine receptors). In the presence of these latter 3 drugs (so-called "APS" system), serotonin inhibited ^{3}H-LSD binding by 50% at about 30 nM; in the absence of these 3 drugs the serotonin IC_{50}% was about 1000 nM, as has usually been found by others (Bennett & Aghajanian, 1974; Lovell & Freedman, 1976; Burt et al., 1976; Bennett & Snyder, 1976; Fillion et al., 1978).

Thus, by blocking dopamine- and adreno-ceptors, the selectivity of ^{3}H-LSD for serotonin receptors was greatly enhanced. We further found that ergots inhibited the binding of ^{3}H-serotonin and of ^{3}H-LSD (APS system) at similar concentrations; tryptamines had a higher affinity for ^{3}H-serotonin than ^{3}H-LSD sites. The results support the concept of two serotonin receptors, one pre- and the other post-synaptic. (Funded by MRC of Canada and the Ontario Mental Health Foundation).



1690 FREQUENCY RESPONSE ANALYSIS OF RECEPTOR POTENTIALS FROM PRIMARY ENDINGS IN ISOLATED MUSCLE SPINDLES OF CAT. R.S. Wilkinson* and C.C. Hunt. Dept. Physiology and Biophysics, Washington Univ. Sch. Med., St. Louis, MO 63110.

Isolated decapsulated spindles were mounted in a chamber containing Locke's solution with one end tied to a force transducer and the other to an electromechanical stretcher. The primary axon was drawn on a recording pipette into oil. After impulse block by tetrodotoxin, tension, displacement and receptor potential were recorded and subsequently analyzed by a computer. The first through fifth harmonics of steady state responses were measured over frequencies, f, from 0.01 to 100 Hz. The gain, A_{RP} and A_T, and phase, ϕ_{RP} and ϕ_T, of the receptor potential and tension (with respect to displacement) of the first harmonic response were used to construct Bode plots. Measurements were performed under linear response conditions, as described below.

For f below approximately 10 Hz, A_T was constant or showed a slight monotonic increase with increasing f, while ϕ_T was 10^o-20^o at 0.01 Hz, decreased slowly with increasing f, and began to lag the applied stretch above approximately 10 Hz. Receptor potential response differed qualitatively from that of tension and was more complex: A_{RP} was an approximate power-law function of f, $A_{RP} \propto f^\gamma$, with $\gamma = 0.3$-0.5, depending on the particular spindle. ϕ_{RP} was 30-45^o at low f, usually rose gradually with f to a peak at approximately 2 Hz, then decreased with increasing f, becoming negative above approximately 15 Hz. Neither ϕ_T nor ϕ_{RP} showed a tendency to approach 0^o as f approached 0.01 Hz, the lowest attainable measurement frequency.

In the range from 1.0 to approximately 10-25 µM peak-to-peak, A_{RP} and ϕ_{RP} did not depend on the amplitude of stretch and harmonic distortion in the response waveforms was negligible. Frequency response measurements were obtained at constant amplitude within this range, determined independently for each spindle. Higher stretch amplitudes were also investigated. A_{RP} decreased with increasing amplitude as a power-law function, independent of frequency. Harmonic distortion increased with amplitude, and was predominantly second harmonic. Neither tension nor receptor potential responses were consistent with simple linear models employing finite combinations of viscoelastic elements.

1691 THE COMPLEX BINDING OF γAMINOBUTYRIC ACID TO A RAT BRAIN SYNAPTIC MEMBRANE PREPARATION. Marvin H. Winkler*, William J. Nicklas* and Soll Berl (Spon: G. Cohen). Dept. Neurology, Mount Sinai School of Med., New York, N.Y. 10029.

The extent to which γ-aminobutyrate is bound to rat brain synaptic membrane preparations was determined over a wide range of free GABA concentrations. The binding curve defined by the data is complex and characterized by no less than four inflection points. The curve is consistent with either the existence of several sets of independent binding sites or with cooperative binding or with a combination of both. The fact that there is more than one set of binding sites is established by exhaustive iodination of separate aliquots of membrane in the presence or absence of an excess of transmitter. The difference between the binding expressed by the two aliquots corresponds to one sixth of the imidazole acetic acid displaceable binding of GABA expressed by the uniodinated membrane at 0.25 uM free GABA. It is concluded, from this result, that one set of sites can be defined as being composed of members having an accessible iodinatable residue whose integrity is essential to the functioning of that site. The difference in binding expressed by the two iodinated aliquots between 0.03 and 0.25 uM free GABA defines a saturable binding curve which fulfills the Scatchard criterion for a set of independent, homogenous sites thus reinforcing the conclusion that more than one set of sites is expressed on the membrane. The experimentally determined curve is further analyzed in terms of a theoretical discussion which assumes that physical occlusion, specific binding and non-specific binding may all contribute to the apparent observed binding. A method for separating physical occlusion from binding and for determining the maximum number of binding sites without the use of competitive binders is described. The use of this analysis in the determination of possible selective inhibition of specific sets of sites by selected agonists or antagonists is discussed. Supported in part by NIH Grant NS 11824 and the Clinical Center for Research in Parkinson's and Allied Diseases, and Grant NS 11631.

1692 EFFECTS OF CHRONIC ALTERATIONS IN RECEPTOR STIMULATION ON β-ADRENERGIC RECEPTORS IN RAT BRAIN. B.B. Wolfe*, D.A. Staunton*, P.M. Groves and P.B. Molinoff. Dept. Pharmacol., Univ. Colo. Med. Ctr., Denver, CO 80262.

Access of norpinephrine to postsynaptic β-adrenergic receptors can be altered by the chronic administration of various pharmacological agents. The effects of such treatments on isoproterenol-stimulated cAMP accumulation and β-adrenergic receptors were measured respectively in slices or membranes prepared from rat cerebral cortex. Chronic administration of (+,-)-propranolol-HCl (10 mg/kg; twice daily) for 9-12 days caused a 30% increase in isoproterenol-stimulated cAMP accumulation. This increase in cAMP accumulation was accompanied by a 33% increase in the density of β-adrenergic receptors as measured by Scatchard analysis of (^{125}I)-iodohydroxybenzylpindolol (IHYP) binding. No change was observed in the affinity of IHYP following propranolol administration suggesting that no residual propranolol remained in the tissue preparation. Chronic administration of desmethylimipramineHCl (10 mg/kg; twice daily) for 7-21 days caused a 35-45% decrease in both isoproterenol-stimulated cAMP accumulation and in the density of β-adrenergic receptors. No changes in the affinities of either agonists or antagonists active at β-adrenergic receptors were observed. The effect of desmethylimipramine was blocked by the simultaneous administration of propranolol. The increases in isoproterenol-stimulated cAMP accumulation and in the density of β-adrenergic receptors caused by propranolol were not affected by the simultaneous administration of desmethylimipramine. Administration of d-amphetamine sulfate (17.2 mg/kg; twice daily) for 8 days resulted in a 30% decrease in isoproterenol-stimulated cAMP accumulation. These studies suggest that manipulations which increase the availability of norepinephrine at β-adrenergic receptors lead to a decrease in the density of receptors with no change in their properties. Conversely, manipulations which decrease the availability of norepinephrine at β-adrenergic receptors cause increases in receptor density. The changes in receptor density appear to be responsible for changes in the ability of catecholamines to stimulate cAMP accumulation.
(Supported by the USPHS: NS 13289, NS 09199 and AA 03527.)

1693 RECEPTORS FOR NERVE GROWTH FACTOR IN THE NUCLEAR MEMBRANE OF PHEOCHROMOCYTOMA. Bruce A. Yankner* and Eric M. Shooter. Dept. Neurobio., Stanford Univ. Sch. of Med., Stanford, CA 94305.

The binding of β nerve growth factor (βNGF) to nuclei from dorsal root ganglia was observed by Andres, R.Y. et al. (PNAS, USA 74: 2485, 1977). Correlation of this interaction with target tissues for nerve growth factor was well demonstrated. We have studied a continuous line of pheochromocytoma cells (PC12) which responds to βNGF in culture by the outgrowth of neurites. When nuclei were purified from PC12 cells and incubated with ^{125}I-βNGF, specific binding sites were found. Incubation of nuclei in the presence of deoxyribonuclease I resulting in the solubilization of about ninety percent of the nuclear chromatin did not decrease the specific binding. Chromatin was purified from nuclei by the method of Harlow, R. and Wells, J. (Biochem. 14: 2665, 1975) and bound βNGF nonspecifically. The specific binding of βNGF was associated with the nuclear membrane which was purified according to Jackson, R.C. (Biochem. 15: 5641, 1976). Resistance to extraction with Triton X-100 suggests that the nuclear receptor may be localized to the inner nuclear membrane or the pore complex.

The binding of βNGF to purified nuclear membrane is saturable and consistent with two sites; a high affinity site with a K_d in the range of 10^{-11}-10^{-10}M and a lower affinity site with a K_d of 1-3 x 10^{-9}M. The capacity ratio is about 1:13 for the high and low affinity sites, respectively. Neither insulin nor epidermal growth factor could compete for the NGF sites.

When PC12 cells were grown in culture in the presence of 50ng/ml βNGF for 8 days, the receptor capacity of the nuclear membrane increased from about 12,000 sites per cell in confluence to 60,000 sites per cell. Although the DNA content of the nuclear chromatin remained constant, a four to five-fold increase in the protein content of the nuclear membrane was observed.

1694 EFFECTS OF GTP ON BRAIN DOPAMINE RECEPTORS. N.R. Zahniser* and P.B. Molinoff (SPON: M.D. Dibner). Department of Pharmacology, University of Colorado Medical School, Denver, CO 80262.

Guanine nucleotides regulate the hormonal sensitivity of adenylate cyclase in a variety of systems. These nucleotides have also been shown to decrease the affinities of glucagon receptors and β-adrenergic receptors for agonists. The affinities for antagonists were not affected. In the current experiments inhibition of the binding of labelled spiroperidol by agonists was measured in rat brain by a direct in vitro assay in the presence and absence of various purine nucleotides. In striatal membranes GTP (0.3mM) produced a 4-5 fold decrease in the Kd values for dopamine(DA), the DA-receptor agonists epinine and A-6,7-DTN, and the partial agonist apomorphine. Hill coefficients for the agonists showed significant negative cooperativity (0.55 +/- 0.02). These values were shifted slighly closer to unity in the presence of GTP (0.64 +/- 0.03). As shown by Scatchard analysis of binding data, GTP had no sigificant effect on the affinity of spiroperidol for sites on striatal membranes (control: Kd=79+/-6pM; GTP: Kd=85+/-5pM). Similarly, GTP had no effect on the inhibition of spiroperidol binding by the dopamine receptor antagonists (+) and (-)-butaclamol or (α) and (β)-fluphenthixol. Effects identical to those of GTP were found with GMP-PNP, GDP, and ITP while GMP, guanosine, and ATP were ineffective. These observations expand the number of reports of agonist-specific effects of GTP to include another receptor whose effects may be mediated through changes in adenylate cyclase activity. In contrast to the agonist-specific effect of GTP in the striatum, addition of GTP to membranes prepared from frontal cortex had no effect on the inhibition of spiroperidol binding by either DA, DA receptor agonists, or serotonin. These data are consistent with the following conclusions: (1) Spiroperidol binds to DA receptors in the striatum but may be associated with another class of binding sites in the frontal cortex. (2) Agonists for DA receptors can be distinguished from antagonists in the striatum on the basis of the shift in the Kd value in the presence and absence of GTP.
(Supported by NS 05970, 09199, 13289 and AA 03527.)

REGENERATION

1695 OLFACTORY NERVE REGENERATION IN THE AXOLOTL FOLLOWING NERVE GROWTH FACTOR (NGF), ANTI-NGF, AND D-AMPHETAMINE TREATMENT. P.M. Adams, M.W. Mellinger, R. J. Perez-Polo and K. Hall*. Depts. of Psychiatry and Behavioral Science, and Pharmacology, Anatomy and Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas 77550.

In previous work we described the extensive central nervous system regenerative capacity of the axolotl (A. mexicanum). We have studied this regeneration following the surgical removal of the anterior two-thirds of the forebrain. The present study examined the effects of treating the axolotl with NGF, an antibody to NGF or d-amphetamine sulfate. The NGF and anti-NGF were administered intracranially immediately after surgery. The d-amphetamine was administered chronically beginning immediately or Day 2 post-operatively and continued until sacrifice at Day 10. Light microscopic examination of the treatment groups indicated an increased fiber density in both the NGF and amphetamine treated animals. There was increased olfactory nerve diameter and better fiber organization in these two groups. High power examination indicated a predominance of fine fibers to heavy fibers for the NGF and amphetamine treated animals. In contrast, in the anti-NGF treated animals regenerating nerves were not as well organized nor was regeneration as extensive as in either controls, or d-amphetamine or NGF treated animals. The extent of regeneration at Day 10 was least in anti-NGF treated animals, and increasingly greater in controls, NGF-treated and d-Amphetamine treated animals respectively. This investigation supported by Grant Nos. DHEW 5501-RR-05427-16GRS and Research Career Development Award 1-K04-NS-00213.

1696 CELL-TO-CELL SPECIFICITY OF REGENERATED GIANT AXONS IN EARTHWORMS. Stewart C. Birse* and George D. Bittner
Dept. of Zoology, University of Texas, Austin, Texas 78712

Previous experiments in our laboratory have shown that severed giant axons in the earthworm ventral nerve cord (VNC) regenerate with a high degree of cell-to-cell specificity within 2-4 weeks (Birse and Bittner; Brain Res. 113:575, 1976). This regeneration is accomplished by outgrowing axonal processes which may originate in one or both halves of the giant axon and functionally connect it with its severed counterpart. Similarly, VNC's with 1 or 3 ablated segmental ganglia regenerate within 1 to 8 months by the same mechanism with a lesser degree of success and possibly less cell-to-cell specificity (Birse and Bittner; Fed. Proc. 36:554, 1977). It was necessary, then, to determine 1) from which severed axonal stump outgrowing processes arise, 2) if outgrowing processes connect only to their severed counterparts, and 3) whether those processes morphologically fuse with their severed counterparts or form electrotonic synapses. To accomplish this goal, animals suffering either simple VNC transections or ganglion ablations were injected with 0.5M $CoCl_2$, developed with ammonium sulfide, embedded in paraffin, sectioned at 10 microns, and the cobalt stain enhanced with the Timm's procedure. Preliminary results indicate that the cobalt stain does not proceed through the lesion site suggesting that morphological fusion of the outgrowing processes and the severed giant axon may not occur. (Supported by NIH grant NS-14412 and an RCDA to G.D.B.)

1697 RETENTION OF SPECIFICITY FOR APPROPRIATE SYNAPTIC SITES BY REGENERATING FROG OPTIC AXONS. Ronald C. Bohn* and Dennis J. Stelzner. Dept. Anat., Upstate Medical Center, Syracuse, NY 13210

Most studies of regeneration in the visual system have shown that regenerating optic axons retain remarkable specificity in reinnervating normal visual targets. However, certain investigations have demonstrated that by ablation of normal visual targets (tectum) the regenerating optic axons will form connections in anomalous regions. Optic axons in neonate hamsters will even form connections in the medial geniculate body following destruction of superior colliculus and section of the brachium to inferior colliculus (Kalil and Schneider, 1975). The question remains whether regenerating optic axons form anomalous connections because they are forced to do so by removal of their normal targets or because regenerating axons are attracted to denervated sites made available by the ablations. Our experiments were designed to test whether or not regenerating optic axons will reinnervate inappropriate denervated regions in close proximity to visual projection areas without damaging the normal visual targets.

The right optic nerve was crushed in 12 adult Rana pipiens which also received a left hemisection through the isthmal region at the same time. The latter lesion cuts axons which make synaptic contact in regions of thalamus which lie adjacent to contralateral optic nerve terminal sites. After survival periods ranging from 1 week to 6 months, the right eye of each frog was injected with 4 µl of ^{3}H-proline (10 µCi/µl) and brains were prepared for autoradiography. Spread of label from normal optic tract terminal zones into adjacent regions denervated by the isthmal lesion would imply that regenerating optic axons establish connections with inappropriate targets even though the normal optic nerve projections remain intact.

Examination of the distribution of label within contralateral thalamic visual targets at all survival periods indicated that the regenerating optic nerve did not expand its projection to occupy sites vacated by the isthmal lesion. Distribution of silver grains within contralateral visual targets was similar to grain distribution in animals receiving only right nerve crush. These results are most easily interpreted to imply that adult regenerating optic axons retain their specificity for thalamic visual targets and will not form contacts in adjacent denervated thalamic regions. A second possibility is that fibers cut by the isthmal lesion regenerate and return to fill their normal sites in thalamus. To exclude this possibility, regenerating optic axons are being studied in 6 frogs where regeneration of ascending projections to thalamus was prevented by teflon block at the isthmal lesions. (Supported by NIH Grant NS14096)

1698 THE EFFECT OF REPEATED NERVE INJURY ON THE RETINAL GANGLION CELL AND AXONAL REGENERATIVE RESPONSE IN THE OPTIC NERVE OF THE NEWT (Triturus viridescens). T. O. Brock, III and J. E. Turner. Dept. of Anat., Bowman Gray Sch. of Med. of Wake Forest Univ., Winston-Salem, NC 27103.

In the transected newt optic nerve, pioneer regenerating axons enter the distally denervated stump 4-6 days post lesion, before the glial scar is maximally developed (Turner and Singer, J. Exp. Zool. 190: 25, '74). A series of experiments was designed to delay the entrance of these axons into the distal stump to ascertain whether a fully developed glial scar would hinder regeneration (Brock, Anat. Rec. 190: 349, '78). The results from repeated nerve lesion experiments indicated that a well developed glial scar did not offer a hindrance to regenerating fibers, in fact a second lesion appeared to enhance axon outgrowth. In a second series of experiments the lesion sequence was reversed. An initial optic nerve crush was made on all animals at a point midway between the eyeball and the optic foramen and animals were divided into various groups. Two groups were further treated in a manner to test the effect of delayed entrance of axons into the distal stump. In these groups, nerves were transected at midpoint between the initial crush and the eyeball 4 and 11 days post initial lesion and the animals were sacrificed 7 and 11 days respectively, after transection. Control groups were sacrificed 7, 11, and 22 days post initial crush. Comparisons of numbers of axons per nerve cross section and axon densities by EM morphometric analysis indicate that a fully developed glial scar did not hinder axon outgrowth into and within the distal stump. As in the previous studies the second lesion appeared to enhance axonal outgrowth. Examinations of the retinal ganglion cell layer of animals treated to delay axon outgrowth demonstrate prominent nucleoli and chromatin changes in 40-50% of these neurons by day 11.

(Supported by a Basil O'Connor Starter Research Grant from the National Foundation - March of Dimes; the National Society for the Prevention of Blindness made possible through the Alder Foundation and NIH Grant NS 12070 awarded to James E. Turner.)

1699 THE EPENDYMAL CELLS OF THE SPINAL CORD IN RAT AND STINGRAY Richard E. Coggeshall and Robert B. Leonard, Depts. of Anatomy and of Physiology and Biophysics, and The Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550.

In most areas of the vertebrate nervous system, there is a proliferation of neurons very early in development, followed by a precisely programmed cell death. After this, it is generally felt that the number of neurons in any particular part of the nervous system is constant. In recent years, however, this opinion has been modified because it has been shown that a population of small neurons in the forebrain keep proliferating into adult life. Nevertheless, it is still felt that most neuronal populations in vertebrate brain and spinal cord have a constant number of elements throughout the life of the animal. Recently, however, it has been shown that the Atlantic Stingray, Dasyatis sabina, does not have a stable population of neurons in various well defined neuronal groups in the spinal cord in that the number of dorsal root axons and ganglion cells as well as ventral root axons and motor cells increase steadily as the animal ages. This presumably implies that new neurons are added to an already functioning adult nervous system and the fact that these animals retain the capacity to make new neurons in adulthood may well be related to the ability of these animals to regenerate parts of the nervous system.

In an attempt to determine the mechanism underlying the proliferation of spinal neuronal elements in the stingray, the ependymal cells of the spinal cord were examined. The ependymal cells of the stingray can be divided into 2 populations, first a typical columnar cell that forms the bulk of the ependymal lining and second a cell type that is relatively round and has a distinctive nuclear pattern, a basal location, and moderately large numbers of lysosome-like bodies. Synapses end upon these cells, which probably implies that they are small neurons.

The ependymal cells of the stingray were compared with those of the rat, which is stated to be an animal that does not increase the number of spinal neuronal populations as it ages. As expected, there were few, if any, second ependymal cell types as described above. It was of interest, however, that there did seem to be synapses on the typical ependymal cells in this animal. Synapses have previously been described on tanycytes of the 3rd ventricle in mammals, but they are not described, to our knowledge, in the spinal cord. The relation of these findings in stingray and rat to the question of proliferation or non-proliferation of adult neuronal populations will be discussed. Supported by grants NS 07377, NS 11255, NS 10161, The Muscular Dystrophy Society of America, and NIH Fellowship NS 05434.

1700 A QUANTITATIVE ULTRASTRUCTURAL STUDY OF AXOTOMIZED FELINE BETZ CELLS. M.P. Dentinger and K.D. Barron. Department of Neurology, Albany VA Hospital and Albany Medical College, Albany, NY 12208.

Adult cats underwent unilateral left lateral funiculotomy at the second cervical (C2) level. Observations were made on animals surviving 1 to 153 days with several survival times being paired. One hundred and twenty large pyramidal neurons (Betz cells) from pericruciate cortex were analyzed using quantitative stereologic methods. The morphometric observations on organelle composition were evaluated by analysis of variance statistics. Chromatolytic changes appeared exclusively in Betz cells of the cortex contralateral to the spinal cord lesion. Estimation of Betz cell population at the light microscopic level indicated approximately a 50% reduction in the right pericruciate cortex at a later survival time (153 days). Analysis of the neuronal organelle composition showed a decrease in the percent of cytoplasm occupied by mitochondria at survival times of 5 and 10 days. At all survival times, including the time of maximum chromatolysis 10 days postoperatively, reactive neurons showed no depletion of rough endoplasmic reticulum, Golgi apparatus, nor lysosomes. Analysis of somatic bouton coverage showed no definite displacement or stripping of boutons at any survival time. An increased percentage of somatic surface covered by boutons was found for large neurons in the right cortex at later survival times (49 and 153 days). Thus, in normal Betz cells the percent coverage of the somal surface by axosomatic boutons is 22.2% and at 49 days after funiculotomy axotomized Betz cells have a percent bouton coverage of 30.2%. (Supported by VA Medical Research Service and NINCDS Research Grant 08735.)

1701 SELECTIVE RE-INNERVATION OF SLOW MUSCLE FIBERS OF THE FROG. Alejandro Elizalde and Enrique Stefani. Department of Physiology and Biophysics, Centro de Inv. del IPN. Apto. Postal 14-740, México 14, D. F.

Following denervation by crushing the sciatic nerve, fast motor axons regenerate faster than slow motor axons and re-innervate non selectively twitch and slow muscle fibers. Slow axons reach the muscle later and re-innervate selectively slow muscle fibers (Schmidt & Stefani, 1976). The initial non-selective re-innervation of slow muscle fibers could be explained by the fact that fast motor axons reach the muscle earlier than slow motor axons. To test this hypothesis experiments were performed to determine the selectivity of re-innervation by crushing the nerve just before its entrance into the muscle. By reducing the regenerating distance one may expect that slow and fast motor axons will reach the muscle almost simultaneously. Muscle fibers of the piriformis muscle were impalled with two intracellular microelectrodes and were characterized as twitch or slow fibres according to the electrical properties. The piriformis nerve was crushed 1-3 mm from its entry into the muscle. Re-innervation was studied by stimulating the sciatic nerve in two different points. The conduction velocity of an individual nerve fiber was calculated from the difference in latency of the synaptic potential and the interelectrode stimulating distance. Fast and slow axons were distinguished according to conduction velocity and threshold. Functional re-innervation started 9 days after the operation. From the beginning, slow and twitch muscle fibers were selectively re-innervated. 25 slow fibers studied 9-40 days after the operation were re-innervated by slow axons (cond. vel. <5 m/sec, threshold 2-4 V). In the same muscles 53 twitch fibers were re-innervated by fast axons (cond. vel 8-25 m/sec, threshold 0.3-0.8 V). These results indicate that re-innervation is selective for fiber type and that regenerating axons can recognize the corresponding muscle fibers.

1702 SELECTIVITY IN THE LOSS OF SYNAPTIC INPUT TO FROG SPINAL MOTONEURONS FOLLOWING VENTRAL ROOT SECTION. Paul B. Farel. Dept. Physiol., Sch. Med., Univ. N. Car., Chapel Hill, NC 27514

A variety of electrophysiological studies have shown that frog spinal motoneurons receive spatially segregated synaptic input from two sources: (1) fibers descending in the lateral columns (LC) synapse upon soma and proximal dendrites; (2) dorsal root (DR) fibers synapse upon more distal dendritic regions. These results are consistent with the finding that in normal motoneurons the time to peak of the monosynaptic DR-EPSP is approximately 1-2 msec longer than the time to peak of the monosynaptic LC-EPSP. The present investigation indicates that after section of their axons motoneurons undergo a selective desynapsis such that the electrotonic distance from the soma of the DR inputs is now shorter than that of LC inputs.

Slow waves can be recorded from ventral roots of the isolated frog spinal cord which reflect the time course of the monosynaptic EPSPs elicited by activation of different synaptic inputs. Normally, the mean time to peak of the LC-elicited slow wave recorded from ventral root 9 is 2.77±.34 msec ($\bar{X}$±SE) and 3.92±.34 msec to DR stimulation. Following ventral root section 21-42 days previously, these values are now 7.41±.68 msec for LC stimulation and 4.19±.3 msec time to peak for the slow wave reflecting the monosynaptic DR-EPSP.

Intracellular recordings revealed that, after correction for distortion by extracellular field potentials, the time to peak of the monosynaptic LC-EPSP was twice that of the monosynaptic DR-EPSP by 30 days postaxotomy. These changes were progressive in the period following axotomy and were associated with an increase in firing threshold to both synaptic activation and to injected current.

To the extent electrotonic distance as measured by time to peak can be equated with physical distance, axotomy can be seen to disrupt selectively those synaptic inputs from descending fibers while sparing those from primary afferent fibers. These results cannot be attributed to a general somatic to dendritic gradient of desynapsis. Such a gradient would be expected to produce DR- and LC-EPSPs of similar risetimes. An anatomical correlate of these results may be seen in the work of Sumner (1976) who found axotomized rat hypoglossal motoneurons lost boutons only of certain morphological types. Whether similar anatomical distinctions can be made between terminals derived from LC and DR fibers remains to be determined.

Supported by NSF grant BNS 76-24528 and NINDS grant NS 11132.

1703 LECTIN BINDING IN THE REGENERATING GOLDFISH VISUAL SYSTEM. E.L. Feldman*, A.M. Heacock and B.W. Agranoff. Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48109.

Recent reports from this laboratory have described an in vitro approach to the study of optic nerve regeneration in the goldfish. Explant culture of the adult goldfish retina results in vigorous neuritic outgrowth, provided that the optic nerve has been crushed in vivo a week prior to explantation. Histological studies have shown that these neurites derive from retinal ganglion cells and, therefore, may be considered regenerating optic nerve fibers. Since glycoproteins and glycolipids have been postulated to play a role in cellular recognition and in retinotectal specificity, it was of interest to investigate the nature of the cell surface carbohydrates of the retinal explant neurites. By means of fluorescence microscopy, we studied the binding to neurites of lectins, plant proteins which recognize specific sugars or sugar linkages. Of the fluorescent lectins applied to the retinal cultures thus far, two have been found to bind to the neurites--ricin and wheat germ agglutinin. The binding of ricin, a lectin specific for terminal galactose residues, was inhibited by pre-incubating the lectin with 50 mM lactose. Wheat germ agglutinin shows an affinity for the linkage (N-acetyl-β-(1,4)-D-glucosamine)$_2$. Its binding to neurites is blocked by 20 mM N,N',N"-triacetylchitotriose. Two other lectins, from Ulex europeus (fucose) and from Dolichos biflorus (N-acetyl-α-D-galactosamine), do not appear to bind to the retinal neurites.

We have also examined lectin binding to the goldfish optic tectum. Frozen sections were taken with a cryostat through the tectum of goldfish whose optic nerve had been crushed 14 d previously. The sections were dried and either used directly or fixed by a variety of methods. Lectin binding specificity for the tectum was the same as that found for the retinal neurites. The characteristics of ricin and wheat germ agglutinin binding depend upon the method of fixation.

These studies offer a combined approach toward characterization of cell surface carbohydrates of the regenerating goldfish visual system. The tectal sections are useful in that, in addition to the optic nerve, membranes of adjacent cell types can be examined. In contrast, the retinal explant preparation permits analysis of the carbohydrate nature of optic nerve membranes in the absence of adjacent structures.

1704 HISTOLOGICAL EVIDENCE FOR AXONAL REGENERATION IN BRAINS OF ADULT RATS. Anne P. Foerster. Dept. of Physiology, Univ. of Toronto, Toronto, Ontario, Canada, M5S 1A8.

Precisely placed brain lesions were made with a cutting device consisting of a 90μ diameter cross-wire, 1-2 mm long, supported between two long parallel 90μ wires which extended 1/2-1 mm beyond the horizontal cutting wire. The device was lowered through the brain (adult male rats, 350-600g) by a micromanipulator, and the two protruding vertical wires cemented to the upper skull. The device was left in situ until after the brain was fixed; it was then removed from the ventral surface, leaving two vertical "marker" channels between which the cutting wire had passed through the brain tissue. Survival times ranged from about one minute to many months. Brains were block silver stained (Ranson), and horizontal sections usually counterstained with Luxol Fast Blue and Nuclear Fast Rubin. Because of their predominantly parallel-fibred alignment, the horizontal parasaggittal tracts of midbrain-hypothalamus-subthalamus were studied in detail.

In brains fixed immediately after insertion of the device, masses of severed axons were clearly defined along the borders of the incision; a few intact fibres in the path of the device deviated for variable distances (never exceeding 200μ) around the holes left by the support wires. By 3-6 days, there was degeneration and a loss of fibres on both sides of the lesion, and terminal enlargements were found on many of those remaining, some of which appeared to have turned along the line of the lesion and even to have passed around the vertical holes, where axon numbers were now increased. By 18 days terminal enlargements were scarce, and a massive bundling of axons was visible, parallel and close to the lesion, on one or both sides; in addition, and related to this, there was a striking increase in the number of axons coursing around one or other of the holes. These surrounding axons turned obliquely to assume the normal orientation and position of the particular lesioned tract within 500μ or less of the cut. These appearances were not observed after implantation of two perpendicular wires minus a cutting cross-wire. When a tract lesioned 18 or more days earlier failed to have detoured in this characteristic manner, measurements indicate that the cut had extended more than about 200μ outside its borders.

The results seem most readily explained by regeneration of the cut axons around the lesion (only in 15% of sections did a few axons appear to have crossed the cut). The eventual restoration of normal appearances of lesioned tracts at distances of about 500μ from the cut emphasizes the usefulness of unambiguous markers of the boundaries of the original lesion.

1705 EFFECTS OF NERVE GROWTH FACTOR, ANTIBODIES TO NERVE GROWTH FACTOR AND D-AMPHETAMINE ON FOREBRAIN REGENERATION IN THE AXOLOTL. Kimberley Hall*, Perrie M. Adams, Melvyn W. Mellinger and J. Regino Perez-Polo. Depts. Human Biological Chemistry and Genetics, Psychiatry and Behavioral Sciences, Pharmacology and Anatomy, Univ. Texas Med. Br., Galveston, TX 77550.

The axolotl (Ambystoma mexicanum) is a neotenic amphibian with an extensive regenerative capability within the central nervous system. In control animals, bilateral removal of the anterior 2/3 of the forebrain results in a sequence of morphological events which involves the forward growth of the regenerating forebrain and inversion of the lateral walls to complete ventricle closure, followed by the functional reinnervation of the caudal forebrain stump along the midline by the olfactory nerve, with hemisphere regeneration complete by six weeks postsurgery.

A single intracranial injection of 15,000 biological units of Nerve Growth Factor (NGF) at the time of forebrain removal resulted in an increased cellular density within the anterior cavity of the brain and olfactory nerve reinnervation of the forebrain stump by day ten, a time course significantly earlier than that seen in controls. The olfactory nerves also showed a greater cellular density with NGF treatment as determined by light microscopic analysis, and hemisphere lengths were significantly longer by day ten than controls, with anterior closure of the ventricles. Animals kept in daily changes of 2500 cc dechlorinated water with .5 mg d-amphetamine from time of surgery until sacrifice showed earlier contact of the olfactory nerve with the regenerating forebrain, earlier closure of the lateral ventricles, and an increase in cellular density and organization of nerve fibers in the olfactory nerve. Treatment with antibodies to NGF at the time of surgery resulted by day ten in a distinct cellular disorganization within the olfactory nerves, with no reinnervation of the caudal forebrain stump and a marked eversion of the lateral walls of the hemispheres showing no signs of ventricle closure. Supported by PHS grant NS14034, RCDA to J.R.P. K04 NS 00213, DHEW 5502-RR-05427-16GRS.

1706 FURTHER STUDIES ON THE BIOCHEMICAL CONSEQUENCES OF OPTIC NERVE CRUSH IN THE GOLDFISH. A.M. Heacock, H.R. Burrell* and B.W. Agranoff. Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48109.

This laboratory has been engaged in studies of the biochemical correlates of optic nerve regeneration in the goldfish. We have previously shown, by means of a double-label in vitro incubation of control and post-crush retinas, that tubulin labeling is selectively augmented within 5 d after optic nerve crush and remains elevated for several weeks. Colchicine binding studies indicate that the retinal tubulin content does not show a comparable increase, suggesting that the altered labeling pattern represented increased tubulin turnover, or that there was increased transport of newly synthesized tubulin out of the retina.

Axonally transported protein was examined following intraocular injection of ^{3}H-proline into control fish or into fish whose optic nerve had been crushed 14 d previously. Contralateral tecta were removed at various times, from 8 h to 30 d following isotope injection, homogenized and separated into soluble and membrane-bound fractions by high speed centrifugation. One and two dimensional acrylamide gel electrophoresis patterns were examined by fluorography and by scintillation counting.

At each time point examined, the amount of radioactivity transported by the regenerating nerve was increased 6-8 fold over controls. Comparisons of the labeling patterns revealed changes during regeneration in several transported proteins. Prominent among these was tubulin, which as expected was confined to the slow phase of axonal transport. Thirty days following injection of precursor, tubulin comprised 19% of the labeled soluble protein in contralateral tecta from control fish, whereas, in the post-crush fish, 36% of the labeled soluble protein comigrated with tubulin.

Recent studies (Burrell et al, in preparation) have demonstrated that tubulin mRNA is increased in the post-crush retina. This finding suggests that the observed alterations in tubulin labeling in the regenerating goldfish visual system are regulated in the ganglion cell nucleus.

1707 A COMPARATIVE STUDY OF CNS NEURON REGENERATIVE ABILITIES. Claire E. Hulsebosch* and George D. Bittner (SPON: Robert G. Grossman). Dept. of Zoology, University of Texas at Austin, Austin, Texas 78712.

One way to interpret current data on nerve cell body regeneration in central nervous systems (CNS) of metazoans is to hypothesize that the ability of a species to regenerate nerve cells correlates with the ability of that species to add CNS neurons during ontogeny. As a corollary to this "neuronal addition" hypothesis, those species in which the CNS nerve cell numbers are constant or decreasing in number during ontogeny would be expected to lack the ability to regenerate ablated nerve cells. To test this corollary, several species in the phylum Annelida were examined for the ability to regenerate ablated CNS nerve cells.

Ganglion, quarter ganglion and single cell ablations were performed in several species of leeches (Hirudo medicinalis, Haemopis grande and Macrobdella sp.), a class of annelids in which the CNS nerve cell numbers are constant within a species. In agreement with the neuronal addition hypothesis, no nerve cell body regeneration occurred in the species of leeches examined.

To test this corollary further, a second species in which we found the CNS nerve cell numbers to be constant, Clymenella torquata, was examined for CNS nerve cell regenerative ability. In this species, regeneration of nerve cell bodies does occur in response to CNS ablations. Consequently, in this case, the ability to regenerate entire CNS neurons would not have been predicted by the neuronal addition hypothesis since this species has a constant number of CNS nerve cells.

However, in agreement with the neuronal addition hypothesis, other species which do add CNS neurons during ontogeny may demonstrate CNS nerve cell regeneration.

(Supported by Kappa Kappa Gamma Fellowship to C.E.H. and NIH grants NS-11861 and NS-14412 and RCDA NS-00070 to G.D.B.)

1708 FURTHER EVIDENCE THAT 4S RNA IS AXONALLY TRANSPORTED IN REGENERATING OPTIC NERVES OF GOLDFISH. Nicholas Ingoglia. Dept. of Physiol. and Neurosci., N.J. Med. School, Newark, N.J. 07103

Following the injection of ^{3}H-uridine into the eye of goldfish, ^{3}H-RNA is found within regenerating optic axons in the optic tectum (Gambetti, et al., 1978, Brain Res., in press). It is likely that this RNA was synthesized in retinal ganglion cell bodies in the eye, and then transported axonally into regenerating optic axons. Indirect evidence indicates that only 4S RNA may be intra-axonal (Ingoglia & Tuliszewski, 1976 Brain Res. 112: 371-381). The present experiments offer two additional lines of evidence that 4S RNA is the predominant, if not the only RNA species transported in this system.

Both optic nerves of goldfish were crushed and 18 ds. later 10.0 ug of cordycepin, an inhibitor of RNA synthesis, was injected into the right eye. ^{3}H-uridine was injected into the same eye 3 hrs. later and trichloroacetic acid soluble and insoluble radioactivity was analyzed from both tecta after 6 ds. The amount of precursor arriving in the tectum was decreased by approx. 60% of control, while the amount of ^{3}H-RNA was decreased by 88%. If 4S RNA is the only RNA species axonally transported, we would predict that the 88% decrease in transported RNA would represent a greater loss in 4S than ribosomal RNAs. Polyacrylamide gel electrophoresis (PAGE) of tectal RNA following the procedures outlined above, showed that ribosomal ^{3}H-RNA was decreased by $\sim$ 70%, whereas ^{3}H 4S RNA was decreased by >90%. These results are interpreted as evidence that the decrease in tectal ribosomal ^{3}H-RNA is due to a decrease in the availability of ^{3}H nucleotides for tectal RNA synthesis, and that the decrease in ^{3}H 4S RNA is due to the same process plus a loss of axonally transported ^{3}H 4S RNA. In the second experiment both optic nerves were crushed in 36 fish and 18 ds. later ^{3}H-uridine was injected into both eyes of all of the fish. One group of 12 fish was killed 6 ds. later and tectal ^{3}H-RNA was characterized by PAGE. The second group of 12 fish had both optic nerves cut 6 ds. after injection and were killed 7 ds. later, allowing for optic nerves in the tectum to degenerate and thus leaving an optic tectum with degenerated optic axons. Group III was killed along with Group II and tectal ^{3}H-RNA was analyzed by PAGE. If 4S RNA is intra-axonal we would predict relatively less ^{3}H 4S than ribosomal RNA in Group II compared to Groups I and III. In Groups I and III approx. 70% of tectal ^{3}H-RNA was 4S, whereas in Group II (tecta in which optic nerves had degenerated) 4S RNA was approx. 25% of total RNA. It is likely that the loss of ^{3}H 4S RNA, in tecta with degenerated optic axons, is due to the loss of axonally transported intra-axonal 4S RNA. Supported by NIH Grant NS 11259.

1709 UTILIZATION OF EMBRYONIC HIPPOCAMPAL IMPLANTS TO PROMOTE REGENERATION OF THE CHOLINERGIC INPUT TO THE HIPPOCAMPUS IN THE ADULT RAT. Lawrence F. Kromer, Anders Björklund* and Ulf Stenevi*. Dept. of Histology, Univ. of Lund, Lund, Sweden.

Embryonic hippocampal (HPC) tissue (taken from 20-30 mm embryos) was transplanted into a cavity produced by transecting the fornix-fimbria at the septal end of the HPC in adult rats. After 6-15 weeks survival in situ, both the implant and host HCP were analyzed for the presence of acetylcholine esterase-positive (AChE) fibers. Only those specimens which possessed a complete transection of the fornix-fimbria were used in the present experiments. Animals with fornix-fimbria transections but no implants served as controls.

At 6 weeks survival, the HPC implant is in contact with the septum-fimbria rostrally and the host HPC caudally. Regenerating AChE fibers enter the implant only at regions where it has fused with the host fornix-fimbria, septum, the contralateral HPC formation, or the anteriodorsal thalamus. An AChE terminal plexus extends throughout the implanted tissue. This plexus is sparse at the early survival time, but by 3 months it has developed a density similar to that observed in the normal adult HPC. AChE fibers are also observed to grow into the adjacent regions of the host HPC. At 6 weeks, cholinergic fibers are present only in the most rostral regions of the host HPC which are immediately adjacent to the HPC implant. However, there is a progressive invasion of the host HPC by these regenerating fibers so that by 3 months, a moderately dense plexus of AChE fibers has formed within the dorsal HPC. This plexus is densest in the hilar region of the dentate gyrus and among the subicular neurons. Cholinergic axons are also present in regions of the dentate molecular layer nearest the implant, in strata oriens and radiatum of regio inferior and the area of regio superior which is adjacent to the subiculum. More caudally, the AChE fibers gradually disappear with no fibers being present in the most caudal regions of the dorsal HPC or within the dorsal fornix, fimbria, or cingulate bundle. AChE fibers are absent from the entire dorsal HPC of control animals.

The results suggest that intracerebrally matured HPC implants are capable of stimulating the lesioned cholinergic neurons of the adult host brain to regenerate a new terminal system within the implant. The HPC implants also provide a tissue bridge and favorable environment whereby regenerating axons can reach their appropriate postsynaptic target neurons within the adult CNS. In addition, our experiments demonstrate that lesioned adult neurons can regenerate new axons that are capable of growing for considerable distances to reach their normal target neurons provided there is a favorable environment for their regeneration.

Supported by PHS grant IF 32 NS 05528-01.

1710 ELECTRON MICROSCOPIC COMPARISON OF THE MECHANISM OF AXONAL ELONGATION IN TRANSECTED SPINAL CORDS AND SCIATIC NERVES. R. Liu* D. Rigamonti, J. Wrathall* and C.C. Kao* (SPON: B. Hamilton). Department of Anatomy, Georgetown Univ. Schools of Medicine and Dentistry, Washington, D.C. 20007.

In studying central nervous systems (CNS) regeneration, much can be gained by comparing events in the CNS to those during regeneration of the peripheral nervous system (PNS). In the CNS, despite a sharp and bloodless spinal cord transection, outgrowth of the transected axons from the cut ends of the spinal cord rarely occurs. In the PNS, in contrast, the transected axons emerge, as a rule, from the proximal cut end of the nerve. The reason for this difference between the CNS and PNS is not well understood.

Recent electron microscopic studies of tissue sections obtained at the cut ends of both the spinal cord and the sciatic nerve within minutes to hours after transection have provided some explanations for this difference. In both CNS and PNS, passive leakage of axoplasm from cut ends of fibers occurred almost immediately after transection, thereby shortening the axon. Consequent to the escape of axoplasm, the fiber near the cut end was devoid of axoplasm. The actual end of the axon was set back 1 to 2mm, or sometimes further, from the cut end of the fiber with an axoplasm-free sheath tube extending to the point of transection. Thus, the first requirement necessary for the proximal axon to emerge from the proximal end of the fiber was its advancement within the 1 to 2mm empty sheath tube, which included at least 1 or 2 nodes of Ranvier, to reach the cut end.

In the PNS, the axon advanced wihin its own sheath tube and successfully reached the proximal cut end and then emerged from the cut end of the nerve. The sheath tube in the PNS, consisting of myelin sheaths, Schwann cell cytoplasm and a basement membrane, provided a durable structure for the passage of the axon that was uninterrupted as the nodes of Ranvier were also ensheathed by the continuous neurilemma basement membrane.

The CNS myelin sheaths were without an outer basement membrane. Furthermore, the nodes of Ranvier were normally bare. Loss of axoplasm at the node was seen to cause actual disconnection of the internodal myelin sheaths. Thus, the spinal cord axons were not only unable to advance within the internodal myelin sheaths due to many mechanisms reported previously (J.Neuropath. Exp. Neurol. 36: 140, 1977) but also were unable to cross the nodal space to enter the next paranodal myelin sheath due to the actual discontinuity at the node. Axonal elongation in the CNS after transection was only seen in the form of naked axons advancing within the interstitial space, towards the cut end of the spinal cord.

Supported by: NIH - NS14413-01

1711 *PROTEIN SYNTHESIS AND FAST AXONAL TRANSPORT IN REGENERATING* GOLDFISH RETINAL GANGLION CELLS: EFFECT OF A CONDITIONING LESION. Irvine G. McQuarrie and Bernice Grafstein. Dept. Physiol., Cornell University Medical College, New York, NY 10021.

Axonal outgrowth following a lesion of the goldfish optic axons (testing lesion) was enhanced if a similar lesion (conditioning lesion) had been made 14 days earlier. With the testing lesion alone, the delay preceding axonal outgrowth was 4.3 days and the elongation rate was 0.34 mm/day; the regenerating axons began to arrive at the contralateral optic tectum 13-17 days after the testing lesion. When the testing lesion had been preceded by a conditioning lesion, the delay decreased to 2.5 days and the elongation rate increased to 0.74 mm/day; axons arrived at the tectum by 7-11 days.

Protein synthesis and fast axonal transport were also altered as a result of a conditioning lesion. With a testing lesion alone, the incorporation of tritiated proline into the ganglion cells and the amount of newly-synthesized labeled proteins entering the optic axons increased together to reach a peak of 5 times normal at 15 days postoperative. When a conditioning lesion had preceded the testing lesion, incorporation at 1 day following the testing lesion remained at the high level that it had reached at the time the testing lesion was made, whereas the amount of fast-transported protein showed a 70% increase. By 8 days after the testing lesion, incorporation had increased a further 60% but the amount of fast-transported protein had declined nearly to the level that would have been seen in the absence of a conditioning lesion. Thus, the improvement in axonal outgrowth resulting from a conditioning lesion is associated with a transient early increase in the amount of newly-synthesized protein entering the optic axons, occurring prior to any increase in protein synthesis.

A sham conditioning lesion (i.e., a lesion of the contralateral axons) preceding the testing lesion had an effect in increasing protein synthesis, but there was little or no effect on fast axonal transport; axonal outgrowth was slower than in the conditioning lesion group.

1712 *THE REGENERATING SUPRAESOPHAGEAL GANGLION AND ITS CONTROL OF* WITHDRAWAL RESPONSES IN EARTHWORMS. Thea Mendelson* (Spon: B. M. Twarog. Psych. Dept., SUNY Stony Brook, Stony Brook, NY 11790.

The influence of the supraesophageal ganglion on the withdrawal responses of earthworms (L. terrestris) was investigated using morphological and behavioral techniques. The withdrawal response was elicited by applying a bright light spot to the anterior 10-15 segments of the animal. Normal animals responded primarily by withdrawing from the light, either by contracting the anterior part of the body, or by moving the entire body backward with a series of alternating tail extensions and body contractions. Extirpation of the supraesophageal ganglion resulted in photic responses that were often anterior extensions followed by forward locomotion. Animals tested during the course of ganglion regeneration exhibited a gradually increasing tendency toward withdrawal responses until the normal level was reached again at 28 to 35 days post-op.

At this time, although the ganglion was structurally discrete entity, it was strikingly different from the original. The normal ganglion contains obvious neurosecretory granules within unipolar neurons and abutting the capillary network in the area between the cortex and neuropil. In the 35-day regenerate the absence of neurosecretory products in both neurons and storage area suggested that neurosecretion was not a factor in the control of withdrawal responses. This was confirmed in animals whose supraesophageal ganglia were surgically separated from the rest of the CNS but left in situ. They responded as though the ganglion had been removed entirely.

The normal ganglion has a broad dorsal cortex containing many distinct cell types: large pale-staining unipolar neurons medium sized granule-filled neurosecretory cells, very small cells with little cytoplasm, and dopamine and serotonin-containing cells located in specific areas within the cortex. The ganglion of the 35-day regenerate contained only small, non-fluorescing neuron somata similar to the small cells in the original ganglion. This difference in cell population was reflected in the 65% reduction in size of the regenerated ganglion. Furthermore, the neuropil was disproportionately decreased in comparison to the cortex. It was not clear whether a decrease in the size or number of neurons, a decrease in arborization of the neuron processes, or a combination of both produced the decreased size. However, the necessary circuitry to control the withdrawal reflex is contained within this minimally regenerated ganglion.

1713 MATURATION OF REGENERATED SPINAL CORD SEGMENTS IN XENOPUS TADPOLES. M. E. Michel* and P. J. Reier. Dept. Anatomy, Sch. Med. Univ. MD., Baltimore, MD. 21201.

During spinal cord regeneration in Xenopus laevis tadpoles, continuity between the rostral and caudal cord stumps is re-established within 10 days following transection. This process consists of ependymal and neuroblast outgrowth from the rostral and caudal cut ends. In the present investigation, the spinal cords of Stage 54 tadpoles were transected within the lumbar region. The morphology of the regenerated cord segment was studied with the light and electron microscope at post-operative intervals between 2 to 6 weeks. Extensive maturation of the reconstituted cord had ocurred by the fourth week; thereafter, only subtle differences in fiber and cell number were observed. Ependymoglia in this region either surrounded a central canal or aggregated into cellular clusters at the blind ends of central canal diverticula. The organization of gray matter in this segment did not conform with the appearance of the mantle layer at more rostral and caudal levels. The parenchyma of the regenerated cord primarily consisted of unmyelinated axons, ependymoglial processes and oligodendrocytes. The population of myelinated axons progressively increased during the period studied but remained substantially less than that seen at levels within 400 μm of the lesion site. Specifically, large caliber, descending axons within the rostral ventrolateral funiculus were less frequent near the lesion site; even fewer of these axons were observed within the regenerated segment. The animals from which cord specimens were obtained had advanced to later prematamorphic and early postmetamorphic stages of development by the time they were sacrificed. As seen in a companion study of normal spinal cord maturation and within more rostral segments of the operated cords, a considerable increase in fiber number and further cellular maturation were seen during the larval periods represented in this investigation. These observations indicate that reconstitution of a cord segment in this system is occurring during relatively dynamic maturational periods. Such further normal development of the spinal cord, however, does not appear to be reflected by corresponding changes in the axonal and cellular content of the restored segment. (Supported by NIH Grant NS-13836 and the Paralyzed Veterans of America).

1714 "HYBRID" SYNAPSES PRODUCED BETWEEN SOMATIC AXON TERMINALS AND AUTONOMIC NEURONS. W. R. Proctor, S. Frenk* and S. Roper. Depts. Anat. and Physiol., Univ. Colo. Med. Cntr., Denver, CO 80262.

To study the factors which govern how regenerating nerve terminals recognize and re-establish synapses with target cells, we have attempted to reinnervate parasympathetic ganglion cells in the frog heart with a somatic motor nerve. Our aim was to determine the influence postsynaptic targets have on the anatomy, physiology, and pharmacology of nerve endings, and vice versa.

Vagal preganglionic axons terminate in boutons on the cell bodies of cardiac ganglion cells. These synapses are cholinergic and are fairly resistant to α-bungarotoxin (α-BuTX). In contrast, motor nerves in the frog form long branch-like endings without marked varicosities. Furthermore, neuromuscular transmission is highly sensitive to α-BuTX. We here report the properties of functional connections which have been formed by cross-innervating denervated autonomic neurons with the hypoglossal nerve.

These results were obtained as follows: the left hypoglossal nerve (1st spinal motor nerve) was cut and its proximal end sutured to the distal end of the sectioned left vagus nerve. The proximal ends of both vagus nerves were tied into the skin to prevent reinnervation. As a further precaution against spurious vagal reinnervation, in some animals the remaining central stump of the left vagus nerve was resected 7-14 days before analysis.

Ten to 18 weeks after the original operation, somatic innervation to the heart was first tested by stimulating the left hypoglossal nerve at its exit from the spinal cord. Successful cross-innervation was shown by a rapid and complete block of the heart beat caused by repetitive stimulation of the hypoglossal nerve. Next, the heart and the entire length of the cross-innervating hypoglossal nerve (which could easily be traced into the heart) was removed and placed in a chamber for intracellular recording. Stimulating the hypoglossal nerve evoked excitatory postsynaptic responses in 86% of the ganglion cells (23% of these received suprathreshold responses). The conduction velocity of axons reinnervating the ganglion from the hypoglossal was about 0.5 M/sec. Light microscopic (zinc-iodide staining) and electron microscopic evidence showed that the hybrid synapses looked more like vagal boutons and unlike motor end-plates. In addition, α-BuTX studies showed that transmission at these hybrid synapses resembled that in autonomic ganglia; they were resistant to concentrations of α-BuTX which completely abolished neuromuscular transmission in the frog (10^{-8}M).

Our studies suggest that in the frog cardiac ganglion, the target tissue has a pronounced influence on some features of reinnervating pre-terminal endings and furthermore, that the postsynaptic cells retain their transmitter receptor characteristics.

1715 PROPERTIES OF INDIVIDUAL SENSORY AND MOTOR NEURONS ISOLATED FROM THE LEECH CNS AND MAINTAINED IN CELL CULTURE. Donald Ready and John Nicholls. Dept. Neurobiology, Stanford Univ. Med. School, Stanford, CA 94035.

Sensory cells, motor neurons and interneurons in the CNS of the leech show remarkable powers of regeneration. Both in the animal and in organ culture axons grow across the site of a lesion to reform synaptic connections with a high degree of precision. As a next step in approaching the mechanisms that cause neurons to sprout and to send their axons towards appropriate targets, we have devised a technique for isolating single cells, and keeping them alive for prolonged periods.

The connective tissue capsule surrounding a ganglion is cut, exposing the neuronal cell bodies and washing away glial cytoplasm. An individual neuron can be readily identified by its shape, size, position and electrical properties. A noose of fine nylon monofilament is slipped over the neuron and pulled, tying off the cell body. The neuron is then removed by gently pulling the nylon thread away from the ganglion. Individual sensory cells responding to touch, pressure or nociceptive stimuli, Retzius cells, motor neurons and interneurons isolated in this way survive for periods of three weeks or more in medium consisting of Leibovitz 15 with 2% fetal calf serum. Such cells continue to give characteristic action potentials, to produce afterpotentials following trains of impulses and to respond to transmitters applied to the soma.

When single neurons or groups of cells are applied to cover slips coated with polylysine they adhere to the surface and send out sprouts. After about four days a profuse arborization develops and in certain instances adjacent cells become coupled by low resistance, nonrectifying electrical junctions.

Such preparations promise to be valuable for studying the ability of identified neurons to form synapses in vitro and the chemosensitivity of invertebrate neurons and their processes.

Supported by USPHS grant NS 11544

1716 PENETRATION OF IMPLANTED ASTROCYTIC SCARS BY REGENERATING AMPHIBIAN OPTIC NERVE FIBERS. Paul J. Reier, Department of Anatomy, University of Maryland School of Medicine, Baltimore, MD. 21201

Previous studies of optic nerve regeneration following surgical transection in amphibian and teleost species suggest that glial scars formed by reactive astrocytes do not impede axonal outgrowth. In Xenopus tadpoles, such scars consist of a loose matrix of astrocytic processes at the time when the first outgrowing neurites enter the degenerating, distal nerve stump. The present investigation was undertaken to determine whether regenerating axonal sprouts in this system are capable of penetrating a dense glial scar. Glial scars were produced in the optic nerves of post-metamorphic, juvenile Xenopus by unilateral enucleations. By 30-40 days the degenerated optic nerve consisted almost entirely of perikarya and densely-packed processes of hypertrophic astrocytes. These degenerated nerves were removed and cut into 0.5-1.5 mm segments. The optic nerves of Stages 54-56 tadpoles were then transected with the entire distal nerve stump being removed. One or more segments of the prepared glial scar was immediately implanted at the cut end of the proximal nerve stump. The tadpoles were sacrificed 2-14 days later, and the astrocyte allograft was examined with the electron microscope; no rejection of the implant occurred within this two week period. The astrocytes within the graft remained hypertrophic; their processes were highly-compacted. By 7-10 days, numerous, unmyelinated axons were present within the implant and appeared to extend the full length of the implanted scar. Growth cones were observed both deep within the implant and at its surface. These observations demonstrate that under the conditions of this experiment, an extremely dense glial scar formed by mature, hypertrophic astrocytes, does not constitute a barrier to axonal outgrowth. In mammals scars formed by reactive astrocytes are believed to inhibit axonal outgrowth. Whether neuroglial scarring in this system is comparable to that occurring in mammals remains to be determined; nevertheless, these results emphasize the need for re-evaluating the significance of astrocytic scarring in the failure of mammalian CNS regeneration. (Supported by NIH Grant NS-13836 and the Paralyzed Veterans of America).

1717 AN INVESTIGATION OF SELECTIVE REINNERVATION OF RAT SKELETAL MUSCLES. Dan A. Riley. Dept. Anat., Sch. Med., UCSF, San Francisco, Calif. 94143.

Most workers examining reinnervation of mammalian skeletal muscles conclude that the process is non-selective. However, Hoh (J. Physiol. 251, 1975) reported that in the rat the soleus nerve cross-reinnervated, at best, 50% of the fast EDL muscle whereas self-reinnervation of the slow soleus muscle was nearly 100%. In contrast, the nerve of a fast muscle was able to reinnervate fully both fast and slow muscles. The apparent inability of the soleus nerve to innervate the EDL muscle was investigated further in the present study. Although EDL muscles are composed primarily of fast fibers, there is a small population of slow fibers. The possibility exists that soleus nerve fibers preferentially reinnervate slow fibers leaving fast fibers denervated. This was checked in 16 rats in which the soleus nerve was directed to reinnervate the EDL muscle under optimal conditions, i.e., no competition from the EDL nerve and the soleus muscles were removed completely to prevent self-reinnervation. Three to 8 wks following surgery, the degree of innervation was assessed by comparing the tension generated by soleus nerve stimulation with that obtained by massive direct stimulation of the whole muscle. Functional innervation of individual EDL fibers was affirmed by the glycogen-depletion technique of Kugelberg and Edström (J. Neurol. Neurosurg. Psychiat. 31, 1968). Serial cryostat sections were stained histochemically with the PAS reaction for glycogen and for myosin ATPase and NADH dehydrogenase activities to identify fiber types and assess the functional state of their innervation. At 3 wks only EDL muscles contracted in response to soleus nerve stimulation. However, 5-8 wks after surgery in over half of the cases neighboring denervated muscles, such as the TA and PL, were innervated by the soleus nerve. In other instances, nerve fibers grew back over the surface of non-denervated muscles without synapsing. Thus, these results indicate that the incomplete reinnervation of the EDL in part accounted for by the failure of soleus nerve fibers to reach the EDL. Even so, tension measurements indicated that on the average 83% of the EDL muscle fibers were cross-reinnervated. Those nerve fibers reaching the EDL functionally innervated both fast and slow fibers. The total number of innervated fibers exceeded the normal number of slow fibers. Therefore, it is unlikely that incomplete reinnervation was due to a failure of soleus nerve fibers to innervate fast fibers. The present results reveal that incomplete cross-reinnervation of the EDL by the soleus nerve is a problem of nerve fiber ingrowth rather than selectivity in forming connections. Supported by a grant from the Musc. Dyst. Assoc. of Amer., Inc.

1718 NEONATAL 6-HYDROXYDOPAMINE-INDUCED NORADRENERGIC SPROUTING IN THE RAT CEREBELLUM: INTRACISTERNAL DOSE-RESPONSE STUDIES. Richard H. Schmidt* and Ranbir K. Bhatnagar. Dept. Pharmacology, University of Iowa, Iowa City, IA 52242.

Treatment of neonatal rats with 100 mg/kg 6OHDA by the subcutaneous route is known to result in derangement of the development of the noradrenergic locus coeruleus system. This is characterized by extensive, permanent loss of norepinephrine (NE) from telencephalic structures and spinal cord, but a marked increase in NE content in the brain stem and cerebellum. As part of a more extensive study of the reasons for this developmental response to 6OHDA, a dose-response relationship for this effect was determined using intracisternal 6OHDA application in order to obviate any influence of the blood brain barrier.

On the day of birth Sprague-Dawley rat pups were distributed 10 per litter and injected intracisternally with 10, 20, 40 or 80 µg 6OHDA or 4 µl vehicle while cold-anesthetized. At 32-35 days of age various brain regions were dissected for NE assay by a radioisotopic method. The cerebellum was cut in a cryostat in .5 mm thick saggital sections from which 7 discrete regions were sampled by micropunching.

At all doses NE content of the cervical and upper thoracic spinal cord was reduced by more than 95%. In several cortical regions NE depletion was 50-75% after 10 µg, and greater than 90% at the 3 higher doses. In the cerebellum 10 µg of 6OHDA resulted in NE levels ranging from 140-190% of control; after 20 µg the range was 190-225%; after 40 µg the range was 120-225%; and after 80 µg the range was 10-20%. At doses of 6OHDA between 10-40 µg, NE content, both absolute and as percent of control, was highest in vermian lobules I-III and IV-VIa and lowest in lobules VIb-VIII and IX-X. There was no tendency for a gradient of NE following proximity to the locus coeruleus.

Intracisternal 6OHDA treatment of 20-40 µg thus duplicates the salient features of 100 mg/kg 6OHDA s.c. At 80 µg NE terminal growth is prevented throughout the brain, but at lower doses occurs to a considerable extent in the cerebellum but not cerebral cortex or spinal cord. The high resistance of the neonatal cerebellar noradrenergic innervation does not appear to be due to a resistance to the degenerative effects of 6OHDA, first because of the lack of a simple dose-response relationship between 10-80 µg, and secondly because 12-48 hours following injection, initial drug-induced depletion of NE exceeds 95%. Regenerative growth and collateral sprouting thus probably account for the recovery and hypertrophy of NE levels in the cerebellum. The neonatal cerebellum may possess some unique attribute which promotes reinnervation from axotomized locus coeruleus neurons, and this attribute is not present in either the cerebral cortex or spinal cord.
(Supported by USPHS grant NS-12121.)

1719 PRELIMINARY STUDIES OF PROTEIN SYNTHESIS IN THE FROG RETINA 1-6 DAYS AFTER SECTION OF THE OPTIC NERVE. Thomas M. Scott and Alen J. Mathewson. Faculty of Medicine, Memorial University of Nfld., St. John's, Newfoundland, Canada A1B 3V6.

While regeneration in the CNS of mammals is limited, reformation of original connections occurs readily in lower animals. Many factors have been cited as contributing to the lack of CNS regeneration in mammals. One of these factors is the ability of the axotomised neuron to synthesise protein necessary for regrowth. These preliminary investigations form part of a larger comparative study of factors involved in CNS regeneration.

The left or right optic nerves were cut, close to the chiasma in 25 rana pipiens. At daily intervals from 1-6 days, each animal was injected I.V. with 30μCi ^{3}H-leucine. Three hours later the animals were decapitated and the retinae removed into 3ml of 0.9% saline. The retinae were homogenised in a glass homogeniser and 50μl were removed for estimation of protein content. 3ml of 10% TCA was then added to the homogenate. 20 minutes later the homogenate was filtered through a millipore filter, rinsed twice with 2ml 5% TCA and once with 3ml 95% ethanol. After drying, the discs were placed in scintillation vials to which 1ml Protosol was added. The vials were then left for 12 hours before adding 10ml Liquifluor and counting in a Beckman LS 9000 scintillation counter. The 50μl of homogenate removed before TCA precipitation were used for protein estimation using the Bio-Rad protein assay.

In this way readings were obtained for cpm/mg protein. This figure was compared for the operated and control retinae and expressed as a ratio. The ratio of operated to control was compared at one to six days. No significant difference was found, in incorporation rate into the TCA precipitable fraction between regenerating and control eyes from one to six days after section of the optic nerve.

While no similar studies have been reported in frogs, Grafstein and Murray (Exp. Neurol. 25, 494-508, 1969) have reported an increase in transport, perhaps reflecting an increase in synthesis, occurring between 6 and 8 days after section of the optic nerve in the goldfish. It is intended to extend these studies to cover longer periods.

1720 BIOCHEMICAL EFFECTS OF NERVE GROWTH FACTOR ON NORMAL AND AXOTOMIZED SUPERIOR CERVICAL GANGLIA FROM ADULT RATS MAINTAINED IN VITRO. D.V. Sinicropi* and F.C. Kauffman. Dept. Pharmacology & Exp. Ther., School of Medicine, Univ. Maryland, Baltimore, MD. 21201.

Conditions were established for the culture of adult superior cervical ganglia to determine if biochemical alterations initiated by axotomy in situ are maintained in vitro. Whole desheathed ganglia from 150-175 g rats (7-8 weeks of age) were placed on stainless-steel grids just below the atmosphere-media interface and cultured in 200 μl of Eagle's minimum essential medium supplemented with 10% (v/v) fetal calf serum. Incorporation of leucine-H^{3} into acid precipitable material of cultured ganglia was linear for at least 48 hours. In contrast, concentrations of ATP and phosphocreatine in the tissue were reduced more than 5-fold after 21 hours in vitro. NAD:NADH ratios, calculated from measured levels of pyruvate and lactate, decreased to approximately 20% of control (not cultured) values within 6 hours and subsequently remained constant for at least 21 hours. Despite this rapid decline in energy status of the cultured tissue biochemical changes elicited by axotomy are maintained.

Enhanced activity of 6-phosphogluconate dehydrogenase (6PGDH) and elevated protein content that occur after axotomy of the ganglion in situ (Harkonen and Kauffman, Brain Res. 65: 141-157, 1974) are maintained in culture as indicated in the table below.

hours culture		6PGDH activity $\mu mol \cdot min^{-1} \cdot g\ protein^{-1}$	protein content $\mu g \cdot ganglion^{-1}$
		NORMAL GANGLIA	
0		7.17 ± 0.15	62.5 ± 2.0
48	-NGF	8.81 ± 0.26	85.7 ± 2.0
	+NGF	10.68 ± 0.53	71.8 ± 3.0
		2-DAY AXOTOMIZED GANGLIA	
0		8.77 ± 0.20	92.9 ± 4.2
48	-NGF	12.62 ± 0.52	117 ± 6.0
	+ NGF	11.35 ± 0.60	105 ± 6.9

The increase in 6PGDH activity that occurs in normal ganglia placed in culture is enhanced by nerve growth factor (NGF). NGF did not enhance the activity of axotomized ganglia maintained in vitro. These results support the hypothesis that selective increases in metabolism via the pentose pathway occur in nerve cell bodies subsequent to axonal injury, and suggest that similar biochemical alterations are elicited in normal ganglia maintained in vitro.

1721 RETINAL GANGLION CELL RESPONSE TO AXOTOMY AND NERVE GROWTH FACTOR IN THE REGENERATING VISUAL SYSTEM OF THE NEWT: AN ULTRASTRUCTURAL MORPHOMETRIC ANALYSIS. James E. Turner and Rebecca K. Delaney*. Dept. Anat., Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27103.

Nerve growth factor (NGF) treatment, given as a single 200 BU intraocular injection at the time of optic nerve transection, was found to significantly alter the retinal ganglion cell response to axotomy in the newt (Triturus viridescens). In the control series the percent of neurons in the retinal ganglion layer demonstrating nuclear reactivity (i.e., chromatin changes) reaches a peak by 14 days post axotomy (14 DPA), plateaus through 21 DPA and falls thereafter returning to control levels by 90 DPA. NGF treatment is shown to significantly accelerate the entrance of responding retinal ganglion cells into the reactive nuclear phase between 1-7 DPA and by 7 DPA nuclear reactivity has reached a peak, in contrast to 14 DPA for control values. Consequently, NGF treatment causes retinal ganglion cells to remain in the nuclear reactive state a week longer than controls but reactivity diminishes after 21 DPA like control values. Electron microscopic morphometric analysis further substantiates these observations by demonstrating that NGF treatment can elicit certain cellular organelle changes a week earlier (i.e., at 7 DPA) than they would normally occur (i.e., at 14 DPA). In addition to eliciting cellular hypertrophy at 7 DPA, NGF treatment significantly increases mitochondrial and Golgi field densities in the neuronal perikaryal cytoplasm as well as a doubling of the number of nucleoli per nucleus and stimulates a significant increase in nucleolar cross sectional areas. A dose response relationship exists between the percent of retinal ganglion cells demonstrating nuclear reactivity at 7 DPA and various NGF concentrations which compares favorably with the dose response study involving the number of regenerating axons per nerve cross section at 14 DPA. Studies to determine if the NGF mediated responses were a specific effect elicited by this protein molecule or whether they are also produced by other peptides which share some properties in common with NGF demonstrate that only NGF is capable of eliciting these responses.

Supported by a Basil O'Connor Starter Research Grant from the National Foundation March of Dimes; a grant from the National Society for the Prevention of Blindness made possible through the Adler Foundation and an NIH grant NS 12070 awarded to Dr. Turner. Dr. Turner is also the recipient of an NIH Research Career Development Award NS 00338.

1722 REINNERVATION OF RAT EXTENSOR DIGITORUM LONGUS MUSCLES AFTER AUTOGENOUS FREE GRAFTING. Kenneth R. Wagner, Bruce M. Carlson*, and Stephen R. Max. Depts. Neurol. & Peds., Univ. Md., Sch. Med., Baltimore, MD 21201 and Dept. Anat., Univ. Mich., Sch. Med., Ann Arbor, MI 48104.

Morphological and biochemical studies were undertaken to determine the time-course and extent of reinnervation of freely-grafted muscles. Extensor digitorum muscles were removed from rats and injected with a solution of 0.75% Marcaine. After incubation in Marcaine solution for 10 min., the muscles were grafted into their original beds (Carlson, Exp. Neurol., 52:421, 1976). Grafts and contralateral control muscles were removed at 4, 7, 11, 15, 19, 25, 31 and 90 days postoperatively. One series of muscles was examined histologically. A parallel series was assayed for the activity of choline acetyltransferase (CAT), a marker for formation of cholinergic synapses. CAT was 2% control by day 4 and 6% by day 7. Between days 7 and 11 there was a 4-fold increase in activity, to 27% control. After this time, CAT rose continuously to 60% control by day 90, an overall increase of 30-fold. Morphological data to be presented indicate that early changes in CAT are closely correlated with the pattern and time-course of reinnervation of the grafts. These results indicate incomplete innervation of grafted muscles which may be one factor accounting for the incomplete recovery of fresh weight (< 50%) and a number of enzyme activities observed previously (Wagner et al., J. Neurol. Sci., 34:373, 1977). For maximal functional development of transplanted muscles, efforts should be directed to facilitation of reinnervation. (Supported by grants from the Muscular Dystrophy Assn., Inc., NIH - NS 13116 and the National Amyotrophic Lateral Sclerosis (ALS) Foundation).

1723 POLYACRYLAMIDE GEL SEPARATION OF AMINO ACID LABELLED PROTEIN FROM BRAIN AND SPINAL CORD AFTER SPINAL CORD HEMISECTION IN THE RAT. M. R. Wells, Dept. of Neurosci., Univ. of Fla., Gainesville, Fla.,

Following spinal cord hemisection in the rat, general increases in amino acid incorporation into protein occur in brain and spinal cord which appear to be associated with operative stress (Wells and Bernstein, Exp. Neurol. 57: 900-912, 1978). Local increases in protein incorporation also occur in the spinal cord at the site of lesion between one and three days postoperative. Proteins mediating these changes have been studied in the present experiments. Male, Long-Evans Hooded rats were given a laminectomy and dura cut (sham) at spinal segment T2, a left spinal cord hemisection, or no operative procedures. One hour prior to utilization at 1, 3, and 14 days postoperative, animals were injected subcutaneously with 200μCi of [^{3}H]-L-lysine and 200μCi of [^{3}H]-L-amino acid mixture. Samples were taken from somatomotor cortex and left side of spinal cord extending 2mm rostral and caudal to the lesion. Samples were prepared for electrophoresis and protein separated on 3.0mm, gradient (7.5-18.75% acrylamide), SDS polyacrylamide slab gels. Gels were fixed in trichloroacetic acid, stained with Coomassie Blue, photographed, cut into slices, and processed for scintillation counting.

In somatomotor cortex there was evidence for a general stimulation of amino acid incorporation at one day postoperative in sham and spinal hemisected animals. At three days proteins in the regions of 70-80,000 molecular weight (MW) and 50-65,000 MW showed transient increases in radioactivity compared to normal, while protein in the region of 35-50,000 MW decreased in both sham and hemisected groups. At 14 days only an increase in radioactivity of heavy molecular weight proteins (>125,000) was present in both operated groups. In spinal cord at one day postoperative, significant increases ($p<0.05$) occurred in the radioactivity of proteins of 10-20,000 MW, 45-55,000 MW, and 70-80,000 MW in both sham and hemisected animals. At three days postoperative, spinal hemisected animals exhibited significant increases in the radioactivity of proteins in the regions 40-55,000 MW. By 14 days postoperative patterns of radioactivity had returned to normal except for an increase in the region of 10-35,000 MW for sham and hemisected animals. The above data indicate that increases in amino acid incorporation into brain and spinal cord of laminectomized and spinal hemisected animals have specific and nonspecific components. (Supported by a grant from NINCDS [NS-06164] and the Paralyzed Veterans of America).

1724 SYNAPTIC REGENERATION IN THE LAMPREY SPINAL CORD. Malcolm R. Wood* and Melvin J. Cohen. Dept. of Biol., Yale Univ., New Haven CT 06520

Spinal cord regeneration in the larvae of lamprey has been previously reported based on the return of swimming and the tracing of axons with light microscopy following spinal transection. Our aim in this study was to determine, ultrastructurally, whether identified regenerated spinal neurons establish synaptic contact distal to the lesion. We find clear ultrastructural evidence that severed spinal axons grow across the lesion and establish synaptic connections.

Larvae of the sea lamprey Petromyzon marinus were anaesthetized and the spinal cord was transected. There is no swimming for several days following spinal transection. By 4-5 weeks coordinated swimming has returned in 90% of the survivors. In the functionally recovered animals, 4% horseradish peroxidase (HRP) was intracellularly injected by iontophoresis into the cell bodies of identified giant reticulo-spinal neurons (Mauthner and MUller cells) at postoperative intervals from 30 to 200 days.

In cleared whole mounts of the brain and spinal cord, axons of the injected neurons often branch and run parallel to each other as they approach the lesion. Some of the regenerating neurites cross the lesion and proceed up to 4mm into the distal region of the cord. Other branches of the same neuron may make a sharp "U" bend at the lesion and return toward the brain, while still other neurites terminate at the lesion. After crossing the lesion, the regenerating axons may also branch several times. Some of these neurites may then turn and grow rostrally to re-cross the lesion and end in the proximal part of the cord. Thick (5μm) serial sections show that branching may start 1-2mm proximal to the lesion. The growing fibers on each side of the cord diverge laterally from their normal mid-ventral location and grow toward the periphery of the cord. After crossing the lesion, they grow distally in the periphery of the cord and show little affinity for the mid-ventral area where the distal axonal stumps of these neurons have degenerated.

Electron microscopic examination of regenerating axons filled with HRP show an overall darkening of the filled processes. Regenerating axons distal to the lesion bear numerous club-shaped spines (up to 1.7mμ in length) that are frequently filled with spherical lucent vesicles. Spines containing these vesicles form typical synaptic junctions consisting of para-membranous densities and a uniform membrane separation characteristic of a synaptic cleft. The regenerated spines are always presynaptic and the postsynaptic element is invariably a small process. Synapses are also found on the regenerating neurite in regions devoid of spines, and here also the marked process is invariably presynaptic.

Our observations provide direct ultrastructural evidence that regenerating spinal axons can form new synaptic junctions distal to a lesion. This preparation provides a model system with identified elements for investigating some of the factors controlling synaptic regeneration in the vertebrate central nervous system. (Supported by NIH grant 5RO1 NS 08996).

1725 FUNCTIONAL REPAIR OF INJURED PERIPHERAL NERVE TISSUE WITH NERVE ALLOGRAFTS BEARING ONLY MINOR TRANSPLANTATION ANTIGENS. Andrew A. Zalewski and Willys K. Silvers*. LNC, NINCDS, NIH, Bethesda, Md. 20014 and Dept. of Human Genetics, Univ. of Pa. Sch. Med., Philadelphia, Pa. 19174.

We previously reported that neurilemmal (Schwann) cells survived longer in allografts which contained minor rather than both major and minor transplantation antigens. Since the neurilemmal cells of a nerve aid nerve fiber regeneration we investigated whether host motor nerve fibers would regenerate through a nerve allograft and reinnervate denervated muscles. Inbred Fischer and Lewis rats which differ only in minor antigens were used. Injury to nerve tissue which resulted in the denervation of the extensor digitorum longus (EDL) and tibialis anterior (TA) muscles was produced by removing a 1.4 cm segment of the host peroneal nerve and replacing it with a 1.8 cm peroneal nerve allograft. After 110 days myelin could be seen grossly throughout the length of nerve allografts and staining of these tissues showed the presence of nerve fibers, myelin and occasional foci of lymphocytes. Stimulation of the peroneal but not the tibial nerve of allografted animals caused a contraction of the EDL and TA muscles which now weighed 70-85% of normal. Sections of muscles revealed the presence of nerve fibers, neuromuscular junctions, and large-diameter muscle fibers which were localized into larger type-groups than normal.

In other studies rats, which had nerve allografts in residence for 110 days, were grafted intramuscularly with a skin or ganglion allograft that bore the same minor antigens as the nerve allograft. As expected these skin and ganglion allografts were rejected, but their rejection was not accompanied by any cellular infiltration of the nerve allograft or by any deterioration of muscle innervation. What apparently had happened was that allogenic cells in the nerve graft survived long enough to permit host nerve fiber regeneration and then they were rejected and replaced by host cells, some of which myelinated the nerve fibers. This seems to be the case since acute myelin degeneration and lymphocytic infiltrations were observed in long-term nerve allografts when previously established tolerance to alloantigens of the nerve donor was broken. It is of interest that allografts cultured overnight at 4° C later permitted host nerve fiber regeneration through them while still maintaining their antigenicity. Our results demonstrate that it is possible to rapidly and functionally repair a large injury to peripheral nerve tissue with a nerve allograft bearing minor antigens in nonimmunosuppressed rats and we suggest such allografts be used in man.

SLEEP

1726 THE EVOKED POTENTIAL SOMNOGRAM - HUMAN AND ANIMAL STUDIES. R.G. Bickford, R. Hajdukovic*, K. Hanson*, Y. Kammer*, C.B. McCutchen, and J. Franke*. Department of Neurosciences, EEG Laboratory, University of California, San Diego, La Jolla, CA and the Veterans Administration Hospital, La Jolla, CA 92093.

The somnogram (K. Hanson, et al., Proc. San Diego Biomed. Sympos., 13:545-548, 1974) is a computer generated (PDP/12 and PDP/1140) data display employed in sleep and coma studies. It allows the sequential relations of EEG, EOG, EMG, respiration to be viewed over periods up to 48 hours in a highly compressed format (3-6 pages). We have recently introduced evoked potential data into this format so that average evoked responses to click, flash or shock are recorded alongside spectral changes of the spontaneous EEG. This display provides increased information in the following areas: Human. 1) Onset of sleep in the human (see figure below) with the remarkable enhancement of the auditory evoked response (AER). This appears to be a sensitive index of sleep onset. 2) In narcolepsy, there are abnormalities of both frequency spectrum and evoked response. 3) In coma, patients may show a) absence of AER, b) monotonic unchanging AER, c) modulation of the AER as seen in normal sleep. Animal. Clearly depicted sequential changes in the rat AER provide a useful index for staging and for sleep and coma studies in this animal.

Supported by NIH USPHS-NS 08962-10

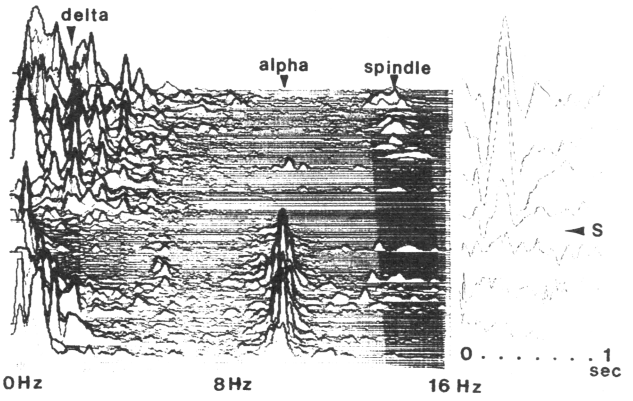


Figure 1. Onset of sleep in normal subject; 30 minute recording.

1727 CIRCADIAN RHYTHMS OF SLEEP, ACTIVITY, AND TEMPERATURE IN THE RAT UNDER ENTRAINED AND FREE-RUNNING CONDITIONS. Charmane Eastman. Sleep Lab., Univ. of Chicago, Chicago, IL 60637.

Long-term recordings of circadian rhythms of three different variables in the rat were made under various lighting conditions to gather normative data and reveal properties of the underlying oscillator(s). Sleep and waking were scored in 30 sec epochs by the computer system of Bergmann et al. (Sleep Res. 6: 206, 1977). Temperature was recorded from thermistors implanted in the intraperitoneal cavity, on the surface of the brain, or cemented to the skull under the temporalis muscle. Tilt-cage activity was registered by a microswitch. All three variables were recorded simultaneously 24 hrs a day and were stored every 30 sec by a PDP-11 computer. Periodograms measured the degree of entrainment to the light-dark (LD) cycles and the period length in free-running conditions. Acrophases, determined by the maximum of the best-fitting cosine wave to the average curves, were used to measure the external phase relationships to the Zeitgeber (LD cycle) and the internal phase relationships between the three rhythms. Data were displayed graphically by computer.

So far, six rats have been run on a 24 hr day (L,D;12,12). After entrainment was established, the rats were assigned to a variety of lighting schedules including DD (dim), LL, increasingly longer days e.g., 26 hrs (L,D;13,13) and 28 hrs (L,D;14,14) and shorter days e.g., 22 hrs (L,D;11,11). Temperature, activity, and waking followed a parallel course, peaking near the middle of dark in the 24 hr day. As predicted by oscillator theory, the phase of all three rhythms advanced relative to the Zeitgeber as the period of the Zeitgeber increased. In constant conditions, the circadian component was gradually diminished as ultradian rhythms in the range of 3 to 6 hrs became more prominent. The period lengths in LL (25.6 hrs) were longer than in DD (24.6 and 24.8 hrs), as predicted by Aschoff's rule.

Some rats in the 22 and 28 hr days showed two frequencies in the rhythm of each variable, one corresponding to the imposed LD cycle and one in the circadian range with period lengths of, for example, 24.8 or 25.4 hrs. The simultaneous presence of two frequencies will give rise to "beats," a waxing and waning in the amplitude of the rhythms. The observed beat frequency matched the predicted beat frequency calculated from the two periods seen in the periodograms. These results suggest that these rhythms are controlled by two oscillators, one of which remains entrained by the LD cycle, and one which breaks away and free-runs through the LD cycle.

1728 EFFECT OF β-(NAPHTHYL-1)-ALANINE, A TRYPTOPHAN ANALOG, ON MONOAMINES IN SPECIFIC BRAIN STRUCTURES AND SLEEP IN RATS. Casimir Fornal*, Walter J. Wojcik, Miodrag Radulovacki, and Hans G. Schlossberger* (SPON: H. K. Proudfit). Dept. of Pharmacol., Univ. Ill. Med. Ctr., Chicago, IL. 60680 and Max-Planck Inst., Martinsried, West Germany.

We showed (Wojcik et al., this meeting) that the administration of L-tryptophan (30 mg/kg, i.p.) to rats affected brain monoamines and sleep. We were interested to see whether DL-β-(naphthyl-1)-alanine hydrochloride (β-NA), a tryptophan analog, had similar effects. In one group of adult rats we analyzed 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA) and norepinephrine (NE) in the cortex (CX), hippocampus (HIPP), pons-medulla (PM) and striatum-thalamus (ST) fifteen and forty-five minutes after a single injection of β-NA (30 mg/kg, i.p.). The animals were sacrificed by decapitation and the monoamines and 5-HIAA were determined by spectrophotofluorometric methods. Fifteen minutes after injection, there was a reduction of DA in the CX by 40% and NE in the HIPP by 16%. In addition, 5-HT levels in the CX were reduced by 10%. 5-HIAA concentration did not change. There was no change in 5-HT, 5-HIAA, DA and NE in all studied structures forty-five minutes after administering the agent.

In another group of adult rats, cortical and neck muscle electrodes were implanted for EEG and EMG recording. One week after surgery the animals were injected with either saline or β-NA (30 mg/kg, i.p.) and polygraphically recorded for six hours. On the following day the same protocol was repeated with the same group of animals but with reverse administration of the agents. Records were analyzed for the amount of time spent in wakefulness (W), slow-wave sleep (SWS), and paradoxical sleep (PS). Furthermore, latencies to first SWS and first PS episodes were determined. Paired t analysis performed at half-hour intervals showed that β-NA increased SWS by 11% and decreased W by 11% during the first half-hour period. We also found that β-NA reduced SWS latency by 50%. There was no change in PS or PS latency. Our data show that SWS latency coincides with the reduction of DA, NE, and 5-HT. Since increased 5-HT turnover has been found to correlate with SWS (Buckingham and Radulovacki, 1975; Kovacevic and Radulovacki, 1976), obtained decrease in 5-HT and no change in 5-HIAA could not account for decreased SWS latency. Therefore, we suggest that the decrease in SWS latency may be due to the reduction of catecholamines in the CX and HIPP.

1729 METHOD FOR INTRACELLULAR RECORDING OF LUMBOSACRAL MOTONEURONS DURING NATURAL SLEEP IN CATS. Loyd L. Glenn, Arthur Poutz and William C. Dement. Stanford University School of Medicine, Stanford, CA 94305.

In order to analyze the changes in motor control during sleep and wakefulness, a method has been developed which enables the intracellular recording of spinal motoneurons in unanesthetized and unparalyzed cats. Cats were chronically implanted for sleep recordings and stimulation of the tibial nerve. The lamina and pedicles of vertebrae L4-L6 were exposed and stainless steel screws inserted into the body of each vertebra. A 1.5 mm opening was drilled 1.8 mm from midline in L5 and the exposed dura mater excised. After a cylinder was placed around the microelectrode opening and knurled nuts placed on each of the adjacent vertebrae, the entire assembly was fused together with acrylic cement. Agar and a cylinder cap temporarily sealed the microelectrode opening. After recovery from surgery, the cats were periodically habituated to simultaneous cranial and lumbral restraint prior to any microelectrode descents. Although it was possible to impale antidromically invaded cells, another identification criterion was used to increase the chances of recording from motoneurons. After either spontaneous discharges or after spikes elicited by short intracellular current pulses, a long and scallop-shaped after-hyperpolarization can be used, according to current knowledge, as a strong indication that the recorded cell is a motoneuron. Ten lumbosacral motoneurons identified by these methods were held 4-15 min covering at least one transition between two different states per cell (wakefulness, NREM, and REM sleep). Three of these cells were recorded continuously through all three states. The main barrier to longer recordings were cord movements during spontaneous postural readjustments by the cat.

Preliminary results indicate little or no change in membrane potential during the transitions from wake to NREM, contrasting with an abrupt hyperpolarization at the onset of REM sleep muscular atonia. This hyperpolarization usually followed a more gradual one just preceding the onset of REM sleep. Although the length of time that individual neurons can be studied is low by comparison to acute studies, this preparation is useful in studies on motor and postural control during sleep and wakefulness.

1730 EFFECTS OF DIAZEPAM ON SLEEP, TEMPERATURE, 5-HYDROXYINDOLEACETIC AND HOMOVANILLIC ACIDS IN CISTERNAL CSF OF CATS. Mindaugas L. Griauzde*, Edward H. Chen* and Miodrag Radulovacki (SPON: L. Isaac). Dept. Pharmacol. Biomed. Prog., Univ. of Illinois, Chicago, IL. 60612.

Administration of benzodiazepines produces a decrease in brain dopamine (DA) metabolism while data on brain 5-hydroxytryptamine (5-HT) metabolism are inconclusive. We have examined the effects of various doses of diazepam (DZ) on sleep, cerebrospinal fluid (CSF) concentrations of 5-hydroxyindoleacetic acid (5-HIAA), a 5-HT metabolite, and homovanillic acid (HVA), a DA metabolite, and rectal temperatures of cats. CSF was obtained from a cannula in the cisterna magna (Radulovacki, 1974). 5-HIAA and HVA were determined by the method of Korf and Valkenburgh-Sikkema (1969). Five days after implantation the animals underwent continuous EEG, EMG and EOG recordings from 9:00 AM until 2:30 PM each day for four days with CSF samples taken at two-hour intervals starting at 10:30 AM and ending at 2:30 PM. Doses of DZ (0.3 mg/kg - 1.5 mg/kg) were administered i.p. at 9:00 AM on experimental days and polygraphic recordings, along with CSF samples and rectal temperature measurements, were taken in the same manner as controls.

Our results show that administration of DZ produced a significant increase ($p < 0.02$) in slow-wave sleep (SWS) with a peak occurring at a dose of 0.9 mg/kg. Further increase in doses of DZ decreased SWS. DZ administration produced no change in REM sleep, rectal temperature, and CSF 5-HIAA and HVA levels. Since no correlation between various doses of DZ and CSF concentrations of 5-HIAA and HVA was found in the presence of an increased percentage of SWS, this suggests a possible mode of action by DZ mediated through pathways other than those associated with normal sleep mechanisms.

1731 THE EFFECT OF SLEEP UPON THE TRANSMISSION OF AFFERENT ACTIVITY IN THE SOMATIC AFFERENT SYSTEM. Gündüz Gücer. Dept. Neurosurgery, Sch. Med., Johns Hopkins Hospital, Baltimore, MD 21205.

Macaque monkeys were trained to fall asleep while sitting in a primate chair with head restrained. A gentle vibratory stimulus was delivered to the glabrous skin of the hand; it did not provoke awakening or change the sleep cycle of the macaque. Postcentral neuronal response to the amplitude of a sine-wave mechanical stimulus and neuronal spontaneous activity were observed repetitively during all the phases of normal night sleep cycles. 106 neurons which could be entrained by a cutaneous mechanical stimulus were studied during both waking and sleep. At threshold, cyclic entrainment of the discharges of postcentral neurons decreased to 81 ± .25% during light sleep (S_1+S_2), to 64 ± .26% during deep sleep (S_3+S_4), and to 9 ± .98% during desynchronized sleep with rapid eye movements (REMs).

The responsiveness of neurons of the primary sensory cortex (Brodmann area 1,2,3) appears to be a balance of the specific thalamocortical input versus the generalized thalamocortical input. During slow wave sleep a progressive increase in the influence of the generalized thalamocortical system is felt to lead to a decrease in postcentral neural entrainment. Superimposed on this decreased entrainment is a further loss of entrainment during REM's of desynchronized sleep which is felt to be secondary to post and presynaptic inhibition at the dorsal column nuclei, in the brain stem.

1732 EFFECT OF HEROIN WITHDRAWAL UPON RAPID EYE MOVEMENT (REM) SLEEP IN HUMANS. Richard C. Howe, Frederick W. Hegge*+ and Jerry L. Phillips*. Dept. of Physiology and Bioengineering, Eastern Virginia Medical School, Norfolk, VA 23501 and +Dept. Experimental Psychophysiology, Walter Reed Army Institute of Research, Washington, D.C. 20012.

The objective of this study was to examine the effect of withdrawal from pure heroin upon various sleep parameters. The data was obtained from young military personnel who were addicted to pure heroin, used no other drugs concurrently, utilized the nasopulmonary route of administration and had short heroin use histories. This setting thus provided a good model to evaluate heroin withdrawal without the many complications associated with statewide drug users.

Electroencephalogram (EEG), electrooculogram (EOG) and other physiological parameters were recorded on a 24-hour per day basis for 5-7 days. Twenty heroin dependent patients and five drug-free controls were studied. Control subjects were non-addicts undergoing the exact same experimental procedures as the addicts. EEG records were manually scored into the standard awake and sleep states. All scored EEG data were transferred to a computer for subsequent analyses.

Preliminary results for REM sleep have been summarized below (see table). Day 1 was not included in the final means because the addicts did not begin withdrawal until midway through Day 1, after they had "nodded out" and/or slept through the first part of Day 1. By contrast, the controls did not sleep well on Day 1 showing the typical "first night" effect. On Day 1, both the controls and heroin addicts averaged approximately the same amount of REM sleep (53-54 minutes). On days 2-5, REM sleep increased for the controls to a mean value of 95 minutes. The addicts during withdrawal (Days 2-5) showed a marked decline in REM sleep to approximately 29 minutes. Also, the heroin addicts during the "nodding out" phase on Day 1 showed less REM sleep (54 minutes) than the control values on Days 2-5 (mean 95 minutes). These results indicate that REM sleep time is significantly decreased during heroin withdrawal. Further analyses of interstate intervals, duration and frequency of REM periods is necessary in order to examine the biological periodicity of this state during heroin withdrawal.

REM
(mean values in total minutes)

DAY	CONTROLS	ADDICTS
1	53.2	53.9
2	73.7	26.0
3	91.3	23.2
4	112.0	30.7
5	103.6	35.1
MEAN+	95.2	28.8*
S.D.	16.6	5.2

+Days 2-5 *p<.005

1733 L-TRYPTOPHAN AND METHYSERGIDE EFFECTS ON SLEEP IN CATS. Herbert L. Jackman and Miodrag Radulovacki. Dept. Pharmacol., Univ. Ill. Med. Ctr., Chicago, IL 60612.

5-Hydroxytryptamine (5HT) has been implicated in the mediation of sleeping behavior (Jouvet, 1969). The exact role of 5HT in sleep mediation probably involves an increased turnover of the amine at critical CNS sites. The administration of L-tryptophan (L-TRYP) is the most appropriate method of increasing 5HT's concentration (Wurtman and Fernstrom, 1975) and turnover (Radulovacki, 1974) in the CNS. It may then be possible to manipulate sleeping behavior by the administration of L-TRYP. Methysergide (ME) is a 5HT receptor blocker and should antagonize the effects of 5HT action.

Two groups of cats were surgically prepared with EEG, EMG, and EOG leads to evaluate sleep states during a 6-hour experimental sleep period. EEG records were evaluated to determine latency to first slow-wave sleep (SWS) and first paradoxical sleep (PS) episode and the percent time spent in either wakefulness (W), SWS or PS was also determined.

The first group of cats was treated with 30 mg/kg L-TRYP, but showed no significant changes in sleep pattern. The second group of cats was treated with 0.5 mg/kg ME and showed large changes in sleep patterns. This group of cats was then pretreated with 30 mg/kg L-TRYP and significant reversals toward normal sleep patterns were seen (Table).

	Placebo + ME	L-TRYP + ME
First SWS Episode (Latency in min.)	160.4 ± 6.5	116.8 ± 6.4*
First PS Episode (Latency in min.)	324.5 ± 16.8	254.1 ± 9.1*
Total % W	67.8 ± 6.8	52.3 ± 3.4*
Total % SWS	30.7 ± 6.3	45.0 ± 3.2*
Total % PS	1.5 ± 0.6	2.7 ± 0.4 ns

The results seen with L-TRYP pretreatment in ME treated cats point to separate and independent mechanisms for SWS and PS. This experiment also shows that sleep patterns disrupted by ME can be partially reversed by L-TRYP pretreatment. This response could be predicted if L-TRYP enhances those parts of the sleep mechanism mediated by 5HT and whose receptors are sensitive to competitive blockade by ME. We conclude that 5HT plays a role in the separate and independent mechanisms of SWS and PS and that 5HT plays a more important role in the initiation of PS than in its maintenance.

*< 0.05 ns = No significant change

1734 LESIONS OF NON-MONOAMINE NEURONS OF THE PONTINE TEGMENTUM ELIMINATE PARADOXICAL SLEEP IN THE CAT. Barbara E. Jones. Depts. Neurol., Neurosurg. and Psychol., McGill Univ., Montreal Neurological Institute, Mtl., P.Q., Canada H3A 2B4.

Previous theories regarding the neural control of the sleep cycle assigned a major role to the monoamine neurons of the brain stem tegmentum (see Jouvet Ergebnisse Physiol. 64: 166-307, 1972). However recent studies by the present author (BR 124: 473-496, 1977) indicated that in the case of paradoxical sleep (PS), major destruction of the noradrenaline locus coeruleus neurons did not eliminate this state. In the present experiments it was found that lesions of the pontine tegmentum which damaged neither the noradrenaline locus coeruleus neurons nor the serotonin raphe neurons greatly disrupted the sleep cycle and eliminated all signs of PS.

These lesions concerned the pontine gigantocellular tegmental field (FTG) and portions of the lateral tegmental field (as defined by Berman). The lesions were successfully performed by a Kopf Radio Frequency Lesion Generator in five adult cats which were chronically implanted for EEG recording and were continuously recorded (24 hours around the clock) one week before and three weeks after the lesion. At the end of the experiment, the animals were sacrificed for histological verification of the lesion and biochemical assay of serotonin and noradrenaline.

The state of paradoxical sleep was totally eliminated following pontine FTG lesions. Muscle atonia, normally typical of this state, was absent for the duration of the survival period. PS phasic activity (REM and PGO spikes) was not observed in association with a desynchronized EEG typical of this state. In fact PGO spikes were virtually absent from the record in the first week and only returned as isolated events in association with a synchronized EEG the second week after the lesion. At this time (16th day post-lesion) the total number of PGO spikes per day was only 15% of the control count. In addition to the elimination of PS, Stage II slow wave sleep was also disrupted (40% of normal amount) after the lesion. Endogenous levels of noradrenaline and serotonin were normal in cortical and spinal cord regions corroborating the histological evidence that neither the locus coeruleus nor raphe neurons were damaged by the lesions.

These results suggest that neurons of the pontine FTG, and not monoamine neurons, are crucial for the initiation and maintenance of both phasic and tonic components of PS and also for the normal functioning of the sleep cycle.

1735 PONTO-GENICULO-OCCIPITAL SPIKES IN RATS: A COMPONENT OF THE ALERTING RESPONSE. Laura S. Kaufman* and Adrian R. Morrison, Dept. Psychobiol., Oberlin Coll., Oberlin, OH 44074 and Labs. Anat., Sch. of Vet. Med., Univ. of Penna., Phila., PA 19104.

Although ponto-geniculo-occipital (PGO) spikes are a prominant feature of paradoxical sleep in the cat, their presence in albino rats has only recently been confirmed (Farber et al., Sleep Res. 5/21, 1976). Our studies of this phenomenon in rats reveal an additional finding which lends support to the recent theory of Bowker and Morrison (Brain Res., 102/185-190, 1976), stating that PGO spikes are an indicator of alerting, and that they can occur during wakefulness and slow wave sleep (SWS), as well as paradoxical sleep (PS).

Five albino rats were chronically implanted for sleep recording, with bipolar electrodes placed stereotaxically in the dorsolateral pontine tegmentum. Sharp spikes (80-100uv) were recorded from 3 of the pontine placements. These electrode tips were histologically determined to be located in the area of the locus coeruleus. 85% of all spikes occurred during PS, 4% occurred during SWS in the 1 min. preceeding PS onset, and 6% in the 1 min. preceeding spontaneous arousals from SWS. The mean frequency of spiking during PS was 15-20 per min. Bursts of spikes averaging 5 spikes per burst and lasting 2-3 seconds were only recorded during PS. During the transition from SWS to PS, no more than 2-4 singularly occurring spikes were observed, and occasionally the rats would pass into PS without exhibiting any PGO spikes during the transition. The above confirms Farber et al.

It was also found that spikes identical in amplitude, shape and duration to those occurring spontaneously during PS could be produced with startling auditory stimulation (1500 and 2500 hz pure tone bursts of 18 msec duration) during wakefulness and SWS. As previous studies have demonstrated in the cat, stimulation at less than 5 sec. intervals resulted initially in activation of the EEG, gross body twitches or orienting movements, and PGO spikes. With continued presentation of the stimulus, EEG signs of alerting gradually disappeared followed by habituation of alerting behaviors and PGO spikes. PGO spiking could not be evoked in the 2 animals which did not exhibit spontaneous PGO spikes during PS.

These data indicate that certain structures which are spontaneously active during PS can also be activated by environmental stimuli in wakefulness and SWS. When analyzed in this context, PGO spikes can no longer be considered as spontaneous phenomena which are limited in their occurrence to paradoxical sleep.

(Supported by Oberlin College and NIH Grant #NS13110.)

1736 SLEEP PATTERNS IN QUADRIPLEGIC PATIENTS Lawrence W. Kneisley MD. From the Research and Spinal Cord Injury Services, West Roxbury Veterans Administration Hospital, Boston, Mass., Department of Neurology, Peter Bent Brigham Hospital, Harvard Medical School and the Department of Neuroscience, Childrens Hospital Medical Center, Boston, Mass.

To determine the effect of cervical spinal cord transection on human sleep, all-night EEG-EOG sleep recordings were measured in 9 quadriplegic subjects aged 19 to 34. The subjects, who had clinical evidence of recent or longstanding spinal transection above the C-8 level, and were taking no sedatives or alcohol at the time of the recordings, were studied for two consecutive nights. Serum samples were collected for endocrine studies from some by an indwelling intravenous catheter. The second night sleep-EEG tracings, scored by the methods of Williams et al (1), were evaluated for this report.

The mean amounts of sleep stages zero (wake), and 1, expressed as percentages of sleep period time (SPT) were significantly greater than those of normal controls ($p<.05$), while, conversely, mean percentages of stages REM (Rapid Eye Movement), 4, and 3 & 4 combined were significantly less than those of normal control subjects ($p<.05$). The sleep efficiency index (SEI), calculated by dividing total time actually spent sleeping (TST) by time spent in bed attempting to sleep (TIB), was .84± .07, which was significantly less than that of normal subjects from this and other laboratories (.95± .04, $p<.01$). One explanation for the increased amounts of waking (stage zero) and light sleep (stage 1), the diminished REM, and the low sleep efficiency may be the periodic body repositioning required by some of these individuals. However, those subjects not requiring these repositionings demonstrated the same abnormalities. Relative inactivity and immobility due to motor paralysis may be an important factor in reducing the "deeper" phases of sleep (stages 3 & 4), since exercise may increase the amount of these stages in the sleep of normals. These studies indicate that the sleep patterns of individuals with cervical spinal cord trauma are dramatically and persistently altered.

1. Williams, R.L., Karacan, I., Hursch, C.J. The EEG of Human Sleep. Clinical Applications. John Wiley & Sons, New York, 1974.

1737 MULTIPLE SLEEP LATENCY TEST IN NORMAL AND NARCOLEPTIC DOGS E.A. Lucas, A.S. Foutz, M.M. Mitler*, D. Brown*, W.C. Dement Univ. of Arkansas for Medical Sciences and Stanford University

The purpose of this study was to evaluate the effectiveness of multiple, serial sleep latency trials to differentiate between normal and narcoleptic dogs on the basis of their sleepiness. Six dogs, (4 miniature poodles and 2 beagles) were prepared surgically for chronic sleep recordings. Following a 2-week recovery period, one affected and one normal dog were placed in separate recording chambers with food and water ad libitum. A 48-hr period of adaptation was followed by an 84-hr observation which included a 48-hr baseline period and a 24-hr test period, during which the animals were removed from the chamber and kept alert for the first 30 minutes of each hour, and allowed to sleep ad libitum in the chamber during the second half hour. Polygraphic recordings included tracings from eye, sensorimotor and visual cortex, neck muscle and lateral geniculate leads at a paper speed of 10 mm/sec on Grass 78 recorders. The data were scored in 30 sec. epochs into six categories: alert, drowsy, light slow wave sleep, deep slow wave sleep, REM sleep and cataplexy. These data were entered into the computer and analyzed for each of the tests. The sleep latency scores were measured as the time in minutes from the closing of the chamber door until the first 30 seconds of sleep states. In comparison to control data, latencies to sleep tended to be shorter in affected dogs, particularly during the first 12 hours of the test. It became progressively more difficult to keep all affected dogs awake preceding each trial. The test clearly differentiated between the affected narcoleptic dogs and the normal dogs. The beagle appeared to be less affected than the poodles. This study needs to be extended to include more animals and the test applied to human narcoleptics and normals.

Mean (N=24 trials) sleep latencies (in min.) following 30 min. wakefulness

	12 h	24 h
Poodle Narc.	1.1 ± 1.1	.81 ± .83
Control	24.6 ± 10.1	19.1 ± 12.4
Poodle Narc.	1.7 ± 2.6	1.1 ± 1.9
Control	28.5 ± 5.3	21.5 ± 12.4
Beagle Narc.	3.3 ± 2.4	3.8 ± 3.6*
Control	10.2 ± 6.1	6.5 ± 5.7

*Not significant at the $p < .05$ level by linked pair t test.

1738 PATTERN ANALYSIS OF NEURONAL SPIKE TRAINS DURING SLEEP AND WAKEFULNESS IN THE CAT. Thaddeus J.Marczynski and Leslie L.Burns*.

Action potentials from single neurons in the dorsal hippocampus and nonspecific thalamic nuclei were recorded chronically in freely moving cats, using "floating" microelectrodes.The long trains of up to 60,000 spike intervals were then subjected to analysis of temporal patterns. The technique was based on inequality testing of intervals in consecutive pairs ; if the second interval in a pair was longer or shorter than the first interval, a (+) or a (-) sign was recorded respectively in sequential bins of computer memory. Subsequently, the trains of signs were analyzed for presence of specific patterns (Marczynski and Sherry, Brain Res.,35:533,1971; Sherry and Marczynski,Intern. J.Neurosci. 3:259,1972). In this manner, the sign permutations composed of 3 through 6 signs were studied. Using chi squared statistics, the empirical distribution of sign patterns was then compared with the theoretical distribution based on the assumption that spike intervals are independently arranged (cf. Brudno and Marczynski, Brain Res.,125:65,1977).

During slow wave sleep (SWS), five neurons in the dorsal hippocampus showed distribution of intervals consistent with the theoretical model. Four of these neurons during REM sleep, and three of them during quiet wakefulness (QW) showed significant deviation from the theroetical model. Similarly, during SWS, five neurons in the region of the nuc. reticularis thalami and two neurons in the pulvinar showed pattern distribution consistent with the theoretical model, and a significant departure from it during QW. However, of six neurons in the centro-median-parafascicular complex (CM-PF), only one fired during SWS in a manner consistent with theoretical distribution of patterns and increased deviation from this model during QW. One neuron showed no changes in patterns, and four neurons showed greatest departure from the theoretical model during SWS, and a tendency toward theoretical distribution of patterns during QW.

The results indicate that 11 of 12 neurons in the dorsal hippocampus, pulvinar and the vicinity of the nuc. reticularis thalami fire in a manner consistent with the hypothesis that SWS is characterized by a relaxation of constraints in neuronal connectivity resulting in an independent distribution of spike intervals. Significant departures from the theoretical model during REM sleep and QW may be associated with integrative processes and information transmission. The results also suggest that neurons in the CM-PF may behave differently.

1739 EFFECT OF NEONATAL ADMINISTRATION OF MSG ON SLEEP IN THE MOUSE, John Metz, William J. Pizzi, June E. Barnhart* and James R. Unnerstall*. Neuropsychology Lab., Northeastern Illinois University.

When monosodium glutamate (MSG) is administered to neonatal mice, it produces permanent CNS damage along with somatic and behavioral dysfunctions. Previous studies have shown a lowered metabolic rate and obesity. Metabolic rate and body size have been related to various aspects of sleep across mammalian species (Zepelin & Rechtschaffen, Brain Behav. Evol., 10: 425, 1974). Of primary interest to this study is the finding that species with higher metabolic rates also have longer daily sleep times. The MSG pretreatment utilized in this study provides an adult animal whose metabolism and body size have been chronically altered, without confounds from short-term drug, environmental, or stress factors. It thus allows an assessment of the relationship between metabolism and sleep within a species.

Six male neonatal mice were given subcutaneous doses of MSG from days 2-11 after birth, according to a dose schedule which started at 2.2 mg/g b.w. and increased to 4.4 mg/g b.w. by the last day of injection. The six control mice received equal volumes of bacteriostatic saline. When the mice reached adulthood they were implanted with skull screws and wires in the neck muscles for chronic recording of the EEG and EMG. Recordings were taken for 20 hrs/day for two days (8 hrs light, 12 hrs dark). The polygraph records were scored by an experimenter who was blind to the treatment condition.

Table 1 summarizes the percentage of time spent in each of the sleep stages during both the light and dark periods. The MSG-treated mice showed significantly more sleep and less waking during the dark period than did control mice (two-tailed t-test, $p < 0.05$). The control animals displayed a marked light-dark difference in W and SWS but the MSG animals did not. In light of the gross physiological abnormalities seen in MSG mice it is striking that sleep is so little affected.

Table 1: Sleep stage percentages for control & MSG mice.

	Control		MSG	
	Light	Dark	Light	Dark
Waking	29.7 ± 8.0	45.3 ± 10.7	33.2 ± 9.1	33.9 ± 6.4
SWS	61.0 ± 6.1	47.1 ± 7.8	60.0 ± 9.0	57.6 ± 7.7
PS	9.2 ± 3.7	7.5 ± 3.6	7.6 ± 2.7	8.4 ± 2.9

These results fail to extend within a species the finding of a direct correlation between metabolic rate and total sleep time. This could mean that the relationship between sleep and metabolic rate across species is regulated genetically and is not subject to postnatal manipulation.

All of the additional sleep in the MSG animals occurred during the dark period, suggesting a specific deficit in the ability to regulate the light-dark distribution of sleep.

1740 STATE DEPENDENT EFFECTS OF PNEUMOTAXIC CENTER LESIONS AND VAGOTOMIES ON BREATHING IN THE CAT. A. Netick*, T. L. Baker*, A. S. Foutz, and W. C. Dement. Psych. Dept., Calif. State Univ., Hayward, CA 94542 and Sleep Res. Cent., Stanford Univ. Sch. Med., Stanford, CA 94305.

Lesions of the pneumotaxic center are best known to produce apneuses (prolongations of inspiration) especially during anesthesia in vagotomized animals. We have examined the effects of these lesions, later combined with vagotomies, on breathing in unanesthetized cats as a function of natural sleep. The purpose was to determine whether sleep produces the same effect as anesthesia on these preparations as well as to determine whether the effect of sleep upon breathing in the intact preparation (slowing during non-rapid eye movement sleep, NREM, and speeding and irregularity during REM) is modified by destruction of rostral pontine structures.

Ten cats were prepared for measuring sleep states; an endotracheal tube inserted into a chronic fistula in the trachea served to measure respiration. Following adaptation to atraumatic restraint, control baseline recordings were followed by bilateral lesioning of the pneumotaxic center under ether anesthesia. Recordings were made during the procedure and at intervals for at least two weeks. Cats were then vagotomized under ether and recorded for two succeeding days.

Apneusis was a frequent but not invariable concomitant of the pneumotaxic lesioning procedure. More commonly observed were prolonged expiratory pauses, or apneas. When apneusis occurred, the breathing pattern usually became apneic upon recovery from anesthesia. In subsequent recordings, the apneic pattern intensified during NREM; apneas sometimes lasted over a minute. During REM, the pattern invariably improved; the post-lesion pattern during REM was similar to the pre-lesion REM pattern.

During the vagotomy, breathing became apneustic. Withdrawal of anesthesia was followed by recovery from apneusis. Two patterns of breathing during sleep were observed. One pattern was similar to that of the nonvagotomized, lesioned cat (apneas primarily during NREM); the second pattern was a reappearance of apneusis during REM but not during NREM nor wakefulness.

The results demonstrate an interactive effect of sleep and pneumotaxic center lesions. REM sleep, like anesthesia, can produce apneusis in the vagotomized, lesioned cat. The effect of state upon breathing observed in the intact cat survives extensive destruction of rostral pontine structures eliminating them as generators of these effects.

(Supported by NIH grant #NS 10727 and NIH Research Scientist Award MH 05804 to WCD.)

1741 STREPTOMYCIN: ELECTROGRAPHIC AND BEHAVIORAL EFFECTS IN THE CAT. R.Pivik, J. Metz* and A. Rechtschaffen*. Dept. of Psychiatry, University of Ottawa Faculty of Medicine, Ottawa, Ont. Canada, and Dept. of Psychiatry, University of Chicago, Chicago, IL 60637.

Electrolytic lesioning of the medial and descending vestibular nuclei has been reported to eliminate bursts of phasic activity (PGO spikes and associated autonomic and spinal reflex modulations) during paradoxical sleep (PS) in the cat. Apart from the difficulty in creating complete, replicable lesions of the vestibular nuclei, this method of lesioning usually destroys a great deal of non-vestibular tissue. The present study sought to create vestibular lesions using an antibiotic -- streptomycin, which is reported to have toxic effects on the vestibular system via the inhibition of the intracellular production of nucleic acids.

Four adult cats chronically implanted for recording EEG, EMG, EOG and LGN activity were administered streptomycin subcutaneously over periods of time ranging from 14 - 25 days. The total amounts administered were 21.5 cc, 27.4 cc, 28 cc and 29.4 cc. Polygraphic sleep recordings were taken prior to and periodically during and after drug administration. The animals were subsequently sacrificed, perfused, and their brains prepared for histological processing (histological results will not be presented since processing is incomplete at this time).

Subsequent to drug administration all animals exhibited vomiting, ataxia, loss of righting reflexes, circling behavior and an absence of response to loud auditory stimuli. The cats experienced a 13% to 16% loss of body weight during the drug administration period. Streptomycin administration was associated with an increase in slower EEG frequencies as demonstrated in increased amounts of drowsiness and slow wave sleep. There was no effect on PS.

In the present study, then, streptomycin effected distinct alterations in behavior and electrographic activity. The behavioral signs indicate malfunctioning of auditory and vestibular mechanisms. Electrographically, increased amounts of slow activity are present in the EEG during sleep, but the effect on PS is negligible. Phasic activity recorded at the level of the LGN is enhanced during slow wave and paradoxical sleep, and bursts of such activity persist.

1742 ACTION OF BROMOCRIPTINE AND α-FLUPENTHIXOL ON SLEEP IN RATS DEPRIVED OF PARADOXICAL SLEEP. Miodrag Radulovacki, Walter J. Wojcik and Casimir Fornal*. Dept. of Pharmacol., Univ. of Il. Med. Ctr., Chicago, IL. 60612

Previous work in this laboratory with L-DOPA and d-amphetamine (Radulovacki et al., FASEB, 1978) has shown that these two agents increase wakefulness (W) and can partially substitute for paradoxical sleep (PS) rebound in PS-deprived (PSD) cats. We have shown in rats a correlation between decreased PS and an increase in brain dopamine (DA) following the administration of a DA β-hydroxylase inhibitor, diethyl dithiocarbamate (Kovacevic-Ristanovic et al., FASEB, 1978). To further delineate the role of DA in the mechanism of PS we utilized the DA receptor agonist, bromocriptine mesylate (CB) and antagonist, α-flupenthixol (FL) in PSD rats. Four groups of adult rats were implanted with cortical and dorsal neck muscle electrodes for polygraphic recordings. One week after surgery, the rats were selectively deprived of PS for twenty-four hours by the "flower pot" technique. To standardize the degree of PS deprivation, all rats were placed on platforms whose surface area corresponded to their body weight. At the twenty-third hour of PSD, animals were pretreated with either vehicle (C) or FL (0.2 mg/kg, i.p.) and placed back on PSD for the remaining hour. All rats, 2 1/2 hours after pretreatment, were injected with either C or CB (5 mg/kg, i.p.).

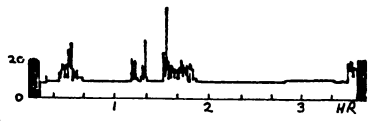
Groups one and two, pretreated with C, received C and CB, respectively. Groups three and four, pretreated with FL, received C and CB, respectively. After all drug combinations were administered, EEG and EMG were continuously monitored for thirty hours. All records were analyzed for W, slow-wave sleep (SWS) and PS. Statistics were performed at five-hour intervals. All observed changes occurred during the first five hours of polygraphic recording.

We found that CB increased W by 65% and reduced PS by 73% in spite of a strong PS pressure caused by PSD. CB administration had no effect on SWS. These results are similar to those that we obtained previously with DDC which significantly increased brain DA while PS was decreased, W increased and SWS remained within control values. It is of interest that the effects of CB were completely blocked by the FL pretreatment. The only effect of administration of FL alone was an elevation of SWS by 28%. From our results we conclude that DA is involved in the mechanisms of both W and PS.

1743 CHANGES IN RESPONSIVENESS OF THE JAW OPENING REFLEX ELICITED BY TOOTH PULP STIMULATION IN THE CAT DURING SLEEP AND BARBITURATE ANESTHESIA. Kenneth H. Reid, George C. Stege III* and S. Wilson. Dept. of Physiology and Biophysics, Univ. of Louisville Health Sciences Center, Louisville, Kentucky 40232.

In REM sleep, muscle tone is lost and the jaw-opening reflex (JOR) is suppressed (Chase & McGinty, Exptl. Neurol. 19: 127, 1970). The suppression is not absolute; a JOR can be obtained during REM sleep if a sufficient stimulus is given to the pulp of the ipsilateral upper canine tooth in the cat. Changes in JOR responsiveness were followed for periods of 4 to 20 hours in 4 cats chronically implanted with bipolar amalgam stimulating electrodes in the upper canine teeth and wire recording electrodes in each digastric muscle. JOR thresholds were evaluated at 30 sec intervals by a computer-driven stimulator doing a binary search in a closed-loop mode, using the peak-peak EMG as the response index. Each cat was also followed through induction and recovery from a surgical level of anesthesia (sodium pentobarbital 35 mg/kg). Representative results are shown below:

Normal sleep:



Anesthesia:

35 mg/kg IP inj at dot

Under barbiturate anesthesia, the JOR threshold rises slowly for 15-30 minutes after IP injection. It then rises, often in abrupt steps, to 3-10 x the initial level, where it stays for 30-120 minutes. Recovery occurs in from 1 to 4 abrupt step changes. During normal sleep the threshold remains near that seen in the waking state except during episodes of REM sleep where it is abruptly elevated to 2-10 x waking level. Stability of the elevated threshold varies greatly from cat to cat during both REM sleep and anesthesia. It is concluded that barbiturate anesthesia and REM sleep may suppress motor responses to noxious stimuli through a common mechanism.

Supported by NIDR grant DE-3644.

1744 SLEEP AND WAKING ACTIVITY OF NUCLEUS RETICULARIS PONTIS ORALIS-CAUDALIS, AND MEDULLARY GIGANTOCELLULAR NUCLEUS CELLS IN UNRESTRAINED CATS. J. M. Siegel, D. J. McGinty and S. M. Breedlove*, Sepulveda V. A. Hospital, Sepulveda, CA 91343.

The nucleus reticularis pontis oralis-caudalis (RPO-RPC) and medullary nucleus gigantocellularis (MNGC) occupy the medial reticular formation areas anterior and posterior to the pontine nucleus gigantocellularis (PNGC). Carli and Zanchetti[1] identified the RPO-RPC region as the area whose destruction eliminated REM sleep. Netick, Orem and Dement[2], recording in restrained cats, recently described 6 cells in the medullary gigantocellular region which discharged selectively in REM sleep. They speculated that these cells might have a role in REM sleep generation. Therefore, it is of interest to determine the sleep-waking discharge correlates of these cell groups in unrestrained cats.

A total of 60 cells were studied. We found that both of these areas contain the same 3 cell types seen in the PNGC area[3]. Type one cells had no spontaneous activity during quiet waking and sleep, discharging only during movements. Type 2 cells had high rates of tonic activity (>4 spikes/sec) during both quiet waking and sleep states with increased discharge during waking movements and REM sleep. Type 3 cells had low spontaneous activity rates (≤4 spikes/sec) during quiet waking and slow wave sleep, but discharged at high rates during both waking movements and REM sleep. Waking discharge related to specific head, neck, back, forepaw, and facial movements. In the MNGC we saw several cells that fired at high rates only during specific waking postures and in REM sleep. These cells might appear to discharge selectively in REM sleep if critical postures were prevented by restraint. This finding may account for previous reports of REM selective cells in this area. It is also conceivable that medullary REM sleep selective cells are rare and were not encountered in our explorations.

In summary, RPO, RPC and MNGC cells, like PNGC cells, exhibit augmented discharge during both waking movements and REM sleep. We have not observed any cells in these areas which discharged selectively in REM sleep. Our results are consistent with an hypothesis of medial reticular formation involvement in the motor activation common to both active waking and REM sleep, but are not consistent with an executive role for these neurons in the triggering of the REM sleep state.

[1]Carli, G. and Zanchetti, A. Arch. ital. Biol. 103:751-788, 1965.
[2]Netick, A., Orem, J. and Dement, W. Brain Res. 120:197-207, 1977.
[3]Siegel, J. M., McGinty, D. J. and Breedlove, S. M. Exp. Neurol. 56:553-573, 1977.

1745 VISUAL PATHWAYS MEDIATING THE EFFECTS OF LIGHT ON PARADOXICAL SLEEP IN RATS. C. L. Sisk and F. K. Stephan. Dept. Psychol., Florida State University, Tallahassee, FL 32306.

In rats, approximately 70% of the daily slow wave sleep (SWS) and rapid eye movement sleep (REM) occur in the light phase of a 12 hr light-12 hr dark cycle (LD 12:12). However, when maintained on very short LD cycles (e.g., LD 0.5:0.5), most REM episodes occur in the dark periods, whereas the distribution of SWS remains relatively unaffected (Borbely, Huston, and Waser, 1975; Brain Res. 95, 89). This investigation was an attempt to determine which component of the visual system mediates the shift of REM sleep into short dark periods. Cortical EEG, neck muscle activity and brain temperature were recorded from rats with bilateral lesions in the primary optic tract, leaving only retinohypothalamic fibers (RHT) and the inferior fasciculus of the accessory optic tract (IAOT) intact. During 48 hrs on a LD 0.5:0.5 cycle, an average of 62% of REM sleep occurred in the dark periods in rats with lesions, compared to an average of 88% for intact rats, with no overlap between the two groups. The functional integrity of the IAOT was assessed by observing a pronounced reduction in water intake in constant light and the entrainment of drinking rhythms to a LD cycle indicated that the RHT was also functional (c.f. Stephan and Zucker, 1972; Physiol. Beh. 8, 315-320).

These results suggest that neither the RHT nor the IAOT is capable of shifting REM sleep into short dark periods to the extent observed in intact rats. Further experiments are in progress to assess the role of the lateral geniculate nucleus, optic tectum and the superior accessory optic tract in the effects of short dark periods on REM sleep.

1746 INDUCTION OF REM SLEEP IN RATS BY PERIPHERAL WARMING. R. S. Szymusiak*, Timothy Schallert*, Evelyn Satinoff, and Ian Q. Whishaw*. Department of Psychology, University of Illinois at Urbana-Champaign, Champaign, IL 61820 and Department of Psychology, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4.

Peripheral warming produces a decrease in tonic postural support followed by REM sleep in infant rats (Schallert, Whishaw, and Teitelbaum, 1977). In the present study, we demonstrate that during slow-wave sleep, peripheral warming decreases muscle tone and induces REM sleep in adult rats as well. Rats were implanted with neck EMG and cortical EEG electrodes. Hypothalamic (T_{hy}) and subcutaneous (T_{sc}) temperatures were continuously recorded. A redbulb infrared heat lamp (total power dissipation 182 W) was placed 35 cm above the animals. Following a one minute period of continuous cortical synchronization, the heat lamp was turned on, and the animals were warmed until they either woke up or entered REM sleep (30 sec-2.5 min). An increase in T_{sc} of 1-3°C, accompanied by only a slight change in T_{hy} (less than 0.3°C), resulted in REM bouts in 78% of the experimental trials, compared to 21% of the control trials when the animals were not warmed. Warming decreased tonic EMG activity while the cortex remained synchronized. Then, as cortical desynchronization occurred, EMG activity completely disappeared. The REM bouts outlasted the warming stimulus, having a duration similar to control (non-warming) bouts (about 80 sec). The relationship between temperature-induced changes in tonic postural support and REM sleep is discussed.

Supported by Grants: U.S. Navy #N00014-77-C-0465, NSF #BNS77-03151, and NIH #R01 NS 11671.

1747 EFFECT OF PUSH-PULL TOPIC PERFUSION OF CARBACHOL ON THE MULTIPLE UNIT ACTIVITY OF THE GIGANTOCELLULAR TEGMENTAL FIELD. Marcos Velasco, Francisco Velasco, Carlos Cepeda* and Ranulfo Romo*. Sci. Res. Dept., Natl. Med. Ctr. IMSS. México, D. F.

The effect of chemical stimulation of the pontine reticular formation with various cholinergic compounds on the wakefulness-sleep states of cats has been previously studied with contradictory results (Hernández-Peón et al 1963, Mitler and Dement 1964, Amatruda et at 1975). These apparent controversial results may be partially explained by a lack of control of certain methodological factors as the precise site and extent of perfusion and the type and doses of the injected compounds.

In this work performed on cats with implanted electrodes and cannula guides and immobilized by means of a head holder device, we studied the effect of push-pull topic perfusion of Carbachol (5 ug/50 ul/min) of the gigantocellular tegmental (FTG) field on the local multiple unit activity and the wakefulness-sleep state of animals.

We found a linear relationship between the doses of Carbachol injected and the number of FTG active units within a range between 7 and 30 ug. The effect started and finished immediately after the Carbachol push-pull injection. Doses larger than 30 ug produced an immediate blockage of the active units (saturation doses). During Carbachol injection, animals remained with their eyes open and with a variable degree of EEG synchronization but with a constant and progressive muscular hypotonia. No other motor or vegetative signs were present and the number of ocular movements, the EKG and respiration frequencies and rectal temperature were unchanged.

1748 THE EFFECT OF L-TRYPTOPHAN ON MONOAMINES IN SPECIFIC BRAIN STRUCTURES AND SLEEP IN RATS. Walter J. Wojcik, Casimir Fornal*, and Miodrag Radulovacki, Dept. of Pharmacol., Univ. of Ill. Med. Ctr., Chicago, IL 60612.

The administration of L-tryptophan (L-TRY) reduces sleep latency and waking time in humans (Hartmann, 1977). Similar results in reduction in sleep latency have also been observed in rats (Hartmann, 1972). According to the monoamine sleep theory, this hypnotic effect of L-TRY was attributed to the increased 5-hydroxytryptamine (5HT) turnover in the brain. While there is abundant evidence for the effects of L-TRY on 5HT metabolism in the brain, there is little information on L-TRY effects on brain catecholamines. We decided to analyze dopamine (DA) and norepinephrine (NE), as well as 5HT and 5-hydroxyindoleacetic acid (5HIAA) in the cortex (CX), hippocampus (HIPP), striatum-thalamus (ST) and pons-medulla (PM) of adult rats fifteen and forty-five minutes after L-TRY (30 mg/kg, i.p.) administration. The animals were sacrificed by decapitation and the monoamines and 5HIAA were determined by spectrophotofluorometric methods. Fifteen minutes after the injection, DA levels in the CX and the HIPP were reduced by 27% and 18%, respectively. At the same time, 5HIAA levels were elevated by 38% in the PM, while 5HT and NE concentrations did not change in any of the mentioned structures. Forty-five minutes after administering L-TRY, 5HT levels were elevated by 18% and 5HIAA concentrations showed an increase, although not statistically significant, in the PM. In all studied structures, NE and DA remained unchanged. In another experiment, adult rats were implanted with cortical and neck muscle electrodes for EEG and EMG recordings. One week after surgery, the animals were injected with either saline or L-TRY (30 mg/kg, i.p.) and polygraphically recorded for six hours. On the following day the same protocol was repeated with the same group of animals but with reversed administration of agents. All recordings were analyzed for wakefulness (W), slow-wave sleep (SWS) and paradoxical sleep (PS). Paired t analyses performed at half-hour intervals showed that the administration of L-TRY increased SWS by 11% and PS by 7%, while W was reduced by 19% during the second half-hour period. However, these effects occurred without changing either SWS or PS latencies. Since the increase in SWS and PS (see also Jackman and Radulovacki, this meeting), occurring in the second half-hour period, coincides with the forty-five minute increase in 5HT and 5HIAA in PM, we suggest that this effect of L-TRY on sleep is mainly due to the increased 5HT turnover.

SOMATOSENSORY SYSTEMS

1749 DEVELOPMENT OF SEGMENTED CORTICOTHALAMIC PROJECTIONS TO THE VENTROBASAL COMPLEX OF THE RAT. R. M. Akers and H. P. Killackey. Dept. Psychobiology, Univ. of Calif., Irvine, CA 92717.

Studies based on succinic dehydrogenase histochemistry have demonstrated that trigeminal projections to the ventrobasal complex of the rat are organized in discrete, segmented clusters which can be related to individual peripheral receptors. This pattern of segmentation is well developed in the seven day old rat but is much less discrete in adults (Killackey and Belford, Anat. Rec., 1976). An autoradiographic analysis of cortico-thalamic projections in neonatal rats suggests that trigeminal and cortical afferents to the ventrobasal complex are organized in a complementary fashion in young animals. Following cortical injections of ^{3}H-leucine or proline, bundles of labeled axons can be seen in the ventrobasal complex as early as the first post-natal day (birth=P0). During the next three days of development, the grain density over the nucleus increases, and a highly organized pattern of corticothalamic projections emerges. By the second postnatal day, contrasting zones of high and low grain density are visible. The low density areas are organized in wide, curving bands which are oriented along the ventrolateral-to-dorsomedial axis of the nucleus. Narrow strips of much higher grain density are intercalated between adjacent low density bands. These strips of higher grain density undoubtedly represent collections of corticothalamic axons, which invade the adjacent bands of lower grain density on the third postnatal day. The growth of corticothalamic axons into the low density bands is discontinuous and further subdivides each band into a curvilinear row of lightly labeled patches separated by narrow zones of high grain density. This pattern is not fully developed until the fourth postnatal day and is most distinct in animals younger than ten days of age. In the adult, patches of lower grain density can be distinguished within the ventrobasal complex following cortical injections of tritiated amino acids, but the contrast between these patches and adjacent strips of high grain density is much lower than in the neonate.

When the pattern of labeling following cortical injections is compared with material stained for succinic dehydrogenase activity, it appears that the corticothalamic axons are preferentially localized in portions of the ventrobasal complex which do not receive dense trigeminal projections. Further, the development of segmentation in these two afferent systems follows a similar time course, beginning on the second postnatal day and being virtually complete by postnatal day four. Finally, it is of interest that developmental processes after the perinatal period obscure the discreteness of segmented organization in both cortical and trigeminal afferents to the thalamus. Supported by NSF grant #GB41294 and NIMH grant #MH14599-02.

1750 NORMAL AND ABNORMAL SEGMENTATION IN THE TRIGEMINAL COMPLEX OF THE YOUNG RAT. G.R.Belford* and H.P.Killackey (SPON: E. Davis). Dept. of Psychobiology, Univ. of Calif., Irvine 92717.

In the young rat anatomical correlates of the facial vibrissae are seen in the trigeminal complex of the brainstem, the ventrobasal complex of the thalamus, and layer IV of somatosensory cortex. Rows of dense clusters of succinic dehydrogenase (SDH) activity within the neuropil of each structure mimic the pattern of vibrissae on the face. Further, neonatal damage to a row of vibrissae follicles results in an aberrant organization within these structures. We have previously reported on the time course of development of the normal and abnormal organization in the ventrobasal complex (Belford, Anat. Rec., '78) and somatosensory cortex (Killackey and Belford, Neurosci. Abst., '76). In the present experiments we extend our study of the development of vibrissae representations to those in the trigeminal complex.

In the first part of the present study, littermate rats with no vibrissae manipulations were sacrificed on postnatal Days 0 through 5. In the second part, littermate rats had a row or rows of vibrissae damaged at birth. These rats were also sacrified on Days 0 through 5. Finally, in the third part, littermates had a row or rows of vibrissae damaged at Days 0 through 5. These rats were sacrificed on Day 7. The brains of all animals were processed with SDH histochemistry.

The trigeminal complex contains three separate representations of the vibrissae, one each in the subnucleus caudalis and subnucleus interpolaris of the spinal trigeminal and the third in the principal trigeminal nucleus. These representations are present on the day of birth. Removal of vibrissae at birth results in abnormalities that are apparent by Day 2 in all three representations. The portion of each representation that is related to the damaged vibrissae shows a density of SDH activity much lower than normal. Further, the row of clusters normally seen in this area is sometimes replaced by a band of activity, but more often the area is amorphic. Finally, removal of vibrissae at different postnatal ages results in variations in the pattern as seen at Day 7. The density of SDH activity progressively increases from a low level with damage on Day 0 to one of almost normal density with damage at Day 5. Removal on Days 0 through 2 leads to the pattern described above for removal at Day 0, with an increased tendency towards bands at the later ages. Day 3 removal results in either a band or clusters. For Days 4 and 5, clusters of below normal density are seen.

These results indicate that the vibrissae representations in the trigeminal complex are plastic for the first few days of postnatal life. (Supported by NIMH MN 14599-02 and NSF GB 41294.)

1751 PSYCHOPHYSICAL COMPARISONS OF ANOMALOUS AND NON-ANOMALOUS REPORTS TO CUTANEOUS COOLING STIMULI. Sherry L. Berg* (SPON: J. E. Aschenbrenner). Depart. Psychology, Florida State University, Tallahassee, Florida 32306.

The results from five male subjects were compared with one anomalously reporting male subject using the psychophysical methods of magnitude estimation and fractionation. Contact thermal stimuli were presented to the right dorsal forearm after the site had been preadapted to 32.6°C. Rates of temperature change varying from 0.1° to 2°C/sec were administered for a 3 sec duration producing stimulus intensities that ranged from 0.3° to 6°C. Five stimulating surfaces (1,2,4,7.5 and 18cm^{2}) were introduced for both magnitude estimates and fractionation measures.

In addition, bilateral, warm-cool and forearm-forehead comparisons along with the neurological pinprick test and subjectively different verbal reports all argue for a unique anomalous effect and sufficient reason for exclusion of this subject from the combined magnitude estimation and fractionation analyses. Furthermore, closer examination of the anomalous and nonanomalous data demonstrates the discreteness of the effect as opposed to one which might have ranged along a continuum. (Supported by USPHS Grant NB-02992 and Training Grant MH 11218)

1752 UNIT RESPONSES FROM THE ISOLATED RAT CORNEA. R.W. Beuerman and D.L. Tanelian, Stanford University Medical Center, Stanford, CA 94305.

Anatomical studies have shown that all axons entering the cornea terminate in the epithelium as so called free nerve endings. The purpose of the present experiments has been to determine whether or not these receptors are similar physiologically by examining their response characteristics. In this preparation the eye is quickly removed from a deeply anesthetized rat (280-300 g) and mounted in a lucite chamber by compressing the bulbar conjunctiva between a stainless steel ring and a plastic disc. This holds the globe in a manner similar to the intact state and assures that the tissue of interest is not stressed mechanically. After incising the sclera around the optic nerve, the ciliary nerves are obtained intraocularly. As many as five nerve bundles (100-150 μm) spaced around the cornea can be dissected in one preparation. The iris is removed with fine tweezers. These bundles are further dissected to obtain identifiable unit activity with a suction electrode. The posterior surface of the cornea and nerves are perfused by a bicarbonate-buffered ringer at 35°C, while the cornea is perfused by a HEPES ringer at 35°C. All units are tested for their receptive fields with a calibrated hair and are tested with the following: an ascending NaCl series, pressure and thermal stimuli delivered by a saline jet (200 μm) dia.) Conduction velocity has not been measured. All units found have been rapidly adapting; receptive fields have varied from spot size to more than a quarter of the corneal surface. Two general types of units have been found: those responding to mechanical stimuli and those excited by temperature changes. Many of the mechanically sensitive units have receptive fields located at the periphery of the cornea, and they respond to a brief punctate mechanical stimulus with a stereotyped burst of action potentials. Most of these fibers show no response to NaCl even at 0.5 M, or to moderate temperature change (±5°C). The receptive fields of the temperature units are generally small (1-3 mm dia.) and located more toward the center of the cornea; they have little or no responsiveness to the calibrated hair but do respond to concentration changes. It is concluded that there is functional evidence for more than one unit type in the rat cornea.

(Supported by NIH Grants EY 02108 and EY 00051).

1753 ACTIVITY FROM NERVES INNERVATING THE PERIPHERAL VASCULATURE IN SQUIRREL MONKEY. Bruce E. Bradley. Dept. of Oral Biol., School of Dentistry, Univ. of Michigan, Ann Arbor, MI. 48109.

Several functions of the innervation of the peripheral vasculature have been suggested by a number of indirect experimental observations, but these functions have seldom been substantiated by detailed electrophysiological studies. Peripheral vascular afferent nerves thus provide an interesting substrate for experiments in sensory neurophysiology which might potentially contribute to a broader understanding of the reflex control of the cardiovascular system.

To identify peripheral nerves innervating blood vessels and to begin to investigate their functions, neuronal activity was recorded in six squirrel monkeys anesthetized with ketamine and pentobarbital.

In one example, whole intact nerve activity was inhibited within 2-3 sec following intra-arterial (IA) injection of 10 µg of phenyl diguanide (PDG). Activity returned gradually and was again abolished by proximal section of the nerve trunk. Subsequent IA injection of PDG elicited neuronal activity. The inhibition of presumed sympathetic activity corresponded closely to a transient depressor response. This observation would tend to substantiate the reflex nature of responses to IA injection of veratrum alkaloids proposed by Krayer and Acheson (Physiol. Rev. 26:383,1946). The location of the nerve corresponded to the distribution of the nerves to the superficial femoral artery described by Potts in human material (Anat. Anz. 47:138,1914).

Thirteen seconds after occlusion of the femoral vein, several units were identified which fired slowly and adapted prior to release of the occlusion. The nerve trunk had been divided centrally prior to venous obstruction and thus the neuronal response might correspond to afferent reflex activity suggested by Haddy and Gilbert (Circ. Res. 4:25,1956).

One single unit was activated by punctate stimulation of an apparently rapidly adapting mechanoreceptor located on the saphenous artery 1.2 cm from the origin of the vessel. The unit appeared to have a single receptive field and was excited neither by occlusion of the saphenous artery several cm distal to the receptive field nor by IA injection of 100µg PDG.

It is tentatively concluded that the nerve fibers innervating the peripheral vasculature have been identified and can be isolated to yield electrical recordings of their neuronal activity.

(This research was sponsored in part by grants-in-aid from the Michigan Heart Association).

1754 DISTRIBUTION OF MECHANORECEPTORS IN SQUIRREL PAWS. G.L. Brenowitz* (SPON: E.F. Domino). Neurosci. Prog. and Dept. Zool., Mich. State Univ., East Lansing, Mi. 48824.

The morphology and distribution of sensory endings in the glabrous paw skin of Fox squirrels (Sciurus niger) and Thirteen-lined ground squirrels (Spermophilus tridecemlineatus) were examined in material prepared with Sevier and Munger's (J. Neuropathol. exp. Neurol. 24:130) silver staining method. Techniques were developed to estimate receptor density, examine dispersion patterns and determine the degree to which different receptors are segregated from each other.

Fox squirrels use their forepaws in skilled climbing and tactile exploratory behaviors and ground squirrels use theirs in excavating extensive burrow systems. Also, I recently showed that Fox squirrels depend upon tactile input from the forepaw more than do ground squirrels in food handling tests. Therefore it was predicted that the density of receptors in the Fox squirrel's forepaw would be relatively greater (forepaw density/ hindpaw density ratio would be greater) than in the ground squirrel's.

Fox squirrels have intraepidermal fibers, dermal free endings and simple and Pacinian corpuscles. Ground squirrels have intraepidermal fibers, dermal free endings and simple and Meissner's corpuscles. As predicted, the forepaw density/ hindpaw density ratio is significantly greater in Fox squirrels (3.3 ± 0.5) than in ground squirrels (1.3 ± 0.3). Unexpectedly, the density of receptors in the palmar region is significantly higher than in the digits for Fox ($95/mm^2$ vs. $16/mm^2$) and ground squirrels ($48/mm^2$ vs. $26/mm^2$). The proportions of different receptor types (corpuscular vs. non-corpuscular) do not differ between species. Receptors were randomly dispersed (Coef. of Dispersion = 1) and different types of receptors were intermingled (Index of Segregation = 0.3) in the skin of both species. These results indicate that the relative densities of receptors may vary with the particular use of the forepaw in different species, but that the basic pattern of the receptor array remains similar.

1755 A SINGLE UNIT STUDY OF CORTICAL AREAS ADJACENT TO THE SECOND SOMATIC SENSORY CORTEX IN THE CYNOMOLOGOUS MONKEY. H. Burton and C.J. Robinson. Depts. of Anat. & Neurobiol., and Elec. Engr., Wash. Univ., St. Louis, MO 63110

The boundaries of the second somatic sensory cortex (SII) in primates are difficult to define physiologically because cutaneous stimulation activates several regions around SII that do not receive projections from the ventroposterior nucleus of the thalamus. In this study, the locations of somatically activated neurons within the lateral sulcus were correlated with retrograde labeling of the thalamic nuclei projecting to these regions and/or with cortical cytoarchitecture in the vicinity of the electrode tracts.

The granular insular cortex (Ig), which receives projections from the supragenicular region of the thalamus, contains neurons that primarily respond to stimulation of hairs over large (>100 cm^2), generally bilateral receptive fields. These borders were often not well defined.

Neurons within the retroinsular cortex (Ri), which receives projections from the posterior nucleus (PO), primarily respond to light tactile stimulation of rapidly adapting skin receptors. Less than 10% responded to moderate or high threshold cutaneous stimulation. Receptive fields were of moderate size (<100 cm^2), with fairly precise borders, and were generally on the contralateral side of the body. The response properties of Ri neurons closely resemble those of SII (Robinson and Burton, this vol.). The transition between SII and Ri was often marked by sudden changes in the location of receptive fields and not in response properties.

The part of area 7 bordering SII and Ri in the lateral sulcus receives a projection from ventral portions of the anterior pulvinar. Cutaneous stimulation reliably activated over half of the neurons isolated in this region. Large, often bilateral receptive fields, with imprecise borders, frequently involved the whole body. Although rapidly adapting inputs predominated, a greater number of neurons than in SII showed slowly adapting discharges to light touch. This part of area 7 was also the region within the depths of the lateral sulcus containing the largest number of neurons with noxious drive characteristics.

Frequently, our designation of cortical areas could only be made after correlating the recording sites with connectional analyses in the same animal because of the great similarity in the somatic response properties especially in the awake and unparalyzed animal. Consequently, previous physiological studies may have designated some of the somatic areas seen here as SII proper. (Supported by PHS NS-09809 and GM-01827)

1756 ORIGIN OF PROPRIOCEPTIVE FIBERS INNERVATING THE TEMPOROMANDIBULAR JOINT CAPSULE. Norman F. Capra*, John H. Romfh* and Glenn B. Gatipon. Depts. Anat. and Pharm., Univ. MS Med. Center, Jackson, MS 39216.

That the mesencephalic nucleus of the trigeminal nerve (MNV) mediates proprioceptive impulses for the head region is generally accepted. In cats, we are unable by electrophysiological methods to identify MNV neurons that respond to passive movements of the temporomandibular joint.

Unilateral injections of the retrograde axonal transport marker horseradish peroxidase (HRP), were made in the joint capsule of 8 animals to identify the location of the first order neurons which innervate the temporomandibular joint. Four of the eight animals also received unilateral injections of HRP in the contralateral masseter and temporalis muscles. Following 48-72 hours of survival the animals were killed and selected tissues processed according to Mesulam (J. Histochem. - Cytochem. 24:1218, 1976).

Careful examination of serial transverse frozen sections throughout the rostrocaudal extent of MNV revealed no labelled neurons in animals receiving unilateral injections of HRP in the temporomandibular joint. Labelled neurons were found only in the MNV of animals receiving muscle injections. In 6 of 8 cats, the trigeminal ganglion ipsilateral to the HRP injected joint capsule contained a substantial number of labelled neurons ranging 35-60 micrometers in diameter. These cells were located in the most caudal part of the mandibular division of the ganglion.

The somatotopic localization was striking when compared with the distribution of labelled cells in the contralateral trigeminal ganglia of the animals receiving muscle injections. In the latter, labelled cells were distributed in more rostral portions of the mandibular division.

Preliminary microelectrode studies of the trigeminal ganglion have allowed identification of neurons with specific response patterns to jaw opening, jaw closing and intermediate angular excursions.

1757 DEVELOPMENT OF SOMATIC SENSORY FUNCTION IN NORMAL AND BRAIN LESIONED INFANT RHESUS MONKEYS. Mary Carlson. Dept. Psychiatry, Harvard Medical School, Boston, MA 02115.

In previous studies of adult rhesus monkeys, surgical lesions of the hand area of primary somatic sensory cortex (SI) produced severe and permanent deficits in the acquisition of manual tactile discriminations. Partial lesions of single hand representations in cytoarchitectural subdivisions of SI produced selective discrimination deficits and recovery of function was not found despite some redundancy of physiological input to remaining subdivisions.

As infant animals are generally believed to have a greater capacity than adults for functional recovery following cortical damage, studies of the correspondence between physiological input to SI and tactile discrimination capacity of normal and lesioned infant monkeys were designed. The results of both electrophysiological and behavioral studies in the normal infant monkey suggest that SI is functionally mature as early as 2-10 weeks of age. Single cortical cells in the hand and arm area of SI responded to either joint or cutaneous stimulation in recording studies of awake infants as young as 2 weeks of age. Furthermore, infants as young as 10 weeks of age (when first developing the motor control necessary for performing the task) were able to master tactile discriminations at a level similar to the adult.

The consequences of SI lesions in infants differed from those found in adult monkeys. Infants receiving partial lesions (Brodmann's area 3, or areas 1 and 2) at 3 weeks of age, made more errors and learned more slowly than normal infants, but performed at normal levels within 14 weeks of training. Animals with a total SI hand area lesion (areas 3, 1, and 2) had a normal discrimination capacity as well, but learned at the same rate and with comparable numbers of errors as normal infants. This difference in the time course of recovery for the two infant groups suggests that the remaining SI cortex in the partial lesioned animals disrupts rather than facilitates the recovery process. Juvenile monkeys receiving either partial or total lesions at 18-24 months of age show a significant impairment in discrimination performance comparable to the adults in previous studies.

These studies are the first to demonstrate recovery of normal function following lesions of sensory cortex in infant primates. Although SI is functionally mature in the first weeks of life, it has a capacity for compensation of tactile discrimination function lacking in the adult and juvenile cortex.

(Supported by PHS grants NS 12090 and NS 14261).

1758 SPINAL PROJECTIONS FROM THE MIDBRAIN OF RAT. A. J. Castiglioni, S. P. Wise, E. A. Murray and J. D. Coulter, Marine Biomedical Institute, and Depts. of Physiology and Biophysics and Psychiatry and Behavioral Sciences, University of Texas Medical Branch, Galveston, Texas 77550.

Horseradish peroxidase was injected into the spinal cords of rats into either the lumbar enlargement, thoracic cord, cervical enlargement, upper cervical cord, (C2-C4) or spinal trigeminal complex. The brains were cut at 50μm and reacted either with diaminobenzidine, o-dianisidine or tetramethylbenzidine to visualize retrogradely labelled neurons. Caudally in the midbrain, beginning at the level of the trochlear nucleus and extending rostrally to levels of the medial geniculate body, large numbers of cells in the medial reticular formation were labeled. These cells were found mainly ipsilateral to injections at all levels of the cord. Scattered cells throughout the central gray were also labeled and were most numerous laterally in that part adjacent to the medial reticular formation which contained labeled cells. The central grey also contained labeled cells in similar locations following injection of the spinal trigeminal nuclear complex. Rostrally many labeled cells were found in the region of the nucleus of Darkschewitsch, the nucleus of Edinger-Wesphal and the interstitial nucleus of Cajal, mainly ipsilateral to injections at all spinal levels. The red nucleus contralateral to injections contained labeled cells with a somatotopic distribution. Those projecting to the cervical cord were located dorsomedially while those projecting to the lumbar cord were found ventrally. HRP labeled cells were also found in the deep layers of the superior colliculus after cervical injections contralaterally. Ipsilateral to injections of the upper cervical cord many labeled neurons were found in the zona incerta. Cervical spinal injections also resulted in labeled cells scattered in the ventral midbrain tegmentum surrounding the decussation of the brachium conjunctivum

Rapid Golgi studies of the medial portion of the rat midbrain reticular formation, in those regions which send axons to the spinal cord, reveal both rounded and elongated neurons with cell diameters of 10-25μm. Most have 3 to 4 primary dendrites which branch to various degrees. Dendrites of nearly all neurons possess varicosities of 2μm x 5μm along the length of the dendrites. Dendrites extending as far as 300μm from the soma have been seen in transverse sections, but do not extend far from the soma in the rostrocaudal direction. Cells in the medial reticular formation can be seen to send their dendrites across the border of the central gray, and far into the central gray substance itself.

This work was supported by NS 12481 and NS 05736.

1759 TOPOGRAPHIC ORGANIZATION OF THE LATERAL CERVICAL NUCLEUS IN CAT. A.D. Craig, Jr., and H. Burton, Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110

The topographic organization of cells in the lateral cervical nucleus (LCN) with ipsilateral somatic receptive fields (RFs) was examined with extracellular unitary recordings. Closely-spaced (100 μm grid) microelectrode penetrations were made, angled along the transverse axis of orientation of the LCN (dorsolateral to ventromedial) in the dorsolateral funiculus (DLF). RF location, stimulus sub-modality, antidromic latency (from the thalamus), and unit position were recorded for 371 LCN neurons in six recording loci in four cats. LCN neurons with hindlimb (H) RFs were located dorsolaterally (superficially), those with forelimb (F) RFs were located more ventromedially, and those with facial RFs were most medial within the LCN. Most units with thoracic RFs were located between H and F units. Some interspersion of H and F units was observed; it was thus not possible to distinguish whether the underlying organization is "segmentotopic" or "somatotopic". A special subpopulation of units (17%), i.e. those which had widefield RFs, and/or had deep, visceral, or noxious input, and/or did not project to thalamus, was histologically verified to lie in the most ventromedial LCN. This group may contain interneuronal elements.

A corresponding topography has been observed in the contralateral projection of LCN neurons to ventrobasal thalamus with the horseradish peroxidase (HRP) technique. Cells in the lateral LCN (hindlimb area) were labeled after HRP injections in VPL, pars lateralis; medially-located (forelimb area) LCN cells were labeled from VPL, pars medialis. Some of the most medially-located cells could be labeled from all parts of VPL, and from VPM: this result is consistent with the physiological identification of a special subpopulation of neurons in the LCN.

These results together indicate a consistent pattern in the organization of the spinocervicothalamic pathway, analogous to that of the dorsal column system, albeit with less discriminable precision in its topography. The analogous organization is further underscored by the identification of a special subpopulation of LCN neurons, which is separable from the main population of projecting neurons and which probably contains interneurons.

Supported by USPHS 5-T01-DE00090-15, NS07505, NS09809, and 5-T32-NS07057-02.

1760 LOCALIZATION BY THE HORSERADISH PEROXIDASE TECHNIQUE OF MEDULLARY NEURONS PROJECTING EFFERENT FIBERS TO THE CAROTID SINUS NERVE OF THE CAT. William C. de Groat, Charles Morgan, Irving Nadelhaft, Thomas Schauble*. Dept. Pharmacol., Univ. of Pittsburgh, School of Medicine and Veterans Administration Hospital, Pittsburgh, PA 15261

The carotid sinus nerve (CSN) of the cat contains efferent axons which are thought to provide an inhibitory input to the carotid body. In the present investigation the technique of retrograde axonal transport of horseradish peroxidase (HRP) has been used to identify the location within the central nervous system of the cell bodies of this efferent pathway. In anesthetized cats the CSN was sectioned near the carotid sinus and the central end of the nerve was exposed to 25% solution of HRP for 6-12 hours. In two experiments the central end of the sectioned glossopharyngeal nerve was also exposed to HRP. After an additional 12-40 hours transport time the animals were sacrificed and perfused with fixative. Frozen sections (42 μ) of the medulla, caudal pons and petrosal ganglion were processed in benzidine and examined with darkfield illumination. Neurons containing HRP reaction product were detected in 5 of 10 preparations following application of HRP to the CSN. The cells were round or spindle shaped, 10-20 μ in diameter and located in the region of the ipsilateral nucleus ambiguus and the adjacent reticular formation (nucleus parvocellularis). A few cells were also observed in a more dorsal position in the reticular formation. Usually only one or two cells were detected in a section. The maximum number of cells identified in single preparation was 58. These occurred over a rostro-caudal length of 1.4 mm. In different animals cells were detected from AP coordinates -8 to -10. The number of labelled afferent neurons identified in petrosal ganglion varied in different preparations from 175 to 613 (average 367). Afferent axons in the medulla were also labelled with HRP. These were evident as lines of fine granules within the central projections of the CSN nerve. These HRP labelled axons could be traced to the nucleus tractus solitarius. Afferent labelling and larger numbers of labelled cell bodies were observed after application of HRP to the glossopharyngeal nerve. Labelled cells were distributed in the nucleus ambiguus and more widely in the reticular formation.

It is concluded that efferent fibers in the carotid sinus nerve are derived from small neurons in the medullary reticular formation adjacent to the nucleus ambiguus.

Supported by a grant from the Western Pennsylvania Heart Association and USPHS grant NS07923.

1761 EFFECTS OF L-DOPA ON DORSAL HORN UNIT RESPONSES TO MECHANICAL STIMULI. Jonathan Delatizky, Charles J. Hodge and Charles I. Woods*. Dept. of Neurosurgery, Upstate Medical Center, Syracuse, NY 13210.

Studies of the effects of L-Dopa on sensory transmission have suggested an inhibitory role for norepinephrine (NA) in the spinal cord. (Anden et al, Acta Physiol. Scand. 67:373, 1966)

In contrast, studies of morphine and stimulus produced analgesia show an elevation in nociceptive thresholds following depletion of NA and a decrease in analgesia with increased NA. (eg Akil & Liebeskind, Brain Research 94:279, 1975). Thus there is evidence that NA has both inhibitory and facilitatory roles. NA endings in spinal cord all originate supraspinally, and can release transmitter after intravenous L-Dopa (Anden et al, op. cit.). We have investigated the effects of L-Dopa on transmission of innocuous mechanical stimuli to lumbar dorsal horn units in acute spinal cats.

Cells were characterized using extracellular single unit recordings on the basis of response to innocuous stimuli only (group I) or to innocuous as well as noxious stimuli (group II). A repeated identical deflection of skin or hair was presented at 5 second intervals and the responses were recorded for 5-15 minutes before and at least 60 minutes after intravenous L-Dopa (10-25 mg/kg). Average spikes per stimulus and average spontaneous activity were determined for each 5 minute block. Cell locations were marked by dye ejection after recording.

Results were 1) Most group I cells were facilitated. 2) Most group II cells were inhibited, sometimes with late facilitation. 3) Most facilitated cells were in lamina IV, while most that were inhibited were in laminae I and V-VI. 4) No change in receptive field size or response type occurred. Since L-Dopa can cause serotonin release, experiments were repeated in cats pretreated with p-clorophenylalanine (PCPA) to deplete 5-HT. In these animals most cells were facilitated without regard to group or laminar location. About one third had an increase in receptive field size. Responses of group II cells in PCPA treated animals were significantly different from those of the untreated cats. In chronic spinal cats all but one of nine cells showed no changes from control following L-DOPA, confirming the supraspinal origin of the effects.

The results suggest 1) Metabolites of L-Dopa (presumably NA) facilitate transmission of innocuous stimuli at the spinal cord level. 2) L-Dopa causes overflow of 5-HT in a descending pathway selective for cells with nociceptive inputs. Attempts to confirm NA as responsible for the changes, as well as extension to noxious stimuli, are in progress. (supported by PHS grant NS 12761-01)

1762 CODING OF STIMULUS LOCATION AND INTENSITY IN SOMESTHESIS: A NEURONAL POPULATION RESPONSE MODEL. G.S. Doetsch and R.P. Erickson. Depts. Surg. (Neurosurg.) & Physiol.,Med. Coll. Ga., Augusta, Ga. 30901 & Dept. Psych., Duke Univ., Durham, NC 27706.

The broad sensitivity of individual neurons to several stimulus parameters (e.g. location, intensity, quality) presents difficulties for any model of sensory coding which considers single neurons to serve specific, unitary functions. The large receptive fields (RFs) of somatosensory neurons alone are problematic in explaining the precision of tactile localization and discrimination. This was evident to Adrian (1931) who showed that individual somatosensory RFs on the frog's skin range from 4-100 mm^2 and to Tower (1940) who observed that RFs on the cornea and sclera of the cat range from 50-200mm^2. Many investigators, using suprathreshold stimulation, have confirmed the existence of RFs that are large relative to tactile discriminability. Adrian hypothesized that stimulus location is signaled by the "particular combination of fibres in action, together with the relative intensity of the discharge in each;" a similar coding mechanism was suggested by Tower. Their similar theoretical positions have gone largely unnoticed in those modern theories of coding which suggest that the identity and location of a stimulus are signaled by individual neurons specifically labeled in their meaning to the organism.

The problems raised by the large RFs and multiple sensitivities of somatosensory neurons are analogous to the problems posed in other sensory systems with broadly-tuned neurons. However, a neuronal population response model, consistent with the ideas of Adrian and Tower, provides a solution. Application of the model shows that precise information about taste quality, limb position, sound localization, temperature, color, etc. can be signaled by the profile of impulse frequencies in a responding population of neurons (an "across-fiber pattern," AFP) rather than the discharge of individual neurons per se. Intensity can be signaled by the total discharge frequency in the responding neuronal population. For example, it is shown that taste stimuli which are similar in quality produce similar AFPs.

Preliminary studies on the somatosensory system of the rat (Cassel, Anz; unpublished data) indicate that the AFP model is applicable to the problem of tactile localization. Analysis in terms of AFPs suggests that localization on the rhinarium exceeds that on the forepaw, which in turn exceeds that on the hindpaw--in this analysis, innervation density (neurons/cm^2) is more relevant that RF size. The breadth and complexities of the RFs, which may be troublesome for labeled-line coding, are advantageous in the AFP model. More detailed applications of the model to somesthesis are given by Ray and Doetsch (these proceedings). (Supported by grants from U.S. Army, NSF, and NIH)

1763 NON-SPECIFIC THALAMIC PROJECTIONS TO SENSORY-MOTOR CORTEX. John P. Donoghue and Ford F. Ebner. Neurosciences Section, Brown University, Providence, RI 02912.

The central intralaminar nucleus (CIN) of the opossum (D. virginiana) thalamus is located dorsal and medial to the ventrobasal complex. CIN receives inputs from at least spinal cord, cerebellum, and dorsal column nuclei (Walsh and Ebner, '73). Lesions of CIN result in a widespread projection from this nucleus to layer I of somatic sensory-motor (SSM) cortex (Killackey and Ebner, '72; '73). The present results demonstrate several additional features of these non-specific thalamocortical projections. Following injection of horseradish peroxidase (HRP) into physiologically defined zones of SSM cortex, retrogradely labelled neurons are present in CIN as well as in the ventral nucleus (VB & VL.) Injections of HRP into hand area leads to labelling in more lateral parts of CIN, while injections in the face area label more medial parts. This topography is crude compared to that seen in VB. Electrophysiological criteria can be used for placement of injections or lesions in CIN. CIN neurons respond at a long (20-50 msec) and variable latency after electrical stimulation of the contralateral body surface. They fail to respond consistently at repetition rates above 1 per 2 sec. Subsequent to localization of CIN and injections of H^3 leucine and proline, silver grains are concentrated in the outer 1/3 of layer I, over a wide area of SSM cortex. In cases with no spread of label from the injection into VB, there is no concentration of silver grains in any other cortical layer. These injections confirm the HRP topography. EM autoradiography and degeneration procedures reveal CIN terminals in the same subdivision of layer I, with only rare terminal degeneration in other layers. CIN terminals contain round synaptic vesicles and are associated with an asymmetrical membrane differentiation. Approximately 80% of these synapses contact dendritic spines. These studies demonstrate the properties of one type of non-specific thalamocortical projection that are clearly different from the "specific" VB projection. Based on connectional information, CIN appears comparable to some part of the posterior complex in other mammals. (Supported by USPHS grant NS-13031).

1764 VELOCITY DEPENDENCE OF A DIMENSION OF CUTANEOUS SENSIBILITY. D. Dreyer, M. Hollins, B. Whitsel and M. Young. Dent. Res. Ctr. & Depts. of Physiol & Psychol, U.of N.C., Chapel Hill, NC 27514.

Three different psychophysical experiments were performed in order to assess the effects of stimulus velocity on perception of the distance traversed by a moving tactile stimulus in normal human subjects. In all experiments, constant velocity stimuli were applied to the dorsal surface of the left forearm; 10 different velocities between 1 and 250 cm/sec were employed. All stimuli moved from distal to proximal and the length of skin contacted by the brush was fixed by a plate having a 4 X 0.5 cm aperture. The 16 subjects were naive as to the design of the experiments. Subjects were required to compare the distance traversed by a test stimulus delivered 2 sec after a standard stimulus. In Exp. 1, the velocity of the standard stimulus was 15 cm/sec. In that experiment, each subject was instructed to assign to his sensations of the distance moved by the standard stimulus a value of 50, and to rate his estimate of the distance moved by the test stimulus accordingly. In Exp. II, subjects were asked to use a drawing of the stimulated region of the arm as the basis for their reports of stimulus distance. The drawing illustrated the region on the arm as a linear array of 9 segments that were numbered sequentially. The most distal segment was assigned the number "0" and the most proximal "8". Subjects were required to identify the segments traversed by the test stimulus delivered 2 sec after the standard (15 cm/sec) which they were told moved continuously from segment "2" through segment "6". Exp. III utilized the same design as II except that sessions were run using standard velocities of 2.5, 15, 75 and 150 cm/sec. The data from this experiment indicate that the velocity dependence of estimated stimulus distance (ESD) is not influenced by the velocity of the standard stimulus. Several observations are common to the data from all three experiments. First, although the actual length of skin contacted by the brush was 4 cm for all stimuli, the ESD is a decreasing function of stimulus velocity. Second, the function relating ESD to velocity is relatively flat in the range of 10-30 cm/sec (ESD is independent of velocity over this range) but possesses an appreciable negative slope at both lower and higher velocities. It is of some interest that the flat portion of the function (where ESD remains constant in the face of changes of velocity) occurs over a range of velocities that is identical to the range of velocities required for optimal performance in a direction discrimination task. The present study has identified dimension of cutaneous sensibility that exhibits a pronounced dependence on stimulus velocity and provides the basis for subsequent investigations of the underlying central neural events.
Supported by grants NS10865, DE02668, RR05333, MH14277 & DE00011.

1765 THE SEGREGATION OF SUBMODALITIES IN PRIMARY SOMATOSENSORY CORTEX OF THE CAT. R. W. Dykes, D. D. Rasmusson*, P. B. Hoeltzell*, Department of Physiology & Biophysics, Dalhousie University, Halifax, Nova Scotia.

In 15 adult mongrel cats the primary somatosensory cortex was studied with glass-coated tungsten microelectrodes which effectively recorded from single neurons and small clusters of units in most layers of cortex. Special attention was given to (1) the details of somatotopy and (2) the nature of the stimulus adequate for eliciting a response. In two animals numerous vertical penetrations throughout SI allowed reconstruction of a precise somatotopic map. In 13 of the cats, electrodes were introduced into the forelimb region of SI in long slanting penetrations which crossed several cytoarchitectonic boundaries. This allowed detailed analysis of both the sequence of adequate stimulus submodalities and the sequence of body parts without the errors of electrode placement which accompany repeated vertical penetrations.

Electrolytic lesions placed at points of interest allowed reconstruction of the path taken by the electrode and permitted function to be related to cytoarchitecture.

Our results suggest that somatosensory submodalities are localized in narrow bands of cortex that stretch along the mediolateral extent of SI. These functional bands are more distinct in some animals than in others, but in all cases it was possible to identify bands of deep and cutaneous modalities. In most cases the cutaneous band could be further divided into regions with either rapidly adapting or slowly adapting characteristics.

This functional segregation within SI cortex may reflect the terminal stage of a sensory system containing separate but paralled relay paths for different receptor classes or it may arise as the result of different kinds of processing mechanisms within the cortex, operating on the same afferent information in completely different ways.

(Supported by the Medical Research Council of Canada)

1766 DEGENERATIVE CHANGES IN DENDRITES OF INTERNEURONS IN LAYERS II AND III OF THE DORSAL HORN OF THE MEDULLA IN DEVELOPMENTAL STUDIES IN NEWBORN KITTENS AND FOLLOWING PULPOTOMIES IN ADULT CATS. William Falls and Stephen Gobel. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD. 20014

Most of the small caliber primary trigeminal axons entering the substantia gelatinosa of Rolando in the caudal medulla synapse in layers II and III on dendrites of two major kinds of interneurons, stalked cells and islet cells. At birth, numerous dendrites radiate in all directions from the cell bodies of the most immature forms of both of these cells. During maturation, a few of these dendrites continue to elongate while many others bead up and disintegrate. Within the beads, small vesicles collect in aggregates and begin fusing with each other to form small cavities. These cavities continue to enlarge and hollow out the beads. Their membranes fuse with the plasma membranes of the beads and the cavities ultimately open to the intercellular space. Finally, the beads fragment and disintegrate. Disintegration of beaded dendrites takes place despite synaptic input from non-primary axons. However, beaded dendrites have never been observed in synaptic contact with a primary axon.

In adult cats, the tooth pulps, which contain the distal ends of large numbers of primary (Aδ and C) trigeminal neurons, were removed from all mandibular teeth on one side and prevented from regenerating. This procedure results in degeneration of axonal endings of affected primary neurons in layers I, II and III (Gobel and Binck, Brain Res. 132(1977)347-354) and transsynaptic degenerative changes in stalked cell and islet cell dendrites 30 and 60 days after surgery. The process of dendritic degeneration proceeds through the same sequence in this experimental situation as in the normal developmental situation with numerous cavities developing in layer II and III dendrites. These cavities hollow out the dendrites, open to the intercellular space and lead to fragmentation of the dendrites. Cavitation of dendrites occurs following a partial loss of primary input despite appreciable synaptic input from non-primary axons.

These studies show that the presence of synapses from non-primary axons on stalked cell and islet cell dendrites is not sufficient to assure their continued elongation during development nor are they sufficient to assure their survival in the adult should even part of their primary afferent input be lost. These studies suggest that the establishment of synaptic connections between primary trigeminal axonal endings and stalked cell and islet cell dendrites is essential for these dendrites in terms of their progressive elongation during postnatal development as well as for their survival in adulthood.

1767 COLLATERAL AFFERENTS TO THE INFERIOR OLIVE AND SUPERIOR COLLICULUS ORIGINATING IN THE SPINAL TRIGEMINAL NUCLEUS. Anthony Frankfurter, John Persing, and Oswald Steward.Depts. of Neurosurgery and Physiology,Univ.of Virginia School of Medicine,Charlottesville,VA

Horseradish peroxidase in conjunction with poly-l-ornithine was used as an anterograde and retrograde tracer to identify the connections of the superior colliculus with midbrain, brainstem, cerebellar, and spinal cord cell groups in the rat. For comparison, the superior colliculus in each of a control series of rats was injected with tritiated proline, and the brains of these animals were processed for autoradiography. The anterograde labeling of fibers and their terminations with HRP is comparable to that seen in the controls. The most significant findings of this study are the extremely large number of labeled neurons in the contralateral interpolar division of the spinal trigeminal nucleus, a lesser number of labeled cells in the contralateral cuneate nucleus, the axon collaterals associated with these cell groups, and the presence of terminal fields in the inferior olive not demonstrable in the tissue which was processed for autoradiography.The labeled cells in the interpolar division of the spinal trigeminal complex are predominantly of the large multipolar type. Two fiber bundles can be identified originating from these neurons. One bundle can be traced exiting from the ventral aspect of the cell group and coursing dorsomedially across the midline and rostrally. The other bundle can be traced exiting from the medial aspect of the cell group and coursing ventromedially through medial levels of the ipsilateral inferior olive, and terminating within the contralateral principal and dorsal accessory nuclei. A smaller contingent of trigemino-olivary fibers can be traced to caudal levels of the inferior olive, where they appear to terminate within a medial segment of the medial accessory nucleus which is coextensive with neurons which receive direct afferents from the superior colliculus. Fibers could also be identified exiting from the rostral portion of the cuneate nucleus and joining the trigeminoolivary bundle. Although these fibers could not be traced in their entirety, terminal label and axon fragments are present in laterally located cells in caudal portions of the contralateral medial accessory nucleus. This segment of the inferior olive is known to receive dorsal column afferents. In summary, this preliminary evidence suggests that the same neurons which provide the cerebellum, via the inferior olive, with cutaneous input from the face and neck also contribute to the somatosensory map contained within the deep layers of the superior colliculus.

Supported by NIH grants NS12333, and T NS0701302.

1768 BODY SOMATOTOPY IN THE SECOND SOMATIC SENSORY CORTEX OF THE MONKEY. D.P. Friedman* (SPON: E.G. Jones). Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

The topography of the connections between the first (SI) and second (SII) somatic sensory areas was examined in cynomolgus and rhesus monkeys in an attempt to define the body representation in SII. Anterograde labelling patterns were studied in SII following injections of tritiated amino acids into parts of the representation in SI. It was assumed that an injection in SI would label a corresponding part of the representation in SII.

Groups of small injections into different parts of the SI representation in each case labelled obliquely oriented bands within SII. In the case of the forelimb area the label ran from posterolateral to anteromedial across an extensive region of the frontoparietal operculum. The evidence indicates that the face is represented anterolateral and the hindlimb posteromedial in relation to the forelimb representation outlined in this way. Portions of the lower trunk and leg representation were traced onto the upper part of the insula. Injections of horseradish peroxidase into SI which retrogradely labelled cells in SII tended to confirm this pattern for the reciprocal projection from SII to SI.

The larger bands of label seen in the autoradiographic experiments were composed of smaller columns approximately 0.25 to 0.5 mm wide. Each of these anterogradely labelled columns in SII involved layers I through IV with the densest label concentrated in layer IV.

Injections in SI with an effective width of about 0.5 mm labelled columns in SII also about 0.5 mm wide. The fact that SI is larger than SII and that its constituent fields (areas 3b, 1 & 2) were each shown to project onto SII indicates that SI projections converge onto specific areas of SII.

Contrary to previous reports, no projection could be traced from the motor cortex to SII, though a projection was confirmed passing from SII to the motor cortex.

(Supported by Grants No. NS 10526 and No. F32-NS05884 from the National Institutes of Health, U.S. Public Health Service.)

1769 EXCITATORY AND INHIBITORY INTERACTION OF MULTIPLE POINT STIMULI IN THE SOMATOSENSORY CORTEX OF ALERT MONKEYS. Esther P. Gardner and Richard M. Costanzo. Dept. of Physiology, NYU School of Medicine, New York, N.Y. 10016.

To determine principles of sensory coding of spatial information on the skin, we examined how single neurons in primary somatosensory cortex (SI) of alert monkeys integrate information from multiple points on the skin. Brief (10 ms) airpuffs were presented both individually, and simultaneously at 3 points 15 mm apart on forearm hairy skin along the longitudinal axis of cortical receptive fields, and the number of spikes produced at each position were measured. Each pattern was tested with a resolution up to 2.5 mm on the skin in order to reconstruct the spatial distribution of activity in SI. Cortical response profiles of the number of spikes per stimulus as a function of field position evoked by simultaneous stimulation of 3 closely spaced points were unimodal: peak activity occurred when the middle airpuff was directed close to the center of the receptive field and the lateral airpuffs straddled the field center. The response profile gently curved across the span of the airpuffs and was much broader than the profile produced by only one airpuff of the same intensity. Most cortical neurons in alert monkeys showed no increase in the overall amplitude of the three probe response profile compared to the single probe profile, but instead displayed a broadening and spreading out of its peak. Responses to 2 of the 3 airpuffs straddling the field center were bigger than responses to separate presentation of any of them, but the lateral airpuff representations were suppressed and smaller than the response to single airpuffs on the field center. Summation of excitation from multiple stimulus sites is non-linear, suggesting occlusion of the three inputs.

To test the role of in-field inhibition on non-linear summation of excitation from multiple stimulus points within cortical receptive fields, strong test airpuffs were delivered near the field center 30-40 ms after conditioning single and 3 point stimuli at various positions within the field. Test response suppression paralleled excitation; in-field inhibition was maximum at points producing the most excitation. Three point stimuli elicited slightly more inhibition than single point stimuli, suggesting occlusion in the inhibitory as well as excitatory pathways.

(Supported by USPHS Research Grant No. NS11862, USPHS RCDA No. NS00142 and the Irma T. Hirschl Foundation.)

1770 INTERACTION OF ORTHO- AND ANTIDROMIC ACTIVITY IN HAIR FOLLICLE AFFERENT UNITS. M.D. Goldfinger and V.E. Amassian. Dept. of Physiology, SUNY, Downstate Medical Center, Brooklyn, N.Y. 11203.

Activity of a single primary afferent axon is recorded with a glass-insulated tungsten microelectrode in the cuneate fasciculus of Nembutal anesthetized, Flaxedil immobilized cats during mechanical stimulation of one or more guard hairs of the axonal receptive field. During continuous airjet stimulation, the evoked spike train is largely random (ref.1) with a mean frequency which is non-linearly proportional to the number of driven innervated receptive field hairs (ref.2).

The primary afferent axonal branches are myelinated distally until near the receptor. To assess the effect of antidromic propagation, the parent axon was stimulated electrically some 5 mm central to the edge of the receptive field with one or a train of 0.1 msec suprathreshold shocks. Antidromic stimulation at 10/sec during continuous airjet stimulation of the entire receptive field at either strong or weak intensities revealed in the Post Stimulus Histogram a brief (2-3 msec) silent period subsequent to the antidromic spike. Following this silent period, a steady-state level was reached either rapidly (~1 msec) or slowly (~5msec or more), depending upon whether the unit fired at a high (> 60/sec) or low (< 60/sec) rate, respectively, during airjet stimulation alone of the whole field at maximal intensity. Significantly, for each unit, such pattern of recovery and steady-state level during mixed anti-dromic electrical plus orthodromic airjet stimulation closely resembled that observed in the Expectation Density function of the spike train evoked by an airjet alone at the same intensity. Thus, excitability appeared to recover rapidly when tested with an airjet stimulus.

By contrast, the threshold amplitude of a fast (~2 mm/msec) triangular displacement of a single innervated hair was increased for longer periods (eg. 8 to 25 msec) following one or a train of electrically evoked antidromic spikes (cf analogous changes in velocity threshold following an antidromic spike:ref.3). No sign of such long recovery was observed during airjet alone or airjet plus antidromic electrical stimulation.

Evidently, even weak airjet stimulation soon overcomes the excitability changes caused by electrically evoked activity, and any excitability changes that might be caused by mechanically evoked activity in the branched complex of the parent axon.

(1) Goldfinger & Amassian (1976). Neurosci. Abst. II:911.

(2) Goldfinger & Amassian (1977). Neurosci. Abst. III:482.

(3) Tuckett,Horch,& Burgess (1978), J. Neurophysiol.41:138-149

(Aided by USPHS, NIH Grants NS11219 and 10987.)

1771 VIBROTACTILE SPATIAL RESOLUTION OF THE QUICKLY ADAPTING FIBER POPULATION. A.W. Goodwin*, M.E. Pierce* and B.D. Youl* (SPON: I. Darian-Smith). Dept. Physiol., Univ. of Melbourne, Parkville, Victoria 3052, Australia.

Psychophysical studies have indicated that vibrotactile resolution is better at 250 hz than it is at 40 hz. It has been suggested that this information is conveyed by the Pacinian corpuscles as they are more sensitive at 250 hz than the Meissner endings which serve the Quickly Adapting fibers (QA's). However Pacinians seem ill suited to convey spatial information because of their large receptive fields whereas the QA's, with their more confined receptive fields, are more likely candidates for this task. We have studied the ability of the QA population in the glabrous skin of the monkey to represent a simple spatial pattern consisting of two probes vibrating in phase. The features of this representation were compared at stimulating frequencies of 40 hz and 200 hz.

The stimulus consisted of two 1 mm diameter lucite probes, separated by 2 mm or 3 mm between centers, vibrating in phase. Single QA's were isolated and their response measured as a function of vibratory amplitude, frequency and position of the probes in the receptive field. Response and stimulus amplitude were related by a piecewise linear function of ramps and plateaux, as for single vibrating probes. Individual fibers differed only in their sensitivities which defined the corners of the function. The effect of position in the receptive field was quantified at both frequencies, independently of fiber sensitivity. Thus for our stimulus a QA's response was determined by a fiber-independent field factor plus the sensitivities of the particular fiber.

From this general description the response of any QA at any position with respect to the probes could be calculated for any stimulus amplitude at either frequency. This allowed us to reconstruct the response of the whole QA population to two vibrating probes and examine the effect of various parameters. The spatial configuration of the stimulus was represented in the QA population even at 200 hz. Moreover, at an amplitude of 100 microns, this representation was more precise at 200 hz than at 40 hz. The reconstruction allows ready visualization of the effect of different QA innervation densities and the effect of variation of sensitivity within the QA population. Population responses were compared for vibrating stimuli and vibrating stimuli scanned across the skin such as occur with the Optacon.[1] The results show that the QA population is capable of transmitting spatial information used by blind people reading with the Optacon.

[1]A prosthetic reading aid for the blind

1772 RESPONSES OF JOINT AFFERENT NEURONS TO CONTROLLED AXIAL LOADINGS OF POSTERIOR JOINT CAPSULE.
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Recordings were made from joint afferent neurons innervating sections of posterior joint capsule from the cat knee. The joint capsule section and its innervation, the posterior articular nerve (PAN) were excised from the knee and studied in vitro using an apparatus which produced controlled loadings in 2 axes of the section. Displacements were produced in the femur-to-tibia axis and in the medial-to-lateral axis. Capsullar afferents were activated in response to loadings in both axes. Thresholds for activation were very low, and the frequency of response of afferents was roughly linearly related to the applied stress in either axis. Only slowly adapting discharge was observed. These results are in sharp contrast to the results of similar experiments done on knee joint medial capsule, where the majority of afferents are sensitive to compressive rather than axial loadings.

Supported by NIH grant NS-10783.

1773 A NORMAL AND ALTERED CORTICAL COLUMN: A QUANTITATIVE AND QUALITATIVE (^{14}C)-2 DEOXYGLUCOSE (2DG) MAPPING STUDY. Peter J. Hand, Joel H. Greenberg, Richard R. Miselis, W. Lee Weller, and Martin Reivich. Depts. Animal Biology and Neurol., Sch. Vet. Med. and Med. and Inst. Neurol. Sci., U. of Pa., Phila., PA 19104.

A previous qualitative 2DG metabolic mapping study of rat vibrissal - cortical barrel system (Neurosci. Abst., 3, 1977) confirmed the topographical organization of the SI barrel field and demonstrated the one-to-one vibrissal-barrel relationship. The laminar distribution of 2DG labeling in cortical barrel column #3, row C was also determined. This investigation examined, quantitatively and qualitatively (Sokoloff et al., J. Neurochem. 28, 1977), the laminar distribution of 2DG labeling in normal and developmentally altered cortical column #3, row C in 8 unanesthetized, restrained adult rats. All vibrissae, excepting #3, row C, were clipped acutely. Vibrissa #3 was stroked 2-3x/second for 35-50 minutes, using a hand-held brush, to increase activity and uptake of 2DG in neurons of cortical barrel column #3, row C. The single column extended from Lamina I(deep) through VI, with densest labeling occurring in layer IV (laminae were determined in counterstained serial sections). The column, not of uniform diameter, was candle-pin shaped. Lamina IV was its widest portion. As the column extended through the remaining superficial and deep cortical layers, a narrowing of its diameter occurred. The laminar analyses of the mean values of the local cerebral metabolic rates for glucose (LCMRG) confirmed the qualitative findings of 2DG labeling density in the individual laminae. Within cortical column #3, row C, the LCMRG (μ mol/100g/min) were determined for each of the cortical laminae: I-83.8; II-III-98.3; IV-143.8; V-107.2; upper VI-93.8; and lower VI-73.3. The LCMRG for laminae of the barrel field, which surround column #3, row C were as follows: I-76.1; II-III-72.7; IV-77.2; V-77.2; upper VI-67.2, and lower VI-58.3. The normal laminar distribution of 2DG labeling in column #3, row C of the adult rat was significantly altered by vibrissal follicle removal (sparing #3, row C) one day postnatally. At 90 days post-follicle removal, labeling in layer IV remained focal, but was of reduced density and size. The oval, focal pattern of labeling (tangential sections) was not discernable in laminae I-III or V-VI. Their labeling appeared diffuse; extending over a wide area of the surrounding barrel field. Thus the metabolic (functional) alterations in lamina IV, the major recipient layer of thalamocortical projections, are minimal, but become accentuated in the remaining cortical laminae presumably via changes (unknown) in local corticocortical connectivity resulting in an "hour-glass"-shaped cortical column. (Supported by Grants NS-06716 and NS-10939).

1774 PRE-INTRALAMINAR NUCLEI IN THE OPOSSUM BRAINSTEM. James C. Hazlett, Dept. of Anatomy, Loyola University, Stritch School of Medicine, Maywood, IL 60153

Brainstem nuclei whose efferents ascend to or through the thalamic intralaminar area have been identified using the retrograde transport of horseradish peroxidase. Unilateral hydraulic injections of 0.02 μl-0.2 μl (25%-50%) of HRP were centered in the thalamic intralaminar nuclei (parafascicular complex and paracentral nucleus) as delineated by Oswaldo-Cruz and Rocha - Miranda '68. While brain slices were exposed to one of several substrates, we found that o-dianisidine yielded exceptional results in our material. Retrograde neuronal labelling subsequent to small intralaminar placements (.02μl - .05μl of HRP) was observed in the following locations: ipsilaterally in the superior colliculus and areae cuneiformis; contralaterally in the interstitial tegmentum, caudal portion of the nucleus pontis caudalis, nucleus gigantocellularis and deep cerebellar nuclei and bilaterally in the nucleus pontis oralis and nucleus locus coeruleus. A few labelled cells were also present in the nucleus raphe magnus. The larger intralaminar placements (0.1 or 1-0.2μl of HRP) resulted in retrograde labelling in the above mentioned areas but additionally included reactive neurons in the pars reticulata of the ipsilateral substantia nigra and in the contralateral spinal trigeminal complex (oralis and interpolaris), dorsal column nuclei, paratrigeminal nucleus and a portion of the lateral reticular nucleus. Retrograde neuronal labelling in these latter nuclei also is present following HRP injections centered in the VB and VL complexes. Interestingly, several of the larger intralaminar injections have resulted in retrograde labelling of neurons adjacent to the abducens nucleus, in the nucleus prepositus hypoglossi and Roller's nucleus. This work supported by NIH Grant number 05384-16.

1775 STUDIES ON THE TONIC EXCITABILITY OF SINGLE C-FIBERS' CENTRAL TERMINALS. Ian D. Hentall and Howard L. Fields. Depts. of Neuro. and Physiol., UCSF, San Francisco, CA 94143

Single C-afferents, recorded with NaCl-filled micropipettes in L6 or L7 dorsal root ganglia of cats, were stimulated through intraspinal microelectrodes in the region of their terminals. With each stimulus, pulse width was adjusted in the direction of the preceding threshold by a single uniform step. The computer program performing this threshold-tracking procedure was set to deliver test pulses with a 2 s. period, initial width of 200-300 μs., and width adjustment of ±7.5 μs. Antidromic thresholds, minimized by placement of the stimulating electrodes, were 4-270 μA (median 66 μA) at 300 μs. The 78 units studied were either classified as high threshold mechanoreceptors (10%), polymodal (15%), or C-mechanoreceptors (28%), or else they possessed no identifiable receptive field (32%).

All units had increased thresholds after being orthodromically active. The time course of this effect (4 min. to recover after orthodromic electrical stimulation of 30 Hz for 10 s.) resembled that of the hyperpolarization due to sodium pumping seen in peripheral nerve (Rang and Ritchie, J. Physiol. [1968], 193, 183). Various natural cutaneous stimuli were applied for 10-20 s. in the vicinity of a receptive field without causing the unit to fire. Significant increases (I) or reductions (R) of threshold occurred as follows: Noxious mechanical stimuli, I-41% and R-3%; innocuous mechanical stimuli (brushing), I-3% and R-32%; noxious heat, I-35% and R-18%. No qualitative effect of the class unit, of the exact site of the conditioning stimulus, or of the 3 kinds of preparation (low spinal/decerebrate, decerebrate, and anesthetized with chloralose-urethane) was discerned. It seems therefore that more than one class of primary afferent can affect the excitability of C-fibers' terminals.

Morphine (2 mg/kg) or naloxone (0.1 mg/kg) did not change thresholds in 10 units. However, this does not preclude opiates having some presynaptic action, not affecting excitability, which influences post-synaptic responses.

Trains of electrical pulses (20-50 Hz, 50-100 μA) in nucleus raphe magnus or adjacent reticular formation in 25 units gave I-56%, in both chloralose-urethane and decerebrate cats. Temporary cold block of the thoracic spinal cord produced R-2, I-2, and 8 without effect. Thus descending influences are either tonically active and of opposite effect from different brainstem regions, or not tonically active and mainly causing decreased excitability.

We conclude that terminals of C-fibers are modifiable by both segmental and descending influences.

1776 THE INTRACORTICAL AND INTERHEMISPHERIC CIRCUITRY OF THE FUNCTIONAL SUBDIVISIONS OF THE SI CORTICAL REGION IN THE RACCOON. Paul Herron*(Spon: J.L. Zacks). Dept. of Psychology, Michigan State Univ., E. Lansing, Mich. 48824

The intracortical and interhemispheric connections of functional subdivisions of SI was investigated. Behavioral data have shown that the SI and SII cortical regions are critical for the interhemispheric transfer of tactile habits. Anatomical data have shown that certain areas in both SI and SII are devoid of or receive relatively sparse projections from the contralateral SI and SII cortical areas. Those areas devoid of interhemispheric projections correspond to those areas that receive electrophysiological projections from the densely innervated regions on the animal's body surface such as the forepaw in the raccoon. The purpose of this study was to determine and compare the topography of intracortical and interhemispheric afferents and efferents of the functional subdivisions of SI which receive interhemispheric projections with those functional subdivisions of SI which are devoid of interhemispheric projections.

Injections of horseradish peroxidase and tritiated adenosine restricted to a particular functional subdivision of SI such as the forepaw were used in this study.

The results show that:

1. The forepaw area of SI is reciprocally connected with the ipsilateral forepaw area of SII. The terminals from SI as well as the cell bodies in SII which send efferents to SI are located primarily in layers three and five of the forepaw area in SII. The forepaw area in SI did send efferents nor did it receive afferents from the contralateral SI and SII.
2. The hindpaw area of SI is reciprocally connected with the ipsilateral hindpaw area of SII. The origin and termination of these reciprocal connections within the hindpaw area of SII is very similar to that decribed for the forepaw above.
3. The hindpaw area of SI is reciprocally connected with the contralateral hindpaw area in SI. The cell bodies in the hindpaw area which sends efferents to the contralateral SI area are located primarily in layers three and five. The terminals are located in all layers but primarily in layers three through five.

The present results indicate that the intracortical and interhemispheric projections of SI subdivisions are to homofunctional areas within SI and SII.

(Supported by NSF Research Grant GB 43236 and the Neuroscience Program).

1777 RECORDINGS FROM CAT SPINAL CORD NEURONES WITH THALAMIC CONNECTIONS. James A. Holloway, Richard E. Fox*, Ainsley Iggo* and Sukhbir S. Mokha*. Department of Veterinary Physiology, University of Edinburgh, Summerhall, Edinburgh EH9 1QH. U.K.

Adult cats were anaesthetized with α-chloralose,subsequently paralyzed with gallamine triethiodide, and artificially ventilated. Following a lumbar laminectomy, glass micropipettes containing 3M KCL and pontamine sky blue dye were utilized for recording and marking single unit electrical activity from the spinal cord dorsal and ventral horns. An array of 8 concentric bipolar stainless steel electrodes, for antidromic electrical stimulation (150μa, 0.3 msec duration) of spinal cord cells, was stereotaxically placed in the following contralateral thalamic nuclei : ventralis lateralis, ventralis postero-medialis, and centrum medianum. These nuclei are thought to receive spinothalamic tract axon terminals. Quantitative mechanical and thermal stimuli were applied to the skin of the hind limb contralateral to the site of thalamic stimulation. Receptive fields of neurones responding to thalamic stimulation varied considerably in size from an area of a few mm^2 to the entire limb surface. Proximal fields tended to be larger than distal fields. Units were observed in the dorsal horn in laminae I-IV that responded to : a) low threshold mechanoreceptive afferents, b) low threshold mechanoreceptive afferents and nociceptive afferents, or c) nociceptive afferents exclusively. Some units were activated antidromically by thalamic stimulation, and may be described as spinothalamic tract neurones; other units excited post-synaptically from the thalamus were in spinal cord laminae VI and VII. Post-synaptically activated responses were by far the most frequently recorded in response to thalamic stimulation. It is likely that at least part of this post-synaptic information is relayed via excitatory thalamo-reticulospinal neurones[1]. The results emphasize the diversity of response properties in some spinal cord neurones.

1. Mancia, M., Margnelli, M., Maurizio, M., Spreafico, R., and Broggi, G. Brain stem - thalamus reciprocal influences in the cat. Brain Res. 69, (1974), 297-314.

Supported by grants : SRC G/RA/2136.8 and NRS Award IF 34 GM 06519-01.NIGMS.

1778 DEVELOPMENTAL CHANGES IN THE DISTRIBUTION OF THE THALAMOCORTICAL RELAY CELLS OF THE VENTROBASAL COMPLEX OF THE RAT. G.O.Ivy* and H.P.Killackey. (SPON: M. Snyder). Dept. of Psychobiology, Univ. of Calif., Irvine, CA, 92717.

During the first few days of life, the pattern of the trigeminothalamic afferents to the rat ventrobasal complex undergoes a marked transformation. At the time of birth these fibers are distributed in a uniform fashion, but by the fourth postnatal day they are discretely organized into rows of clusters which replicate the topographical organization of the mystacial vibrissae on the snout of the rat (Belford, Anat. Rec., '78). Further, the corticothalamic afferents to the ventrobasal complex show a similar discrete organization, which develops along a similar time course (Akers and Killackey, this volume). The purpose of the present investigation was to determine if the thalamocortical relay cells of the ventrobasal complex were themselves organized in a discrete fashion, and if a developmental time course comparable to that of the afferents could be observed.

In the present experiment, one "early" group of rats received parietal cortex injections of horseradish peroxidase on Day 0 (day of birth), and at postnatal Day 1, before the development of the discrete afferent segmentation. A second, "late", group received similar injections on postnatal Days 5 and 7, after the development of afferent segmentation. After a survival of 4 to 24 hours, the animals were perfused and the brains processed according to the technique of Mesulam (J. Hist. Cyt., '76).

These experiments reveal that the distribution of labeled neurons in the ventrobasal complex differs markedly between the early and late groups. In the early group of animals, labeled neurons do not appear to be segregated into clusters, and the visible dendrites of labeled neurons can be seen radiating from the cells in all directions. However, although the neurons appear to be uniformly distributed throughout the extent of the ventrobasal complex at Day 0, there is some tendency for these neurons to form rows by Day 1. This corresponds well to the observed trigeminothalamic pattern at this age. In the older group of animals, the distribution of labeled neurons is even more clearly related to the distribution of trigeminothalamic afferents. Neurons are segregated into rows with distinct row boundaries and the dendrites of labeled neurons are oriented inward, toward the center of a row, thus respecting the row boundaries. Further, there is a noticeable tendency toward clustering of neurons within a row, although within-row boundaries are not as clear as those between rows. Thus a developmental sequence can be detected in the thalamocortical relay neurons of the ventrobasal complex, and this sequence parallels that of the afferents to the nucleus. (Supported by NIMH MH 14599-02 and NSF GB 41294.)

1779 FUNCTIONAL ORGANIZATION OF NEURONS IN AREA 2 OF MONKEY SOMATOSENSORY CORTEX(SI). Yoshiaki Iwamura, Michio Tanaka* & Okihide Hikosaka* Dept. Physiol., Toho Univ. Sch. Med., Otaku, Tokyo Japan.

The receptive field(RF) properties of the neurons in the first somatosensory cortex(SI) were studied in alert monkeys. Monkeys were surgically prepared for chronic single unit recording from the region of SI representing the hand and fingers, and were trained to permit hand manipulation during experiments. The cortical unit recording was done with glass-coated platinum-iridium micro-electrodes. Well isolated initially-negative units were recorded. All electrode tracks were identified histologically and the cytoarchitectural area of each recording site was determined. Units were classified into several categories according to their RF characteristics: 1) simple skin units, 2) complicated skin units, 3) units responding specifically to joint manipulation, 4) units responding to both skin stimulation and joint manipulation, 5) units undrivable by ordinary skin stimulation or joint manipulation. In area 3, the majority of units were of simple skin type with small RFs. In contrast, in area 2 the RFs of skin units tend to be larger, and the number of simple skin units decreased. The category of complicated skin units included those responding preferentially to the moving probe over the skin, or those for which narrowness of the stimulated area was most important: they were activated most remarkably when certain elongated shapes of objects were passively contacted on the monkey's hand. Many units with definit skin RFs also responded to joint manipulation. The most adequate stimuli for this type and some other non-skin units were often the complex patterns of stimuli produced by certain natural movements of monkey's hand such as scratching the table surface with fingers or grasping of objects of certain shapes. When the RFs and other properties of sequentially recorded units were compared with each other, it was found that a set of neurons with similar skin RFs were arranged vertically and that the largest RFs included other smaller ones in the vicinity. In addition, within the same vertical organization non-skin units as well as skin and deep units were intermingled. It is suggested that the hand and finger region of area 2 of the monkey SI is organized not in simple somatotopy but in terms of multiple clusterings of neurons each with particular RF characteristics and functional role such as processing complex sensory information concerning special features of tactile objects, or hand and finger movements.

1780 SOMATOSENSORY REPRESENTATION IN THE ANTERIOR WULST OF THE OWL (Speotyto cunicularia). H. J. Karten, M. Konishi and J. Pettigrew, Dept. Psychiatry, S.U.N.Y., Stony Brook, NY 11794 and Cal. Inst. Tech., Div. Bio., Pasadena, CA 91110.

The posterior (visual) wulst of the owl is both anatomically and physiologically similar to the striate cortex of mammals (Karten, et al., 1973; Pettigrew and Konishi, 1976). In contrast, the smaller quadrilaminate anterior wulst (Wa) was noted to be distinct by Karten (1971), projecting to thalamus, red nucleus, medial reticular formation, medial pons, cuneatus-gracilis and contralateral dorsal funiculus. Burrowing owls were anesthetized, the anterior wulst exposed and tungsen electrodes inserted stereotaxically into Wa. Brisk unit responses were elicted only to extremely localized stimulation of the toes of the contralateral hindlimb. No responses were observed to stimulation of the legs immediately proximal to the toes, ipsilateral foot, wings, trunk, head including beak, cornea, visual stimuli, auditory stimuli or tilting of the head. A series of units lying in contiguity along a single penetration often responded to contiguous points on the ventral aspect of a single toe. Receptive fields were often 0.5 mm or less in diameter and responded to extremely light pressure of von Frey hairs. Occasional units responded to brushing movements over 1-3 mm, with evidence of preferred direction of movement. Due to obliquity of the electrode tract relative to the pial surface we could not construct an absolute map of the topography of projections of the individual toes upon the Wa. Each of the four toes was represented, with responses to the ventral surface, single hair-like modified feather cartridges on the dorsum of the toes and the base of each talon. Injection of HRP into the Wa labeled cells in only a single dorsal thalamic cell group, the nucleus dorsalis intermedius ventralis anterior (DIVA) caudomedial to the visual OPT complex. In pigeons, DIVA receives input from the contralateral nuclei cuneatus-gracilis via the medial lemniscus, suggesting that the Wa receives somatosensory input via the medial lemniscal system. Study of efferent projections of Wa with tritiated amino acids confirmed the earlier report of Karten (1971) though indicated a heavier projection to the cuneatus-gracilis than previously appreciated. Spinal cord projections could be followed only to rostral cervical levels. No evidence of projections to lumbosacral levels was observed.

The similarities to the somatosensory system of mammals are evident. The refined use of the lower extremity by owls to strike and manipulate prey is well known and may involve the Wa.

Supported by NS 12078 to Harvey J. Karten.

1781 RECEPTIVE FIELD PROPERTIES OF PRIMARY AFFERENTS IN THE ATLANTIC STINGRAY, DASYATIS SABINA. R.B. Leonard, D.R. Kenshalo, Jr. and W.D. Willis. Marine Biomedical Institute and Depts. of Physiology & Biophysics and Anatomy, U. of Texas Med. Br., Galveston, TX 77550

Previous work has shown that the peripheral somatic nervous system in the stingray is composed of two groups of myelinated fibers with a virtual absence of unmyelinated fibers. On the basis of their conduction velocities and fiber diameters, these two groups correspond to Aαβ and Aδ fibers of other vertebrates. As this distribution stands in marked contrast to other vertebrate groups, it is of interest to determine the types of receptive fields in this animal and to correlate these with the fiber diameter distribution.

The discharges of single primary afferent fibers were recorded from the dorsal roots of decerebrate, curarized and artificially ventilated stingrays using 4M NaCl glass electrodes with resistances of 25-40 MΩ. Electrical stimulation of exposed peripheral nerves was the search stimulus, and the spike latencies were used to determine the conduction velocities.

Among the fastest conducting fibers in our preliminary survey are many which respond to proprioceptive stimuli. These units are characterized by a very regular discharge pattern that is modified by elevation or depression of the pectoral fin or a portion of it. The patterns are very similar to those of muscle stretch receptors recorded in isolated preparations. These fibers are not activated by cutaneous stimuli which do not move the fin. In contrast, other fibers have small cutaneous receptive fields, several mm in diameter, overlying 1-3 cartilaginous rays. These fibers have von Frey threshold ranging from 4.5 mg to 2 g, the majority being less than 1 g. The response is graded with stimulus intensity and is rapidly adapting. Large movements of the fin are relatively ineffective in activating these fibers.

Our preliminary survey includes several fibers with slow conduction velocities that appear to have low von Frey thresholds and small receptive fields. This group also includes fibers with higher von Frey thresholds which appear to be activated by stimulation of larger areas.

We conclude that the peripheral receptor population in the elasmobranch includes cutaneous mechanoreceptors and perhaps nociceptors as well as proprioceptors. It is possible that some of the proprioceptors correspond to the Poloumordwinoff ending reported by others.

This work supported by NIH grants NS 11255, NIH postdoctoral fellowships NS 05434 (to R.B.L.) and NS 05698 (to D.R.K.), and by a grant from the Muscular Dystrophy Foundation of America.

1782 THALAMIC PROJECTIONS TO S-II CORTEX OF RHESUS MONKEY. P. R. Loe, B. L. Whitsel, D. A. Dreyer, E. A. Cooper, and C. B. Metz. Dept. Physiol., Sch. Med., U. of N. Carolina, Chapel Hill, N. C. 27514.

Most of the neurons in the rostral part of the second somatosensory cortical area (S-II/r) possess bilaterally symmetrical cutaneous receptive fields. We were interested in determining whether or not this property is accounted for by the convergent projection to S-II/r of inputs from the somatosensory thalamus of both sides, and in describing the distribution of the thalamic neuron populations projecting to S-II/r. Horseradish peroxidase (HRP, 0.2 to 0.6 ul of a 30% solution) was injected into regions of S-II/r whose neurons had been characterized according to modality and receptive field location by means of single unit microelectrode recording in the absence of general anesthesia. HRP was visualized, after a 48 hr survival period, by the use of the Hanker-Yates reagent mixture.

Injections of HRP into S-II/r resulted in the labelling of thalamic neurons only ipsilateral to the injection site. Labelled neurons were concentrated in the medial and oral pulvinar (according to the nomenclature of Olszewski, 1952), as well as in the ventral posterior inferior (VPI) nucleus. A few labelled neurons were observed in the ventral posterior lateral (VPL), ventral posterior medial (VPM), and medial dorsal (MD) nuclei. The data suggest that, to a large extent, the thalamic input to S-II/r derives from neurons other than those within the ventrobasal complex that project to S-I. It appears that neurons in the pulvinar and VPI, some of which are known to have bilateral receptive fields, constitute the major source of thalamic input to S-II/r. By the use of a double labelling technique (Hayes and Rustioni, 1978), we are attempting to elucidate further whether S-I and S-II/r receive their thalamic input from wholly distinct neuronal populations or also receive input from thalamic neurons that send collateral branches to both S-I and S-II/r.

(Supported by NIH research grants NS11737 and NS10865, with additional support from DE02668, RR05333, and DE00011.)

1783 MULTIPLE UNIT ANALYSIS OF CUTANEOUS RECEPTORS USING PHASED BINARY CORRELATION. F.J. LOOFT and M.S. FULLER* Bioelectrical Sciences Laboratory, University of Michigan, Ann Arbor, MI 48109

Binary, phased correlation (Heetderks W. and Williams W., Science 188:373,1975) was used to separate single units from population responses in peripheral cutaneous nerves.

Adult cats, anesthetized with sodium pentobarbital, were used for preliminary experiments. The saphenous nerve was exposed from the knee joint dorsally for approximately 8 cm. Proximal and distal sections of the nerve were separated from surrounding tissue and elevated on silver hook electrodes in a mineral oil pool. Electrode separation was between 4 and 6 cm.

A punctate stimulator was drawn across the skin at a constant velocity in a raster scan covering 1 to 4 square cm. Stimulus force was 1 gm.

Recorded data were analyzed by a combination of hardware binary correlation and computer software. Topographic plots of single unit responses were reconstructed from the phased latency histograms(fig.1). Up to six active units have been separated using this technique.

Reconstructed single unit responses were compared to receptive field maps generated from single unit recordings (Looft F. and Williams W. IEEE Bio-Med. Engin.,to be published). Field, type I, and type II receptors produced similar topographic maps using single or multiple unit analysis (fig.2).

Binary correlation appears to be applicable to the analysis of complex spatial-temporal stimuli coding in peripheral cutaneous nerves. (supported by USPHS grant NS 08470)

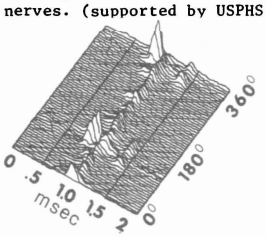


FIG.1 Phased latency histogram. The X direction represents conduction latency, Y the phase or position of the stimulator, and Z the number of action potentials.

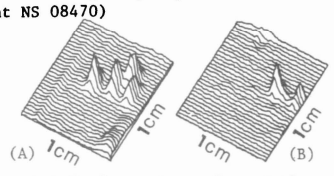


FIG.2 Comparison of a single unit (a) and reconstructed (b) topographic plot of type I mechanoreceptors. The reconstructed map is made by processing data from 32 phased latency histograms all produced from a single raster scan. This particular unit had a conduction latency of .875 msec. Electrode separation was 5 cm.

1784 AFFERENT PROJECTION TO THE NUCLEUS CENTRALIS LATERALIS IN THE CAT AS DEMONSTRATED BY RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE. Charlotte M. McGuinness* and George M. Krauthamer (Spon: S. Rosner). Dept. of Anatomy, CMDNJ-Rutgers Medical School, Piscataway, New Jersey 08854.

As part of a series of experiments to define the anatomical connections of the intralaminar thalamus of the cat, horseradish peroxidase (HRP) was injected into the nucleus Centralis Lateralis (CL) and the brain examined for retrogradely labeled neurons. Electrophoretic injections through glass micropipette electrodes were used to ensure a small, dense, well localized deposit of HRP. Tissue from the brain and spinal cord was processed by a method utilizing Tetra Methylbenzidine after thirty-six to seventy-two hour survival times.

HRP labeled neurons were found in the ipsilateral superior colliculus in both the stratum griseum intermedium and the stratum griseum profundum; in the dorsal median nucleus of the raphe; and bilaterally in the cuneform nucleus, and pontine tegmental fields. HRP labeled neurons were also located in the cerebellum, in the dentate nucleus, and in the cervical spinal cord, bilaterally in lamina 5.

These results, considered in conjunction with previous studies in the intralaminar thalamus, indicate notibly different systems of afferent projections, and therefore of functions for these nuclei. Further studies on both the anatomy and, importantly, physiology are clearly indicated.

(Supported by NIH grant NS10922)

1785 EFFECTS OF GENERAL ANESTHETICS ON THE BODY REPRESENTATION IN S-I. T. McKenna*, B. Whitsel, D. Dreyer and C. Wong* Dept. of Physiol. & Dent. Res. Ctr., U. of N.C., Chapel Hill, NC 27514

It is known that the dynamic properties of somatosensory cerebral cortical neurons are altered by the administration of central depressants, but these agents are generally considered to exert only minor influences on the static properties of the neurons of the S-I cortex. Consequently, the results obtained from analysis of the topographic organization of S-I performed in the presence of general anesthetics are routinely regarded as providing a relatively undistorted view of the cortical representation of spatial information in the conscious animal. The purpose of this study was to examine the actions of several commonly employed general anesthetics on the static properties of S-I neurons. Neurons in S-I of Macaque monkeys and cats were initially studied in the absence of general anesthesia. Receptive field (RF) dimensions, submodality, and the threshold stimulus at various positions within the RF (using calibrated von Frey hairs) were determined. In addition, measures (PST histograms) of the response evoked by moving tactile stimuli were obtained. Measures were repeated during and after recovery from i.v. injection of the general anesthetics. For neurons in cytoarchitectural areas 1 and 3, general anesthetics led to; (i) progressive reduction in the size of RFs on proximal body parts with progressive increments in anesthetic dose; in addition, there was a general tendency for the most proximal portions of these RFs to be differentially susceptible to depression by anesthetic; (ii) reduction to a small focus limited to one digital surface of RFs that formerly included both the dorsal and ventral surfaces of one or more individual digits; (iii) conversion to a discrete field on one or several digits of RFs that formerly included large portions of a distal limb; (iv) elevation of von Frey thresholds at all locations within a RF, with the thresholds obtained for loci in the periphery of the RF being most susceptible to the effects of the anesthetics. For both cutaneous and deep neurons located in cytoarchitectural areas 3a and 2 the administration of general anesthetic in doses at or below those required for induction of anesthesia reduced stimulus-evoked activity to the point that it became difficult to determine RF and submodality properties. The results suggest that general anesthetics introduce a major bias into any study of somatosensory neuron populations, and recommend cautious use of data obtained under this condition for the reconstruction of the cortical representation of somatosensory stimulus events. Supported by NS10865, DE2668, RR05333 and DE00011.

1786 MULTIPLE REPRESENTATIONS OF THE BODY SURFACE IN POSTCENTRAL PARIETAL CORTEX ("SI") OF THE SQUIRREL MONKEY. R. J. Nelson, M. Sur* and J. H. Kaas. Depts. of Anat., Elec. Eng., and Psych., Vanderbilt University, Nashville, TN 37232.

Four separate architectonic zones have traditionally been included in the "Primary Somatosensory" cortex or "SI" of primates, i.e., Areas 3a, 3b, 1 and 2. In microelectrode mapping experiments in 10 squirrel monkeys (Saimiri), we have extensively explored two of these fields, Areas 3b and 1, and have found each to contain a complete and separate map of the body surface. These studies allow the following major conclusions. (1) In each area, the tail and genitalia are represented most medially, followed laterally in order by the posterior leg, foot, anterior leg, trunk, occiput, ear and shoulder, arm and forearm, hand, and face. Major body parts are represented in parallel in the two representations. (2) The representations of similar body regions in the two areas share a common border and are oriented in mirror-image fashion with respect to each other. (3) The two representations differ in many details. For example, the dorsum of the foot is split in Area 3b with the medial dorsal foot represented laterally and the lateral dorsal foot represented medially, whereas in Area 1, the entire dorsal foot is represented lateral to the digits. (4) Within each field, some adjacent body regions are represented in a non-adjacent manner on the cortical surface. The representations of the posterior and anterior leg are separated, in both areas, by the representation of the foot. The occiput, ear and shoulder representation is separated from that of the remainder of the face by the representation of the hand in each area respectively. Thus, each representation is best described as a composite of somatotopic regions rather than as a continuous map of the contralateral body surface (homunculus). (5) Major differences exist in the organization of the two representations in different New World Monkeys. In the owl monkey (Merzenich et al., J. Comp. Neurol., '78), the trunk is represented in Area 3b with the ventrum caudal and the dorsum rostral; the opposite i. true for Area 1. In squirrel monkeys, the ventrum of the trunk is rostral in Area 3b and caudal in Area 1 with the dorsum of the trunk at the 3b-1 border. These two species also differ in the representation of the face. In owl monkeys, the upper lip forms the common border of the two representations, while the common border is formed by the lower lip in squirrel monkeys. (6) Some features of organization remain constant across simian species. In no simian that we have studied have the tips of the digits of either upper or lower extremities been oriented in a manner other than rostrally in Area 3b and caudally in Area 1.

These studies indicate that "SI" in primates requires redefinition.

Supported by NSF Grant BNS-81824.

1787 HRP MAPPING OF THE TRIGEMINAL GANGLION IN EMBRYONIC AND HATCHLING CHICKS. Drew M. Noden* (SPON: S. A. George) Dept. Zool., Univ. Massachusetts, Amherst, MA. 01003

Sensory nerve fibers emerging from the avian trigeminal ganglion (TG) project peripherally to facial, oral and jaw regions. There are 2 neural cell types in the embryonic TG: small, densely-packed immature cells of neural crest origin found in the medio-dorsal core, and larger, more mature neurons which are mostly of placodal origin and are located peripherally and in the ventrolateral TG. The experiments reported here examine the following: 1) is there a spatial organization of sensory neurons such that all cells projecting to each specific peripheral locus are localized in a particular region of the TG, 2) is the dual embryonic origin of the TG correlated with the different cytological characteristics, locations or functions of TG neurons in either the embryo or the hatchling? To answer these questions horseradish peroxidase (HRP) was injected subcutaneously in 7-day embryos (stages 30-32) or 3-5 day chicks and subsequently visualized in 8 um paraffin sections of the TG.

In the embryo most injected HRP is later localized in the large TG cells. The distribution of labeled cells in the maxillo-mandibular but not the ophthalmic lobe varies depending upon the site of injection, although there are often many labeled cells scattered throughout the TG. In some cases neurons in the trigeminal motor nucleus are also labeled, indicating extensive diffusion of the injected HRP. Application of HRP to areas of maxillary nerve innervation results in labeling of a few core cells.

In the hatchling, injections in regions innervated by branches of mandibular or infra-orbital nerves results in labeling of sensory neurons located exclusively in specific parts of the TG. The location of these cells corresponds to the region of the TG from which nerves to the injection site emerge, and is similar to that seen in the embryo. Labeled cells are always interspersed with unlabeled neurons. Injection of supra-orbital regions results in the labeling of twice as many neurons but these are more scattered. HRP is not preferentially localized in cells of any size class or cytological type, nor are any parts of the TG devoid of neurons with cutaneous projections.

These data prove that there is a patterned arrangement of TG exteroceptive neurons which is correlated with the pattern of peripheral fiber distribution but not with embryonic origin or neuronal cytology.

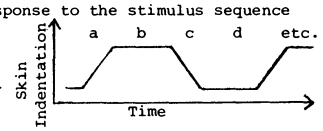
1788 THE PROJECTION OF CERVICAL AND TRIGEMINAL AFFERENTS TO THE DORSOLATERAL SPINAL CORD - MEDULLARY TRANSITION ZONE. Samuel G. Nord, Charles J. Hodge, David E. Rolince* and Charles I. Woods*. Depts. of Neurosurgery and Neurology, Upstate Medical Center, Syracuse, NY 13210.

Clinical evidence reveals that there is a functional overlap between the trigeminal and upper cervical sensory systems. Although both trigeminal and segmental projections to the CNS have been studied extensively, the location of neurons capable of mediating such an overlap has not been established. The area most likely involved is the spinal cord-medullary transition zone where the trigeminal nucleus caudalis is continuous with the morphologically homologous cervical dorsal horn. The present study explored this zone. Unit activity, evoked by graded mechanical stimulation, was recorded from rostral spinal cord and caudal medullary sites in anesthetized, paralyzed cats. Dye, deposited iontophorectically through recording microelectrodes, identified the locations of responsive neurons. Each of the units studied had an ipsilateral mechanoreceptive field. The numbers of cells which had trigeminal, segmental or combined trigeminal-segmental fields were similar. The trigeminal fields consisted of circumscribed portions of single divisions. Areas of the posterior, or peripheral, face were commonly represented while regions of the anterior, or central face (including oral structures) were encountered infrequently. Segmental fields, in most cases, received input from the C_2 or the C_3 dermatome. The fields were moderate in size and, frequently, were limited to well defined anatomic areas such as the pinna or the posterior neck, or to a part of such an area. Units with exclusively trigeminal, with exclusively segmental and with mixed fields were interspersed at all depths of the dorsolateral gray matter and at every rostro-caudal level of the lower medullary-upper cervical zone studied. All but a few of the fields with both trigeminal and segmental representation were continuous. In these, the ophthalmic trigeminal division usually was associated with the C_2 distribution and the mandibular division with various C_3 areas. Our results demonstrate that facial and segmental afferents project to cells in the transition zone between the nucleus caudalis and the dorsal horn, indicating that this zone is not exclusively a bulbar or a spinal cord entity. The profusion of interrelated upper cervical and posterior (peripheral) trigeminal inputs to the region supports the view that its component neurons are involved in the functional overlap of trigeminal and upper cervical sensibility.

Supported by NINCDS Grants NS10814-04 and NS12761-02

1789 ON VS. OFF RESPONSES OF RACCOON GLABROUS SKIN RAPIDLY ADAPTING CUTANEOUS MECHANORECEPTORS. Benjamin H. Pubols Jr. Dept. of Anatomy, College of Medicine, Pennsylvania State University, Hershey, PA.

The mammalian rapidly adapting (RA) cutaneous mechanoreceptor is defined by its pattern of response to the stimulus sequence depicted in the figure at right. When ramp-and-hold stimuli are applied to the receptive field of an RA unit, the response consists of a discharge during a (the on-response), silence during b, and usually a discharge during c (the off-response). During d, the unit is silent.



a. on-ramp
b. static indentation
c. off-ramp
d. interstimulus interval

A frequent finding in previous studies has been that the response during a has been more vigorous than that during c. However, where specified, d > b, and results may thus be due to the fact that pre-ramp time has been less preceding off-ramps than on-ramps, allowing less time for recovery from fatigue in the former case.

In the present study, the responses of raccoon median nerve RA mechanoreceptive afferent fibers were studied. For any given experimental condition, a = c, and b = d (see figure).

Principal findings were as follows:

1. At stimulus levels well above indentation and velocity thresholds for on-responses, ≈ 85% of units yield a more vigorous on-response than off-response (as measured by the total number of ramp impulses); in ≈ 5%, the reverse is true, while in the remaining ≈ 15%, the off-discharge is absent.
2. On and off indentation thresholds are approximately equal (on median ≈ 45μ; off median ≈ 40μ).
3. On velocity thresholds are significantly lower than off velocity thresholds (on median ≈ 1 μ/msec; off median ≈ 4.5 μ/msec).
4. Exponents of power functions relating discharge rate to ramp velocity are consistently greater for on than for off responses (b = d > 10 sec).
5. On discharge rate remains constant as preramp time (b, d) decreases from 10 sec to zero; off discharge rate also remains constant down to a critical pre-ramp time (100 msec - 6 sec, depending on the unit), but then drops precipitously.

The present results generally confirm previous findings that RA units possess a "linear directionality" favoring on-responses, and suggest that, even though RA units do not discharge during static indentation, such indentation may nevertheless suppress the subsequent off-response.

1790 SCALING OF VELOCITY AND DEPTH OF SKIN INDENTATION BY HUMAN SUBJECTS. Lillian M. Pubols and Benjamin H. Pubols, Depts. Anat., The Med. Coll. of Pa., Pa. 19129 and The M. S. Hershey Med. Ctr., Hershey, Pa., 17033.

Studies of radpily and slowly adapting first-order afferent fibers innervating squirrel monkey and raccoon glabrous forepaw skin (B. H. Pubols and L. M. Pubols, J. Neurophysiol., 39: 773, 1976) revealed that response rate in these fibers is a power function of mechanical stimulus velocity during constant velocity skin indentation. In slowly adapting fibers response rate during static indentation of the skin is a linear, log, or power function of indentation amplitude, but correlation coefficients are usually less than those seen for the power function relating response rate to velocity. The present study was performed to determine whether this ability of first-order fibers to code stimulus velocity and amplitude is reflected in the capability of human observers to make magnitude estimations of these parameters, and, given the higher correlation coefficients for velocity coding, and the larger number of fibers participating in it, whether scaling of velocity is more reliable than scaling of amplitude.

Ramp-and-hold mechanical stimuli were applied to the ventral skin of distal digit 3 of the right hand in 5 adult subjects. Each day's session consisted of 70 trials at a given constant onset velocity (1μ or 100μ/msec) or constant final amplitude (200μ or 800μ), within which amplitude or velocity were varied in 7 steps, evenly spaced on a logarithmic scale (200μ to 800μ, or 1μ to 100μ/msec, respectively). The subject's task was to match the seven stimulus intensities, presented in random order, with a magnitude estimation of 1 to 7. Responses occurred before the offset of the stimulus. Two sessions under each velocity or amplitude condition were presented to each subject in counterbalanced order.

Under all conditions subjects made magnitude estimations that were monotonically related to stimulus intensity. Linear regression analysis yielded highly significant ($p<.01$) correlation coefficients (0.571 to 0.908). For all but one subject, amplitude discrimination was better at 100μ/msec than at 1μ/msec. For all subjects, velocity discrimination was better at 800μ than at 200μ. For three of the five subjects, the best performance was velocity scaling at 800μ, and the worst, amplitude scaling at 1μ/msec. Thus, both velocity and amplitude can be scaled reliably by human observers. For the stimulus values used in this study there is better scaling of velocity than of amplitude by the majority of subjects.

(NIH:NS-12254).

1791 CODING OF STIMULUS LOCATION AND INTENSITY IN POPULATIONS OF PRIMARY MECHANOSENSITIVE NERVE FIBERS OF THE RACCOON. R.H. Ray and G.S. Doetsch. Dept. Physiol. & Depts. Surg. (Neurosurg.) & Physiol.,Med. Coll. Ga., Augusta, Ga. 30901.

The broad sensitivity of individual somatosensory neurons to several stimulus parameters poses a problem for the differentiation of stimulus location, intensity, and quality. However, a neuronal population response model can be used to show how these various stimulus parameters may be differentially encoded (see Doetsch and Erickson, these proceedings). The aim of this study was to describe the coding of stimulus location and intensity on the skin in terms of this model. Recordings were made from single primary mechanosensitive nerve fibers innervating the glabrous skin of the forepaw and hindpaw of the raccoon. The discharges of individual fibers were recorded in response to stimulation of six standard test sites on digit #1 and the contiguous foot pad of each paw. Tactile stimuli of varying intensities were delivered to each test site with calibrated nylon filaments. The receptive field (RF) of each fiber responding to at least one test site was mapped at a standard series of stimulus intensities; discharge characteristics at different stimulus sites and intensities were examined.

The size and functional organization of individual RFs varied dramatically as a function of stimulus intensity--from punctate fields at threshold intensities, to relatively large fields with complex characteristics (including on, off, on-off subdivisions) at higher intensities. Thus an individual neuron cannot unequivocally discriminate between changes in stimulus location, intensity, and other stimulus parameters. However, the various stimulus parameters can be distinguished by differences in the impulse frequency patterns across populations of nerve fibers. This is demonstrated by reconstructing the across-fiber pattern (AFP) produced by stimuli of each intensity at each test site. A specific stimulus location is thus disclosed by an AFP which is unique for that test site; intensity is signaled by the total amount of activity in the responding fiber population. According to this model, discrimination is a direct function of the total difference in impulse frequency produced by any two stimuli across all responsive fibers. The major factor which influences the total difference in AFPs is innervation density (neurons/cm^2) rather than RF size. In this study, differences in innervation density were estimated by comparing the integrated voltages of the compound action potentials recorded from the median and tibial nerves in response to mechanical stimulation. Such estimates gave the expected differences in point localization on distal versus proximal skin regions of any one paw and on corresponding regions of different paws. (Supported by GRS Grant, NIH, 5SO1-RRO5365-16)

1792 A SINGLE UNIT STUDY OF THE SECOND SOMATIC SENSORY CORTEX IN THE CYNOMOLOGOUS MONKEY. C.J. Robinson and H. Burton, Depts. of Elec. Engr., and of Anat. & Neurobiol., Wash. Univ., St. Louis, MO 63110

Recordings were obtained from over 1100 neurons located within the lateral sulcus of awake, unparalyzed or lightly tranquilized monkeys. With the aid of cortical cytoarchitecture and with analyses of connections to this region of cortex (Jones and Burton, '76, JCN 168) from the ventroposterior nucleus of the thalamus or from SI, we assigned 660 neurons to SII by correlating electrode tract locations with the anatomy.

Our findings indicate an anteroposterior organization of the body within SII as suggested by Burton and Jones, '76 (JCN, 168). Units with trigeminal receptive fields are found most rostrally, occupying the deepest parts of the parietal operculum bordering on the granular insular (Ig) cortex and Prokoniocortex (Pk). Immediately caudal, the hand representation is interposed between the SI and SII face representations. Further caudally, a large hand and arm representation occupies most of the parietal operculum extending from Ig to the overlying SI and area 7. A smaller foot representation lies buried in the depths of the lateral sulcus caudal to Ig and is bordered by the retroinsular (Ri) cortex in the fundus, by an overlying SII hand representation in its anterior extent, and by area 7 in its posterior extent.

Of the 501 neurons reliably responding to somatic stimulation, a majority were activated from the skin with a rapidly adapting discharge; approximately 25% were activated by low and/or high velocity hair movement; the major portion of the remaining neurons responded to stimulation of subcutaneous tissue. Over 50% had only contralateral input (c/1); ≈13% had predominantly c/1 fields; ≈33% had symmetrical bilateral fields and, 3% had predominantly ipsilateral input. Neurons with similar laterality properties clustered together, suggesting a possible columnar organization. Receptive field boundaries could be precisely delimited in ≈45% of the cases (with 26% < 10 cm^2), moderately in ≈31%, and poorly in the remainder. In the hand representation, the predominant response appears to be activated by transient mechanical stimulation of the type that would occur during active contact with textured surfaces. (Supported by PHS GM-01827 and NS-09809)

1793 MODULATION OF SOMATOSENSORY CORTICAL NEURONS BY CHANGES IN BEHAVIORAL AROUSAL AND ATTENTION IN THE RHESUS MONKEY. J.R.Roppolo and J.G.Collins. Dept.of Pharmacology,Sch.of Med. U.of Pittsburgh Pittsburgh, PA 15261

Previous studies from our laboratory (Fed.Proc.26:411,1977) have shown that drugs (alcohol and pentobarbital) which induce changes in arousal and attention can profoundly alter spontaneous activity (spon.act.) and stimulus representation in the primary somatosensory cortex (S-I). The purpose of the present study was to examine the effects of "natural" changes in arousal and attention on these neurons. To this end extracellular recordings were made from single neurons in S-I of awake, non-paralyzed rhesus monkeys. Peripheral receptive fields, located on the glabrous skin of the hand, were stimulated by a fine brush moved back and forth across the cutaneous surface. The experimental animals were placed in three behavioral states: (1) awake state-in which the monkey simply sat quietly in a sound attenuating recording chamber with the door open; (2) slow-wave sleep (SWS)-here the door was closed and the animal allowed to fall asleep. (cortical EEG was recorded); (3) attentive state (ATT)-in which the monkey performed a previously trained behavioral task. The behavioral paradigm consisted of pressing a bar(using the hand not stimulated by the brush) for a fruit juice reward with a fixed ratio of 10. A 30 sec. red light on period indicated reward available followed by a 30 sec. green light on period indicating no reward available. The brushing stimulus was presented every 5 sec., but provided no behavioral cue, nor did it disrupt the SWS. Twenty-five stimuli in each direction were averaged. The most dramatic effects were seen with SWS on both driven and spon. act. The spon. act. was more easily influenced by SWS with 52% of the neurons increasing their firing rate while only 28% of the driven units increased their rate. Decreases were seen in 25% of the spontaneous and 28% of the driven. The variability of the firing frequency measured by the coefficient of variation (C.V.) was also increased during SWS; 84% of the neurons studied showed an increase in C.V., while 12% showed a decrease. SWS also increased the number of units which showed directional selectivity (i.e. neurons which respond preferentially to one direction of brush movement). The effects of the behavioral task were more subtle with most units showing little or no change. The driven activity however did increase in 15% of the units in both the red and green light period. No effect was seen on directional selective units in the ATT. These results indicate that changes in arousal and attention may have important influences on the ability of S-I neurons to transmit and process tactile information. (Supported in part by Psychobiology Training Grant MH11114 and Research Grant MH11682).

1794 PATTERN OF AFFERENT SUPPLY TO CANINE SPINAL TRIGEMINAL NUCLEUS. R.J. Schneider & A.L. Itani*. Division of Neurosurgery, Dept. of Surgery, University of MD. Hospital, Baltimore, Maryland 21201.

Electrical activity was recorded from the branches of the trigeminal ganglion in dogs. Wire electrodes were insulated in silastic and secured to the branches exposed in the middle cranial fossa for acute recording or left in place during recovery and used for chronic recording. A conventional electrophysiological system was used to amplify and display the signals. The object of these experiments was to define the boundaries of the opthalmic, maxillary and mandibular receptive fields in dogs.

Previous studies had suggested the spinal trigeminal tract could not be differentiated by the level at which certain cranial skin regions terminated. This contradicted results which reported a more caudal spinal termination for opthalmic fibers with maxillary and mandubular fibers ending progressively less caudad, respectively. An opposing notion, of the terminations being "Zwiebelschalenförmig," onionskin-like, had developed from observations of patients with sensory losses due to syringomyelia. In humans, these onionskin layers are centered around the nose tip to lip region and form concentric lamellae progressing caudad. To determine if the former hypothesis was supported, we had to define the respective receptive fields of each division of the trigeminal ganglion. Thus, the object of this study was to seek evidence for either divisional or onionskin-like segregation within the canine spinal trigeminal nucleus.

The maxillary division of the dog's trigeminal ganglion represented a greater body surface proportion among the branches than was the case for its human equivalent. The behavioral importance of this area in exploration and manipulation of extrapersonal space would seem to account for this. The fields of the other branches were distributed, however, as anticipated.

Conduction velocities indicated that the fibers projecting to the spinal trigeminal tract and nucleus were not the fastest in the sensory groups, averaging 41.5m/sec, i.e., toward the lower range of A-beta & gamma fibers. This was supported by the slowness of the response to receptive field hypoxia in decreasing activity in the spinal trigeminal. However, local anaesthetics applied to receptive fields also had minimal effect.

We conclude that the afferent population which terminates in the spinal trigeminal nucleus cannot be segragated into trigeminal ganglion divisions differing in locus of termination on electrophysiological grounds. However, because we did not sample pain and temperature afferents exclusively, the possibility remains that they can be so segregated. Alternatively, we cannot exclude the possibility of an onionskin arrangement for pain and temperature afferents, but the total population of spinal trigeminal afferents in dogs is not so arranged. (Funded by a Bressler Grant)

1795 SENSORY UNITS WITH AFFERENT C-FIBERS INNERVATING THE SKIN OF THE RABBIT EAR. Virginia Shea* (SPON: E.R. Perl). Neurobiology Program, University of North Carolina, Chapel Hill, N.C. 27514

Recordings were made from fine filaments dissected from the great auricular nerve. When an isolated action potential conducting at C velocity was evoked by electrical stimulation of the nerve, a systematic effort was made to characterize its sensory properties. Of the 114 single C-fibers isolated, 19 could not be activated by mechanical or thermal stimuli. Insufficient data for characterization were obtained for 6 units.

The majority of sensory units (57/89) had the characteristics of polymodal nociceptors (PMN), typically having small, punctate receptive fields (RF), and elevated thresholds to mechanical stimuli. Heating the RF's of these units elicited responses at elevated thermode temperatures (41°-56°); sensitization (increased on-going activity and/or decreased thermal threshold) appeared in repeated sequences of heating in 55 PMN's. Cooling RF's after sensitization often decreased on-going activity. In general, irritant chemicals (acetic acid and/or xylene) effectively activated PMN's.

Two groups of high-threshold receptors differed from PMN's. Four units with punctate RF's and mechanical thresholds similar to those of PMN's, displayed no response nor increased on-going activity to repeated noxious heating (61°), but responded to intense cold. These units are termed "cold-mechanonociceptors". Seven units with punctate or multiple spot-like RF's had mechanical thresholds somewhat higher than those of PMN's and little or no responses initially to noxious heating (61°), but developed ongoing activity and responses to heat when retested (resembling Aδ high-threshold mechanoreceptors of cat and primate).

Fourteen units had larger RF's generally located along the edge of the ear and responded maximally to very gentle, slowly-moving, mechanical stimuli in a fashion typical of C mechanoreceptors. Rapid innocous temperature changes, particularly cooling, also elicited weak transient responses from these elements.

Three units were sensitive to innocuous cooling. Four units responded to innocuous warming and could have been warming thermoreceptors or possibly sensitized PMN's.

It is concluded that the unmyelinated afferent units innervating the skin of the rabbit ear largely can be grouped into categories similar to those established for the cat and primate. The present sample differs in reporting a group that responds only to intense cold and strong mechanical stimuli. Moreover, units similar to Aδ high-threshold mechanoreceptors of the cat and primate may, in part, have C-fibers in the rabbit. (Supported by USPHS, NINCDS grants NS10321, NS11132 and a fellowship from an Alfred P. Sloan Foundation grant.)

1796 INFLUENCE OF PUDENDAL NERVE STIMULATION ON NEURONS IN PERICRUCIATE CEREBRAL CORTEX OF ADULT MALE CATS. J. C. Slimp and A. L. Towe. Dept. Physiol. & Biophysics, Univ. Wash. Sch. Med., Seattle, 98195.

Neurons in pericruciate cortex (field 4γ) may be classified on the basis of their responsiveness to various inputs. Some neurons respond to somatic input from limited regions of the body surface, either contralateral or bilateral, whereas others respond to input from anywhere on the body surface, and even from visual, auditory, vestibular and splanchnic input. The present study examined the influence of pudendal nerve stimulation on pericruciate neurons. The pudendal nerve — a somatic nerve — has two branches, one of which innervates the penis and the other the rectum. The pudendal nerve was exposed dorsally, placed on hook electrodes, and covered with mineral oil. Bipolar needle electrodes were placed in each paw, the contralateral forepaw input serving as hunting stimulus. Extracellular recordings were made of neurons in the contralateral forepaw region of pericruciate cortex in chloralose-anesthetized male cats. Neurons were classed as *sa* if they responded only to one contralateral paw, as *sb* if they responded to both forepaws or both hindpaws, and as *m* if they responded to all four paws. Most *m* neurons (85%) were excited and the remainder were facilitated by pudendal nerve stimulation, whereas few *sb* (18%) or *sa* (3%) neurons were either excited or facilitated by this input. All *sa* and *sb* neurons influenced by pudendal nerve stimulation were facilitated by stimulation of the nonexcitatory paws — suggesting that they share some characteristics in common with *m* neurons. When tested, both contralateral and ipsilateral pudendal nerves were found to be effective. So also were both branches of the contralateral nerve, although the branch innervating the rectum appeared less effective. The average latency to pudendal nerve stimulation was similar to that for contralateral hindpaw stimulation, and the mean number of spikes/response was similar to that for the paws. The coadunate behavior observed previously among the *m* neurons (Towe, Tyner & Nyquist, *Exp. Neurol.*, 1976, *50*:734-756), and found to extend to the responses to splanchnic nerve stimulation (Tyner, in press), was also observed in the responses of the *m* neurons to pudendal nerve stimulation. That is, the position of any *m* neuron with respect to its fellows in the responding population remains about the same for all inputs that do not activate local *s* neurons. For example, an *m* neuron that responds one standard deviation earlier than the mean latency for the responding population after ipsilateral forepaw stimulation also responds about one standard deviation earlier than the mean to each hindpaw input, to each splanchnic nerve input and to each pudendal nerve input. Except for the effect of local *s* neurons, *m* neurons appear to behave similarly to a wide variety of inputs. (Supported by NS00396 and NS05136 from NINCDS.)

1797 POSTCENTRAL SOMATOSENSORY CORTEX IN MACAQUE MONKEYS: MULTIPLE BODY REPRESENTATIONS AND NEURON PROPERTIES. M. Sur*, R. J. Nelson, and J. H. Kaas. Depts. of Elect. Engrg., Anat., and Psych., Vanderbilt Univ., Nashville, TN 37240

Previous microelectrode mapping studies of postcentral parietal cortex in the owl monkey, a New World monkey, revealed that "SI" is not a single representation of the body but includes at least three separate maps of the body corresponding to architectonic Areas 3b, 1, and 2, respectively (Merzenich et al., J. Comp. Neurol., '78). Similar studies of 10 Old World monkeys, Macaca fascicularis, also indicate that each of Areas 3b, 1 and 2 contains a separate representation of the body. All three representations are organized in parallel with the feet and tail medially and the face laterally in the architectonic strips. Common body surfaces are represented at the boundaries between areas, while progressions of recording sites away from a border yield receptive fields that are approximately mirror-images of each other. Thus, the hand representations in Areas 3b and 1 are adjoined along the interdigital pad representations, while the digit tips face rostrally and caudally in the two areas respectively. The hand representation in Area 2 faces rostrally and adjoins that in Area 1 along the digit tips. The dorsal midline of the occiput and trunk is represented along the rostral border of Area 3b and caudal border of Area 1, while the ventral midline is represented close to the 3b-1 border in each area. In the face region, the medial chin and lower lip are represented rostrally in Area 3b and caudally in Area 1; the upper lip and face are represented near the 3b-1 border. Discontinuities occur within the representation, and the detailed organization of the representation in each architectonic area differs significantly and consistently from the others. For example, the dorsum of the hand digits is represented laterally (digit 1) and medially (digits 2-5) to the glabrous hand representation in Area 3b, but in Area 1 it occupies a strip of cortex between the representations of the glabrous digit tips and middle phalanges.

Single unit studies of adaptation properties of neurons in Areas 3b and 1 indicate that both fields receive input from slowly and rapidly adapting receptors. In addition, inhibitory influences markedly affect the response properties of the cortical neurons. Most neurons have a phasic "on" discharge to steady skin indentation, a 50-150 msec. period of almost complete suppression of activity, followed by recovery to the rapidly or slowly adapting "envelope" rate of discharge. The inhibition is sometimes cyclical and many neurons exhibit "off" discharges. Area 2 receives significant input from cutaneous as well as deep body receptors. Pacinian receptor input appears to be confined to Area 1. Supported by NSF Grant BNS-81824.

1798 STRUCTURAL AND FUNCTIONAL POPULATION PARAMETERS DETERMINING INPUT-OUTPUT RELATIONS AT THE CUNEATE. J. P. Walsh and D. Whitehorn. Dept. of Physiol. and Biophys., Univ of Vermont, Burlington, Vt. 05401

The evoked total impulse activity (TOTACT) within a neural population is the product of the number of active cells (NACT) and the number of impulses per active cell (NIPACT). Prior input-output (IO) studies using evoked potentials to measure output provide only the IO relation between TOTACT and input. We have additionally acquired the IO relations for NACT and NIPACT by recording from individual, identified cuneothalamic (CUTH) projection neurons in chloralose anesthetized cats. Electrical stimuli, graded from sub-to supramaximum intensities, were applied to the ipsilateral superficial radial nerve (SRN) at 1 Hz. Input was quantified by digital integration of the compound action potential recorded from a second site on the nerve. The IO relation for TOTACT accumulated over a sample of 63 CUTH cells was identical to that obtained using evoked potentials from the medial lemniscus under the same conditions. The IO relation for NACT closely paralleled that for TOTACT, both rising steeply and displaying saturation. NIPACT increased only slightly with increasing input.

We conclude that the IO relation for TOTACT is primarily determined by the recruitement of CUTH cells into activity (NACT) with changes in the number of impulses per active cell (NIPACT) having a minor role.

The IO relations for TOTACT and NACT are well described by equation 1:

(1) $y=1-\exp(-kx)$ where y=fraction of output cells active and x=fraction of input cells active. The equation is consistent with a population model with more afferents than required for complete activation of the output population. The constant k is the product of the activation ratio (AR: number of output cells activated per active afferent) and the population innervation ratio (PIR: number of afferents/number of efferents). k values of 4.9 result from the IO measurements made here. A value of 2.5 is obtained when correction is made for noncontributing fibers in the nerve or when stimulation is applied to the dorsal columns or within the nuclei.

Supported by NINCDS: #NS09472.

1799 ORGANIZATION OF THE IPSILATERAL AND CONTRALATERAL INPUT TO THE NEURONS OF S-II/r IN UNANESTHETIZED MONKEYS. Whitsel, B., Dreyer, D. and Loe, P., Dept. of Physiol. and Dent. Res. Ctr., U. of N.C., Chapel Hill, NC 27514.

Responses of single neurons and neuron clusters were studied in the course of microelectrode penetrations of the anterior portion of the second somatosensory receiving area (S-II/r). The effectiveness of hand-held tactile stimuli applied separately and simultaneously to bilaterally symmetrical cutaneous body regions was determined for a large population of S-II/r neurons. With the exception of a small population of neurons that could be activated only by unilateral stimulation, most S-II/r neurons could be classified as follows: (i) dominated by contralateral input; (ii) dominated by ipsilateral input; (iii) activated equally from both sides of the body. It was found that neurons located in close proximity to one another usually belonged to the same dominance class. Moreover, tangential penetrations across S-II/r frequently encountered sequences of neurons that exhibited the same dominance properties. Simultaneous bilateral stimulation of symmetrical cutaneous body regions either evoked a response (i) greater (summation) or (ii) equal or lesser (occlusion) than that evoked by the most effective unilateral stimulus. It is anticipated that further aspects of the functional organization of bilateral input to area S-II/r will be revealed by plotting the distribution of each neuron class.

To date, a limited sample of S-II/r neurons has been studied quantitatively using dual servo-controlled mechanical stimulators, each of which moves a fine brush at constant velocity over the skin. In addition to confirming the observations obtained using hand-held stimuli, these studies have revealed that the static and dynamic properties of the responses evoked from a single S-II/r neuron by separate stimulation of the ipsilateral and contralateral components of its receptive field (RF) are "matched" (i.e., exhibit the same directional preference, RF organization, and velocity dependence). The use of controlled stimulation has permitted quantification of the effects of simultaneous bilateral stimulation of the ipsilateral and contralateral receptive field components.
Supported by grants NS10865, DE02668, RR05333 and DE00011.

1800 POSITION AND MOTION SENSE UNDER WHITE NOISE STIMULATION W. J. Williams and R. W. Bossemeyer, Jr.* Bioelectrical Sciences Laboratory, Univ. of Michigan, Ann Arbor, MI 48109

Position and motion sense have been considered to be separate sensory entities since the time of Goldscheider. Under normal conditions of joint movement and positioning the two types of sensation are entwined and difficult to separate experimentally. Preliminary experiments using very low frequency sinusoidal joint motion showed that sensation of motion was lost below 0.01 Hz. Position sense remained, but was discontinuous and new positions were suddenly perceived in a succession of rachet-like jumps as the joint continued to move. Since it is difficult to apply a succession of very low frequency sinusoidal stimuli and obtain reliable results, we have devised a method of evaluation of position and motion sense using band limited (0-0.1Hz) white noise.

The general protocol was similar to that previously reported (Kokmen, et al, Ann. of Neurol., 2: 279, 1977). The MCP joint of the index finger was articulated and the subject pressed buttons that indicated whether the finger was up or down and whether the finger was moving up or down. A binary position signal (up, down) and a binary motion signal (moving up, moving down) were derived from the results. Theorems of communication theory were used to determine the transfer functions between white noise input and position and motion sensation. Reaction time effects were minimal due to the low frequencies involved.

The results are shown in Fig. 1. This represents the average of three subjects responding for a total of 120 minutes. These results indicate that position sensation is nearly constant with frequency up to about 0.03 Hz and declines for higher frequencies. Motion sensation increases with frequency. Motion sensation declines precipitously for very low frequencies below about 0.01 Hz. Supported by U.S.P.H.S. Grant NS 08470.

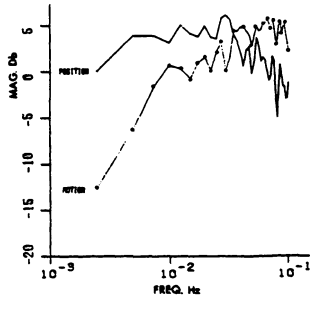


Figure 1
Position and Motion Sensation
Transfer Functions
Under White Noise Stimulation

1801 SOMATOTOPIC ORGANIZATION OF THE RAT CORTICOSPINAL AND CORTICOTRIGEMINAL SYSTEM. Steven P. Wise and Elisabeth A. Murray. Marine Biomedical Institute, Galveston, Texas 77550.

The projection from the rat sensory-motor cortex to various levels of the spinal cord and to the spinal trigeminal complex (SpV) was examined with the horseradish peroxidase (HRP) method. In different animals, injections of HRP involving two to four segments of the lumbar or cervical enlargements or the rostral cervical segments of the spinal cord (0.3-1.0µl; 50% HRP) or SpV (0.5µl) were made through glass micropipettes. Care was taken to avoid damage to the corticospinal fibers which are situated in the dorsal column in rats. Thus, fibers of passage to more caudal levels of the spinal cord were not involved in the injection. Retrogradely transported HRP was demonstrated by the dianisidine and diaminobenzidine techniques.

In both the first somatic sensory area (SI) and the motor area (MI), as well as in two additional sensory-motor areas, the corticospinal and corticotrigeminal neurons are grouped in a clear somatotopic pattern. The somatotopy in SI can be determined by comparison of the location of retrogradely labeled cells in layer V with the overlying dense, granule cell aggregates in layer IV, since Welker (J. Comp. Neurol., 166 (1976) 173) has shown that these granule cell aggregates receive peripheral mechanoreceptive input from specific regions of the contralateral body surface in a somatotopically organized manner. The somatotopic organization of corticofugal cells in the sensory-motor areas adjacent to SI can be determined, although with less precision, by examining the patterns of retrograde labeling in these areas in relation to established maps showing the spatial relationship of MI and the second somatic sensory area (SII) with SI.

The somatotopic pattern is clearest for SI and MI. Corticospinal fibers which extend to lumbar levels of the spinal cord originate mainly from neuronal somata located in the hindlimb representation of the SI-MI cortex. Those neurons projecting to the cervical enlargement have somata mainly in the rostrally and laterally situated forelimb representation of SI and MI. Cortical projections to the rostral cervical spinal segments appear to originate from the posterior head and neck representations of SI and MI. Neurons exclusively within the head, muzzle, and vibrissal representation of SI project to SpV.

Neurons in cortex near the frontal pole and in SII also project to the spinal cord. The corticospinal and corticotrigeminal projections of these areas also appear to be organized in a somatotopic manner. (Supported by Grants NS 12481 and 05736).

1802 VELOCITY DEPENDENCE OF SOMATOSENSORY NEURON RESPONSE TO MOVING TACTILE STIMULI. M. Young, R. Schreiner, B. Whitsel, and D. Dreyer. Physiol. Dept., UNC, Chapel Hill, NC 27514.

Peak mean firing rate evoked by a moving tactile stimulus has been measured as a function of stimulus velocity for single somatosensory neurons with receptive fields on the hairy skin of Macaque monkeys. Discharge activity was evoked by moving a soft brush across the neuron's receptive field at velocities between 0.7 cm/sec and 250 cm/sec by a servomotor. Each neuron was tested with at least 8 different velocities within this range, presented in random order. A minimum of 10 replications of each velocity was delivered. For first-order afferents the relationship between peak response and velocity over the velocity range of 0.7-75 cm/sec could be fit by power functions with exponents less than one (average R^2=.89). At higher velocities the plots tended to saturate and, for a few units, to decline. On the basis of these data it is suggested that individual fast conducting first-order mechanoreceptive fibers from hairy skin are not capable of reliably transmitting information about stimulus velocity (using a peak mean firing rate code) at velocities much above 75 cm/sec. Some first-order neurons were incapable of signaling changes in stimulus velocity above 20 cm/sec. Surprisingly, there were no consistent differences in the velocity response relationships obtained for the different classes of first-order afferents. Two distinct populations of S-I neurons were observed in unanesthetized Macaques. The first exhibited peak mean rate vs. velocity plots whose shapes resembled very closely those seen for first-order neurons, whereas for the second, the plots increase in an approximately linear fashion (average R^2=.84) across the entire range of stimulus velocities used. These data are interpreted to suggest that the latter type of S-I neurons is capable of signaling changes in stimulus velocity over a much wider range of velocities than was observed for first-order afferents. These neurons continued to reliably reflect changes in stimulus velocity at locations along the velocity continuum where the response of the first-order afferent fibers in our sample was saturated. Both classes of S-I neurons show a greater degree of variability in response between individual stimulus sweeps delivered at the same velocity than do the first-order neurons. Velocity transinformation calculations using the stimulus-response matrix method are being utilized to specify more precisely the capacity of both first-order and cortical somatosensory neurons to signal information about stimulus velocity. The laminar distribution of the two populations of cortical neurons is being determined by reconstruction of the electrode tracts.

Supported by NS10865, DE2668, RRD5333, MH14277 and DE00011.

SPINAL CORD

1803 PERIPHERAL INPUTS TO LONG DESCENDING PROPRIOSPINAL NEURONS IN CAT. Robert J. Adams, Robert D. Skinner, and Ronald S. Remmel. Depts. Anatomy and Physiology-Biophysics, Univ. Arkansas for Medical Sciences, Little Rock, AR 72201.

Long descending propriospinal neurons located in the cervical cord project to the lumbar cord (Skinner et al., Neurosci. Abs. 3: 508, 1977). Cats anesthetized with ketamine were spinalized at C1, anemically decerebrated, and the anesthetic discontinued. The cord at L1 was stimulated to antidromically excite cervical neurons, which were recorded from extracellularly with micropipettes. Criteria for antidromicity: predominantly negative spikes with sharp threshold, the ability to follow four shocks at above 300 Hz. Collision with orthodromically-excited spikes was verified. In half of the experiments the intact peripheral nerves (the suprascapular, deep radial, superficial radial, ulnar, medial and musculocutaneous) were stimulated with cuff electrodes.

In segments C6-T1, 236 long descending propriospinal neurons were recorded, with conduction velocities ranging from 12-110 m/sec (63 ± 39 m/sec, av. and std. dev.). Of these, 100 (42%) could be activated by natural stimulation of the forelimbs. The receptive fields ranged from rather small (one digit) to very large (a whole forelimb and part of the other). Of the neurons with peripheral fields, 38% responded to deep pressure, primarily to muscles in the elbow region; 35% to cutaneous touch, heat and noxious pinch; 23% to joint movement, usually from the metacarpophalangeal or interphalangeal joints; and 4% to hair movement. Of these, 9% received multimodal input.

Peripheral fields included the paw (57%), the forearm (43%) and the arm (46%), with 59% of the neurons receiving input from more than one of these regions. Fields restricted to one region were 18% to the paw, 5% to the forearm and 18% to the arm. Inhibition of spontaneous or evoked activity was observed from the ipsilateral limb in 10 cells and from the contralateral limb in 5 cells.

Of 102 cells tested with electrical stimulation to peripheral nerves, 68 were excited by at least one nerve and 43 were excited from three or more nerves. Minimum latencies of the spike as measured from the arrival of the afferent volley at the cord dorsum were 6.6 ± 3.8 msec (mean and std. dev), which indicates a multisynaptic path. Only a few latencies were short enough to suggest a monosynaptic connection.

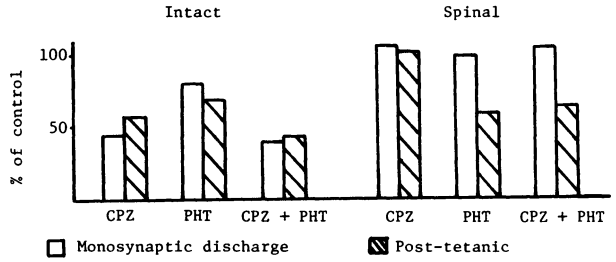
(Supported by NIH Grant RR05350 from DHEW.)

1804 INTERACTION OF PHENYTOIN AND CHLORPROMAZINE ON CAT SPINAL CORD ACTIVITY. R.J. Anderson and J.S. Carp*, Dept. of Pharmacol. Geo. Washington Univ., Washington, D.C., 20037.

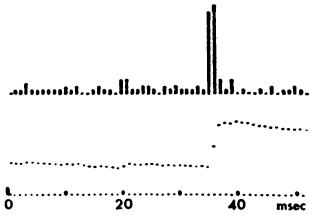
The effects of phenytoin (PHT) and chlorpromazine (CPZ) on spinal cord monosynaptic discharges and post-tetanic potentiation (PTP) were compared in two groups of α-chloralose anesthetized cats: (1) cats with high spinal section and (2) intact cats. PHT produced no significant change in the monosynaptic discharge of either the intact or spinal cats. On the other hand, CPZ produced a dose-related decrease (0.0156-0.125 mg/kg) in the monosynaptic potential in intact cats, a decrease which plateaued at 60%. In spinal cats CPZ produced no change in the monosynaptic discharge. PHT selectively depressed PTP in both intact and spinal animals in a dose-related fashion (5-20 mg/kg). CPZ, on the other hand, had no effect on PTP in spinal animals but significantly decreased PTP in intact cats.

In spinal cats the effects of PHT + CPZ on monosynaptic discharges and PTP was the same as the additive effects of each drug alone. However, PHT + CPZ in intact cats did not produce an additive depression but rather produced a moderate depression of both the monosynaptic discharge and PTP and restored the PTP ratio to the control level. These effects are summarized below for doses of 0.5 mg/kg CPZ and 20 mg/kg PHT.

These results demonstrate: (1) PHT selectively depresses recruitment of subliminal fringe neurons in the spinal cord, (2) CPZ depresses descending excitatory influences on the spinal cord without interfering with recruitment, (3) the effects of CPZ + PHT in the spinal cord are simply additive, and (4) the effect of CPZ + PHT in the intact cat act only to depress slightly spinal cord activity but more importantly to maintain the balance between low and high frequency neuronal traffic through the segmental reflex pathways. This latter effect may be of significance in normalizing spinal cord imbalances due to abnormal descending neuronal activity. (Supported by PMA Foundation).



1805 THE 1A EPSP IN MAN. Peter Ashby, Duane Zilm*, Richard E. Poppele, University of Toronto and Addiction Research Foundation, Toronto, Canada, and Dept. of Physiol., University of Minnesota. Minneapolis 55455, U.S.A.



The spike train produced by a rhythmically discharging human motoneuron was extracted by inserting a needle electrode into a contracting muscle and identifying each occurence of a given motor unit action potential. Alterations in the probability of firing (as a result of afferent volleys) were used to deduce the underlying post-synaptic potentials produced in that neuron.

Homonymous group 1 volleys produced an early peak of increased impulse density in the post-stimulus time histogram (PSTH) of rhythmically firing human tibialis motoneurons which was of appropriate latency to represent the 1a EPSP. We derived the characteristics of this EPSP in 4 stages:

1. The rise time of the 1a EPSP was determined from the width of the early peak in the PSTH (fig.1) as the profile of the PSTH represents the first derivative of the contour of the post-synaptic potential (provided that the combined slope of the membrane potential and the post-synaptic potential remains positive).

2. The amplitude of the 1a EPSP was determined by injecting a group 1 volley at increasing intervals after a spontaneous discharge to find the position (as a percentage of the mean interspike interval) at which the EPSP caused the membrane potential to reach threshold. If the interspike membrane potential trajectory can be considered to be a nearly linear rate of rise from the afterhyperpolarization to a threshold potential about 10 mv less, the amplitude of the EPSP can be obtained by simple geometry.

3. The duration of the falling phase of the 1a EPSP was determined by delivering double group 1 volleys in such a way that the second would fail to bring the membrane potential to threshold without temporal summation from the first.

4. The validity of these methods was tested by inserting the derived EPSP into a computer simulation of a rhythmically discharging neuron in an attempt to reproduce the original PSTH.

We conclude that the method may be used to derive the characteristics of post-synaptic potentials in man. As spike trains can be recorded from CNS neurons in man, the method is not restricted to the study of spinal motoneurons.

We thank the Connaught Foundation. Computer facilities were made available by the USAF; AFOSR 75-2804.

1806 THE EFFECT OF ACUTE EXPERIMENTAL SPINAL CORD TRAUMA ON MONOAMINE OXIDASE ACTIVITY. Neal J. Bannon, Norman Allen and Ronald Yates*, Dept. Physiol. Chem. and Div. Neurol., OSU Coll. Med., Columbus, OH. 43210.

Monoamine oxidase (MAO) activity was measured in microdissected samples of central grey matter (GM) and surrounding white matter (WM) from canine spinal cord at various intervals following 400 gm-cm trauma. Both [^{14}C]-serotonin (5HT) and [^{14}C]-tryptamine (TRY) were utilized as substrates. Non-dissected whole cord sections were also assayed utilizing TRY. In the latter case, no significant differences from control were found within one hour of trauma.

Activities obtained, in terms of nmoles deaminated product formed/min/mg. dry wt., for non-traumatized controls were:

substrate	whole cord	GM	WM
5HT	----------	0.3312±0.022(11)	0.1440±0.010(7)
TRY	0.1341±0.018(4)	0.1810±0.008(12)	0.1395±0.004(8)

mean ± SEM (n)

MAO in traumatized GM dropped significantly (50%) from control levels, with 5HT as substrate, as early as 15-minutes post-trauma and remained so through one hour post-trauma. TRY deaminating activity in GM was not significantly decreased until 30-min. post-trauma (17%) and showed a continued decline (nearly 50% of control) at one hour post-trauma.

MAO activity in WM of traumatized tissue was stable for at least one hour when assayed utilizing TRY. 5HT deaminating activity, however, began to show a significant decrease (33%) at one hour in WM.

Since MAO in the CNS is almost exclusively an outer membrane mitochondrial enzyme, these results suggest a possible GM mitochondrial lesion as an early occurrence in acute experimental spinal injury. These data are consistent with the previous work of Ito et.al.(J.Neurosurg:48,434,1978) in which the inner membrane cytochrome oxidase complex exhibited an early decrease in activity following spinal injury. These data also suggest a heterogenous response by MAO to spinal injury. This may reflect differences in localization of specific isozymes of MAO in the canine spinal cord as well as differences in susceptibility of various cell types to traumatic injury.

Supported by Grant No. NS-10165 from the NINCDS.

1807 LARGE PRIMARY AFFERENTS IN THE SUBSTANTIA GELATINOSA OF THE ADULT MONKEY: A GOLGI STUDY. John A. Beal, Dept. Anat., Sch. Med. in Shreveport, LSU, Shreveport, LA 71130.

It is apparent from recent physiological and certain anatomical studies that the main primary afferent input to the substantia gelatinosa (SG) consists of unmyelinated fibers with additional fine myelinated fibers to the superficial region of the SG. Input from large caliber primary afferents, on the other hand, is "limited and of only a modulatory nature" (Kumazawa and Perl, J. Comp. Neur. 177: 417, 1978). However, Ramón y Cajal (1909) and subsequent authors using the Golgi technique have demonstrated large caliber afferents which enter the dorsal horn from the ventral aspect forming an arbor of considerable complexity within the SG. This apparent contradiction between anatomical and physiological observations prompted the present study. Here Golgi impregnated axonal arrays were serially reconstructed in horizontal sections through the dorsal horn. Confined ansiform axonal complexes, previously described in the monkey (Beal and Fox, J. Comp. Neur. 168: 113, 1976), are shown to be derived from large caliber fibers which enter the SG from its ventral aspect. These axonal arrays are extremely complex entanglements and certainly leave the observer with the impression that they represent a powerful input to the SG. Considering their complexity, however, close inspection reveals that these arrays give rise to surprisingly few boutons. In addition, the boutons on these arrays are generally smaller in the more superficial sections (lamina II) than in the deeper portions of the dorsal horn. Fine caliber afferents to the SG, on the other hand, have relatively simple arbors but are nonetheless predictably more significant physiologically than the large afferents since they are more abundant and give rise to larger boutons.

1808 SYNAPTIC ORGANIZATION OF PRIMARY AFFERENT TERMINATIONS IN THE DORSAL HORN OF THE CAT SPINAL CORD DEMONSTRATED BY ANTEROGRADE INJURY-FILLING OF DORSAL ROOTS WITH HORSERADISH PEROXIDASE. Michael S. Beattie, Jacqueline C. Bresnahan, Deborah L. Lewis and James S. King, Department of Anatomy and Division of Neurosurgery, The Ohio State University, College of Medicine, Columbus, Ohio, 43210.

In order to study the synaptic organization of dorsal root primary afferents to the spinal cord, we have employed a modification of the technique described by Proshansky (Neurosci. Lett., 5: 130, 1977) for the anterograde injury-filling of these axons and their terminals with horseradish peroxidase (HRP). Our procedures have been described elsewhere (Beattie et al., Brain Res., in press). Adult cats were anesthetized and one or more dorsal rootlets from the lumbar spinal cord were exposed to a 40% solution of HRP for 12-18 hrs. Tissue was processed according to established histochemical procedures for the visualization of HRP reaction product.

Filled axons and terminals were readily distinguishable with both the light and electron microscope. Large diameter axons of the dorsal columns were labeled, as were their collaterals, which could be followed to their terminations in the dorsal and ventral horns. The small diameter axons of Lissauer's tract and their ramifications into the dorsal horn were also labeled, and it appeared that at least a sample of the full fiber diameter spectrum of dorsal root axons contained label.

Electron microscopic observations of synaptic terminations in the marginal layer (ML) and substantia gelatinosa (SG) revealed a large number of labeled synaptic profiles. Most terminals contained round, clear synaptic vesicles, while the remainder contained round, clear vesicles as well as large dense-cored vesicles. Terminals with dense-cored vesicles were confined to the ML and outer portion of the SG. The majority of terminations formed the central elements of complex, multi-synaptic arrays (glomeruli) and made synaptic contact with dendritic spines and with small vesicle containing profiles reminiscent of the type 2 spines described by Gobel (J.C.N., 167: 165, 1976) in the SG of the spinal trigeminal nucleus. Some terminals made synaptic contact with larger dendritic profiles located outside of glomeruli, although such observations were more common in regions deep to the SG neuropil. Labeled profiles within the SG tended to be larger and to exhibit more synaptic contacts than those in the ML. (Supported by N.I.H. Grant NS-10165 and Postdoctoral Fellowship NS-05394.)

1809 CROSS CORRELATION ANALYSIS OF CONNECTIVITIES AMONG CELL PAIRS 0.25-2 SEGMENTS APART IN CAT LUMBOSACRAL DORSAL HORN. Paul B. Brown, Robert P. Yezierski, and H. Richard Koerber. Department of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, WV 26506.

Adult cats were anesthetized with urethane (1.1 gm/kg), paralyzed with Flaxedil, and artificially respired. The spinal cord was transected between T_{10} and T_{12}. Pairs of cells were recorded simultaneously from independently manipulated stainless steel microelectrodes. Recording sites were verified to be located in laminae I-VI of L_4-S_1 segments. Of 83 cell pairs, 65 (78%) had flat cross-correlograms, indicating no causal relation in their discharges. Of these, 44 cell pairs were found in which both cells had cutaneous input: 36 (82%) had flat cross-correlograms.

Most cell pairs with non-flat cross-correlograms were indicative of net excitatory causal relations in discharge: that is, their cross-correlograms deviated from baseline only in the positive direction. These deviations were 15-100 msec. wide, and extended into both positive and negative delays. We interpret these as indicating shared input. No signs of monosynaptic excitatory connections (brief positive deviations at monosynaptic delays) were seen.

Inhibitory interactions, indicated by negative deviations from baseline, were rare. Those which were observed were all too broad to suggest a direct inhibitory connection from one cell to the other. All except one were purely inhibitory, the one exception being biphasic.

All cutaneous cell pairs with non-flat histograms had cutaneous receptive fields which overlapped, as would be expected if the implied common input is cutaneous.

1810 AUTORADIOGRAPHIC MEASUREMENT OF CAT SPINAL CORD BLOOD FLOW AFTER MODERATE AND SEVERE IMPACT INJURY. David F. Cawthon*, Franklin C. Wagner, and William B. Stewart. Depts. Neurosurg., Physiol.; Yale Univ. Sch. Med., New Haven, Ct. 06510

Both hyperemia and ischemia have been reported following moderate and severe injuries to the spinal cord. These results have come from several laboratories, using several different injury models and different techniques to measure spinal cord blood flow (SCBF). We sought to test the hypothesis that hyperemia is more common after moderate than severe trauma. For this purpose, we used the ^{14}C-antipyrine autoradiographic method to measure SCBF at 1,4, and 8 hours after moderate (260 g-cm) or severe (500 g-cm) impact injury to the cat spinal cord at T6. We measured SCBF in 2 cats at each interval, for each trauma dose. Control mean SCBF for cord 3 cm or more from the center of trauma in the 260 g-cm group was 11.6 g blood/min/100 g tissue for white and 46.0 for gray. These flows agreed with those obtained by Landau et al. in awake intact cats (63 ml/min/100 g for gray and 14 for white), also using quantitative autoradiography (Trans. Am. Neurol. Assoc. 80: 125, 1955). This report will discuss only white matter SCBF.

At the center of moderate trauma, mean SCBF expressed as percent of control values was 120% at 1 hour and was reduced to 75% at both 4 and 8 hours. Similar values at each time were obtained 2 mm from the center of trauma. At the center of severe trauma, mean SCBF was 83% at 1 hour, 37% at 4 hours, and returned to 71% at 8 hours. In this group, the flows at 2 mm away were similar to flows at the trauma center at 1 and 8 hours, but at 4 hours were significantly greater (62% of uninjured). Longitudinal spread of ischemia and hemorrhagic necrosis were greater for severe than for moderate injury. Also, at 1 and 8 hrs, relative hyperemia was more commonly seen after moderate trauma. Above we report mean white matter SCBF, but this obscures differences between flows within a cord segment. For example, at 1 hour after 500 g-cm trauma, one cat showed hyperemia (18.2 to 23.2) in ventral white and normal flows (12 to 14) in lateral and dorsal white within 2 mm rostral and caudal to the trauma center. At 1 and 4 hours after both severe and moderate trauma, the ventral white matter had higher flows than dorsal or lateral white, regardless of absolute flows. We also noted that mean SCBF was most variable at 8 hours: i.e., in some animals SCBF returned to near control values while in others it did not. We conclude that long tract deficits observed following severe injury (500 g-cm) may be due in part to the greater white matter ischemia near the site of severe trauma compared to moderate (260 g-cm) trauma. The return of function following moderate injury may also be a reflection of greater areas of white matter sustaining normal or relatively hyperemic SCBF.

Supported by USPHS Grants NS10174 and NS05429.

1811 AN ELECTRON MICROSCOPIC STUDY OF THE TRACT OF LISSAUER. Kyung Soon Chung, Arnold E. Applebaum, and Richard E. Coggeshall, Depts. of Anatomy and of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, Texas 77550.

In 1885, Lissauer described a tract of finely myelinated fibers that was located over the dorsal horn of the spinal cord. Lissauer felt that the majority of fibers in this tract were primary afferents. In later years, however, this view has been superceded and it is now felt that the fibers in the tract of Lissauer are predominately propriospinal in nature. The primary evidence for this conclusion is that few fibers in the tract of Lissauer appeared to be affected when the dorsal root was cut. There are 2 difficulties with this data however. First it is often impossible to tell whether or not one is examining a normal myelinated fiber in the light microscope because a myelin figure can often be mistaken for a normal fiber, and second the light microscope does not provide the resolution needed to study the unmyelinated fibers. Accordingly the tract of Lissauer was reexamined, both in the normal rat and after dorsal root section at the T_8 and L_6 levels. Our first results are as follows:

Segment T_8

	Normal Side	Operated Side (dorsal rhizotomy)
Myel. Fibers	2715	575
Unmyel. Fibers	6850	800

These figures suggest that 85% of the axons in the tract of Lissauer are primary afferents. Similar findings have been obtained from an L_6 segment, but the count is not yet complete. If further counts confirm these preliminary observations then the obvious conclusion is that the great majority of axons in the tract of Lissauer are primary afferents.

In addition synaptic terminals were found in the tract of Lissauer. At present we are characterizing the synaptic types. We are also examining the tract of Lissauer after dorsal rhizotomy to determine if the synapses are connections between dorsal root afferents. If so they may provide a morphologic basis for primary afferent interactions which have recently been reported.

Supported by Grants NS 10161, 11255 and Fellowship NS 05430 from the NIH.

1812 PERSISTENCE OF HABITUATION EFFECTS ON FLEXOR WITHDRAWAL RESPONSES AND SYMPATHETIC ACTIVITY MEDIATED BY THE FUNCTIONALLY TRANSECTED HUMAN SPINAL CORD. Marcus J. Fuhrer. Depts. of Rehab. and Psychiat., Baylor Coll. Med., Houston, Tx. 77030.

The study was one of a series concerned with the stimulus parameters that influence the persistence of habituation effects mediated by the chronically transected human spinal cord. The specific goal was to establish whether spontaneous recovery is retarded and retention of habituation facilitated by continuing to apply the habituating stimulus at widely spaced intervals following habituation training. Nine persons with relatively long-standing (16-49 months) transection of the cervical spinal cord participated in two daily sessions. Each individual had been examined neurologically by two examiners, and in no case was voluntary motor functioning or sensation detected below the lesion. The stimulus consisted of a 40-msec pulse train applied unilaterally to the plantar surface. Lower extremity withdrawal activity was assessed in terms of the integrated EMG activity of the tibialis anterior muscle. Phasic sympathetic activity evoked by the stimulus was recorded in terms of skin conductance responses from the volar surfaces. The essential procedure was to: 1) establish baseline responsiveness to test presentations of the stimulus applied at 10-sec intervals, 2) apply habituating stimulation at the rate of 1/sec until EMG responsiveness was extinguished, 3) readminister test presentations of the stimulus at 10-sec intervals, 4) continue to apply a single presentation of the stimulus at 30-sec intervals for a 3-min period, 5) assess the degree of spontaneous recovery and retention of habituation by repeating steps 1-3. The comparison condition was conducted in the same manner except that the stimulus was withheld during the 3-min period following habituation training.

Applying the habituating stimulus infrequently following habituation training resulted in significantly less spontaneous recovery of both withdrawal activity and electrodermal responsiveness. No effect was demonstrable, however, on the retention of habituated withdrawal activity in terms of either the number of stimulus repetitions to extinction or effects on the amplitude of responses. This outcome is consistent with other evidence suggesting that the spontaneous recovery and retention of habituation are distinctive processes that share some but not all of the same determinants. Supplemental analyses revealed a substantial degree of coupling between withdrawal responses and evoked electrodermal activity.

Supported in part by NINCDS Grant NS 07755-09 and by the Rehabilitation Research and Training Center No. 4 (Rehabilitation Services Administration Grant 16-P-56813-6/14).

1813 PROFILE OF MYELINATED AXONS IN THE LUMBAR VENTRAL ROOTLETS OF THE CAT. P.L. GILDENBERG, K. G. CHATMAJIAN* and K. S. K. MURTHY. Department of Surgery (Neurosurgery) and Department of Neurobiology & Anatomy, University of Texas Medical School, Houston, Texas 77030.

Adult cats were anesthetised with sodium pentobarbital and a lumbar laminectomy (L_3-S_1) was carried out. After perfusion of the animal with 12% formalin, the spinal cord was dissected to remove the segments L_4 through L_7 along with the respective dorsal and ventral roots. The ventral quadrants with the respective ventral roots were then carefully removed and mounted on a cardboard with the roots stretched perpendicular to the axis of the cord. Such a preparation permitted the identification of the orientation (in a rostral-caudal direction) of the component rootlets in any ventral root. After fixation for 24 hours in 12% formalin, these roots were impregnated with a buffered solution of osmium tetroxide (Palade, 1952) for 30-48 hours. After dehydration, the roots were embedded in paraffin wax. Serial sections at 5μ thickness were cut transverse to the rootlets and mounted on slides. The rootlets were photographed on 35 mm film (magnification 400) which was then projected on a screen for the measurement of fibre diameters.

The histograms of fibre diameters indicate that within each ventral root, the smaller alpha and the gamma axons are found more frequently in the caudal rootlets (which also join the cord more medially). As one compares the organization of the roots L_4 to L_7 in a caudad direction, the ratio of larger alpha axons increases with respect to the smaller alpha and gamma axons. While the occurrence of gamma axons is of the order of 30-40% in L_5, L_6 and L_7 ventral roots, they outnumber the alpha axons in L_4. It was also observed that near the point of exit from the cord, the gamma axons often are present in small clusters. The significance of this feature is not at present clear.

REFERENCE

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1814 GLUCOCORTICOID EFFECTS ON SPINAL CORD REPETITIVE MONOSYNAPTIC TRANSMISSION AND APPARENT TRANSMITTER TURNOVER. EDWARD D. HALL & THOMAS BAKER. Dept. Pharmacol., Cornell University Medical College, New York, N. Y. 10021

In recent studies, an intensive short term glucocorticoid pretreatment regimen (triamcinolone diacetate 8 mg/kg i.m. daily/7 days) was found to facilitate excitatory spinal reflex function in acute spinal (C-1) cats (Hall et al., Neurosci. Abst. 3:502 and J. Pharmacol. Exp. Ther., in press). The principal findings were an enhanced monosynaptic (2N) post-tetanic potentiation, an increased rate of 2N synaptic recovery after single impulse transmission and an increased polysynaptic discharge.

In the present work, the effects of the intensive triamcinolone regimen were examined on the 2N input-output characteristics and the ability of the primary afferent (Ia) terminals to maintain repetitive transmission at moderate frequencies. Experiments were performed on the first post-treatment day. Stimulation was applied to the triceps surae nerves of one leg. All sensory action potentials and reflex responses were recorded at the L7 dorsal and ventral roots, respectively. First of all, in the treated cats, there was a shift in the 2N input-output relationship such that less primary afferent activation was required to initiate a liminal 2N discharge: 15.7% of maximum Ia activation in the treated vs 27.4% in the untreated ($p<0.05$). The slopes of the input-output curves were identical, however. Secondly, the treated animals exhibited significantly less rundown in 2N response amplitude during trains of ten stimuli at 5 or 10Hz. The response plateau in the treated after rundown was 13.9% ($p<0.01$) higher at 5Hz and 17.0% ($p<0.001$) higher at 10Hz than in the untreated. Comparative analysis of the extent of decline in terms of apparent transmitter release parameters (Capek and Esplin, J. Neurophysiol. 40:95, 1977) revealed no significant effects on the apparent fractional release of transmitter (p) by the Ia terminals, but the rate of replenishment of the available transmitter store (r) was more than doubled in the treated preparations.

The decrease in the Ia afferent activation necessary to just evoke a 2N response, without an increase in apparent transmitter release, suggests that the safety factor for terminal impulse invasion is improved. Furthermore, the enhanced maintenance of repetitive transmission at moderate frequencies reflects an increased rate of transmitter mobilization. These direct facilitatory effects on central monosynaptic function may partly explain the benefits of glucocorticoids in acute spinal cord trauma and certain other central neurologic disorders.

(Supported by NIH grants 5-R01-NS-01447 and 5-S07-RR05396-15).
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1815 CONVERGENCE OF CUTANEOUS, MUSCULAR, AND ARTICULAR INFORMATION ONTO SPINAL NEURONS IN THE CAT.
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The degree of convergence between the sural nerve (SN), the nerve to the medial head of the gastrocnemius m. (MGN), and the posterior articular nerve (PAN) onto single neurons was studied. Extracellular unitary responses were elicited in the lumbosacral spinal cord of the unanesthetized, spinalized, and anemically decerebrate cat in response to electrical stimulation of peripheral nerves. After dissection of the hindlimb, each nerve was cut distally to facilitate the resolution of the compound action potentials.

102 of 173 (59%) units responded (with excitation and/or inhibition) to stimulation of all three nerves. Most of these responded to low levels of stimulation with short duration bursts of activity. Many responded further with late discharges of long duration to levels of stimulation invoking activity in Aδ and C fibers. Very few units responded exclusively to high levels of stimulation. Histological verification of recording sites with iontophoretically applied pontomine sky blue showed that the convergent units lay in laminae IV-VIII, the majority being located in VII and VIII.

Other units were characterized as responding only to SN and MGN (11%), SN and PAN (5.8%), MGN and PAN (1.7%), SN (20.2%) and PAN (2.3%). No units responded only to stimulation of MGN.

The receptive fields remaining after nerve section were mapped for many convergent units in response to natural stimulation. The resultant complex and enlarged receptive fields is suggestive of an "unmasking" effect which reveals peripheral influences extending over several segments.

The great degree of convergence between different peripheral nerves and fiber diameters within each nerve reveals synaptic connections not easily demonstrated by natural stimulation. Because of the convergence of cutaneous, muscular and joint input onto single neurons, it is suggested that ascending sensory and local reflexive pathways may share common interneuronal pools. (Supported in part by NIH grant AM16716)

1816 THE DEVELOPMENT OF SPINAL MONOAMINE SYSTEMS IN THE NORTH AMERICAN OPOSSUM. Albert O. Humbertson, Jr. and George F. Martin. Dept. Anat., Sch. Med., The Ohio State Univ., Columbus, Ohio, 43210.

Pouch-young opossums ranging in size from 15-105mm snout-rump length were subjected to the Falck-Hillarp technique or to glyoxylic acid perfusion and freeze drying (Loren et al., Histochemistry, 49: 177-192, 1976). Observations were made on cervical, thoracic and lumbosacral levels utilizing both techniques. In the youngest animals (15-30mm) there is evidence (fluorescence) for monoamine transport in axons located within the dorsolateral marginal zone. Such axons extend throughout the length of the cord. A few fluorescent varicosities are present also in the ventral marginal zone, but none have invaded the intermediate zone at any level. There is a cranial to caudal sequence to the growth of fluorescent neurites into their definitive terminal territories. At 35mm they have penetrated the dorsolateral intermediate zone at cervical levels and some have reached the area dorsal to the central canal. By 45mm comparable growth has taken place at lumbosacral levels. The fluorescence increases progressively in the intermediate zone at all levels of the cord, and by 55mm it is found in most of the areas expected from adult material. Such areas include: the dorsolateral intermediate zone, the region dorsal to the central canal, the periphery of the ventral horn and the region capping the dorsal horn (presumptive lamina I). In the oldest animals studied (60-105mm) the pattern of fluorescence is like that found in the adult with distinct varicosities. Supported by U.S.P.H.S. Grants NS-07410 and NS-10165.

1817 2 DEOXYGLUCOSE MAPPING OF THE PLANTAR CUSHION REFLEX.
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We have used the method of 2-deoxyglucose(2-DG)autoradiography for mapping metabolic activity in the pathway underlying the plantar cushion (PC) reflex in the cat spinal cord. In four adult cats anesthetized with pentobarbital, the tibial nerve was dissected free of surrounding tissue, left intact, and mounted on bipolar recording electrodes. Following administration of 100 μC/kg of 2-DG, the PC reflex was elicited electrically using 0.5 msec. pulses at 5 or 10 times threshold for 45 min. at 1,3, or 10 Hz. through needle electrodes inserted into the skin of the pad. By varying the frequency parameters of stimulation we were able to examine the pattern of 2-DG uptake in animals in which the reflex was present for the duration of the stimulation and in one animal in which the reflex was habituated prior to injection of the 2-DG. The animals were sacrificed at the conclusion of the stimulation and the tissue processed immediately for 2-DG autoradiography. In three animals in which reflex output was maintained for the duration of the stimulus a discrete, ipsilateral region of increased 2-DG uptake was observed only at the most medial edge of the dorsal horn within Rexed's laminae I-IV in the lumbar enlargement. This uptake pattern was also present in the animal in which reflex activity habituated. No increase in uptake was observed in the S_1 motor nucleus where motorneurons activated during the PC reflex are located. In one control animal prepared identically to the experimental cases, but without stimulation, no focal changes in 2-DG uptake were observed within the spinal gray matter.

The area of increased autoradiographic density at the most medial edge of laminae I-IV following electrical stimulation, likely represents increased activity associated with the termination of primary afferents from the PC. The area of labelling lies within the central distribution of the plantar nerve, in which PC afferents are included, and in part overlaps the region in which dorsal horn neurons activated by PC stimulation are found(Egger & Wall, J.PHYSIOL.(LOND) 216:483, 1971). These results indicate that the 2-DG technique is useful for the localization of the central terminations of primary afferent fibers innervating discrete peripheral sites.

Supported by USPHS grant NS 10174.

1818 MORPHINE ANALGESIA, SUPRASPINAL INFLUENCE ON SPINAL MECHANISM.
Luke M. Kitahata, K. Hanaoka* and A. Taub*. Dept. Anesth. Yale Univ. Sch. Med. New Haven, Conn. 06517.

Recent evidence has delineated both spinal (Anesth. 41: 39, 1974) and supraspinal (Sci. Sinica 13: 1099, 1964) sites for the pharmacological action of morphine analgesia. The interaction between these sites is of major significance for the ultimate understanding of the mechanisms of morphine analgesia. Electrical stimulation in the region of the periaqueductal gray in the rat (Science 164: 444, 1969; Pain 1: 51, 1975) produces behavioral changes comparable to surgical analgesia in man. Such stimulation, particularly in the region of the dorsal raphe nucleus (Exp. Brain Res. 20: 32, 1974) inhibits activity in cells of Rexed Lamina V evoked by noxious stimulation of the skin. Morphine injected in microgram quantities into the region of the periaqueductal gray (Sci. Sinica 13: 1099, 1964) produces an analgesic effect in animals comparable to a 500-fold greater systemic dose. Taken together, these studies suggest that morphine may activate mechanisms in the periaqueductal gray region which, in turn, are suppressive of the activity of cells in Rexed Lamina V of the spinal cord, thus leading to analgesia (Pain 1: 51, 1975). The present study was undertaken to test the hypothesis that intravenous morphine activates supraspinal descending mechanisms which modulate the activity of those spinal ascending mechanisms mediating nociception. A dose-dependent suppression of the activity of Lamina V nociceptive neurons following morphine administration (0.5, 1.0 and 2.0 mg/kg) was demonstrated confirming the work of Kitahata et al. (Anesth. 41: 39, 1974). It was further demonstrated that the degree and duration of this suppression was greater in those animals with an intact spinal cord than in those animals whose spinal cord has been transected and was greater for evoked than for spontaneous activity. The effect was reversed by naloxone. The observation in this study supports the hypothesis that morphine activates brain stem structures, which, in turn, inhibit the activity of nociceptive neurons in Rexed Lamina V of the lumbar spinal cord.

Supported by NIH Grant NS-09871.

1819 EFFECTS OF SOMATOSENSORY STIMULUS MODALITIES ON SINGLE SPINAL UNITS IN RATS; IMPLICATIONS FOR LORDOSIS BEHAVIOR. Lee-Ming Kow, Frank P. Zemlan*, and Donald W. Pfaff. The Rockefeller Univ., New York, NY 10021.

From behavioral (Kow & Pfaff, Brain Res., 1976; Kow et al., in prep.) and dorsal root ganglion (DRG) single unit (Kow & Pfaff, Fed. Proc., 1978) studies, it has been found that the sensory input from pressure stimulation on skin innervated by the lower lumbar spinal cord is necessary and sufficient for triggering lordosis in rats. In the present study, single unit activity (N= 353) was recorded from the spinal cord between lower L_5 and upper S_1 in urethane-anesthetized rats, and unit responses to a series of somatosensory stimuli were studied. 54% of the units did not respond to pressure, but responded to brushing, muscle-joint, and/or visceral stimulation, or none at all. 15% of the units responded only to pressure. The remaining 31% responded to pressure as well as other stimuli. Although the majority of pressure-responsive units were excited by pressure stimulation, a small percentage were inhibited or responded differently depending on the site stimulated. Within the spinal cord most of the units were recorded from laminae IV through VIII. The units responding only to brushing were confined to dorsal horn (laminae IV and V). Those responding only to muscle-joint or visceral stimulation were in the ventral horn. Pressure-responsive units were primarily found at intermediate depths. In contrast to DRG units (Kow & Pfaff, 1978), spinal units have larger receptive fields, higher resting activity, additional and more complex response types, and show greater variability of response. These comparisons indicate convergence of primary sensory information on the units recorded.

We assume that excitation of units responding only to pressure is centrally involved in triggering the lordosis reflex. Those units not responding to pressure are probably irrelevant for this behavior. Presently undetermined are the roles of units with complex or mixed response types.

1820 SYNAPTIC POTENTIALS IN SPINAL MOTONEURONS: EFFECTS OF EXTRA- AND INTRACELLULAR INJECTIONS OF SOME IONS. K. Krnjević, Y. Lamour*, J.F. MacDonald and A. Nistri*. Anaesthesia Res., McGill U, 3655 Drummond, Montreal.

PSPs were evoked in lumbosacral spinal motoneurons of cats (under Dial anaesthesia) by electrical stimulation of dorsal or ventral roots and various peripheral nerves. Extracellular injections of Co or Mn which were sufficient to depress the post-spike hyperpolarization also clearly reduced the amplitude of PSPs. Although the injection sometimes had a depolarizing effect, this was usually too small to account for the depression of EPSPs. Moreover, the EPSPs were clearly diminished even in the presence of no significant change in resting potential or a small hyperpolarization. Under stable conditions of recording this effect of the divalent cations was reversible. Unless these cations can interfere specifically with the ionic movements generated by synaptic activity, the observations suggest that these PSPs are generated mainly by chemical, rather than electrical transmission, although some degree of electrical transmission is not totally excluded.

In contrast to spike potentials, EPSPs were not markedly depressed by intracellular injections of procaine. On the other hand, they proved to be surprisingly sensitive to changes in the internal pH; intracellular injections of protons had a sharply depressant effect. This was probably not secondary to a change in membrane potential and was unexpectedly associated with a fall in cell input conductance. In this respect, it differs from the depressant action of intracellular injections of calcium or strontium. It is not clear whether the effect of protons represents a specific interference with the mechanism of generation of PSPs or whether it may indicate some degree of synaptic uncoupling.

Intracellular injections of Mn and Co were sometimes followed by a marked reversal of IPSPs (or increase in depolarizing IPSPs), but these changes could be ascribed to the concurrent injection of anions that can permeate the IPSP ionic channels.

Supported by the Canadian Medical Research Council.

1821 BRANCHING OF AXONS IN THE L_6, S_1 AND S_2 DORSAL ROOTS OF THE RAT. Lauren A. Langford* and Richard E. Coggeshall, Depts. of Anatomy and Physiology and Biophysics, and The Marine Biomedical Institute, The University of Texas Medical Branch, Galveston, Texas 77550.

In a previous abstract we reported that there were more dorsal root axons in the L_6, S_1 or S_2 dorsal roots of the rat than there were ganglion cells in the corresponding dorsal root ganglia. Our explanation for these data was that many of the dorsal root axons branch in the ganglion or in the dorsal root before they reach the spinal cord. Alternative explanations for the preponderance of axons over ganglion cells are: 1) that there are a number of dorsal root efferent fibers whose cell bodies are located within the spinal cord or 2) that there are a number of postganglionic sympathetic fibers that innervate the blood vessels of the dorsal root in addition to the dorsal root fibers. To control for the possibility of dorsal root efferents, the dorsal root ganglion was removed and the root was then examined in the electron microscope. Any fibers whose cell body is in the spinal cord would survive in the dorsal root whereas those fibers whose cell bodies are in the dorsal root ganglion would die. In one animal 3 days after ganglionectomy, no normal myelinated fibers were found but approximately 290 cell processes that might be unmyelinated fibers were observed in the dorsal root. In another animal which survived 10 days no myelinated fibers were seen but 20 processes that might be unmyelinated fibers were observed. Further studies to provide statistical validity and to determine whether or not the unmyelinated cell processes are unmyelinated axons are underway. For the present argument, however, it is clear that there are not enough dorsal root efferents to explain the discrepancy in axon and ganglion cell numbers. To control for the possibility that there are sympathetic fibers in the dorsal root, a sympathectomy was done and the dorsal root was examined after allowing time for degeneration of postganglionic fibers. In the one rat examined to date, there were 4757 dorsal root ganglion cells and 7422 dorsal root axons.

If further studies support these preliminary observations, the conclusion would be that there are more dorsal root axons than ganglion cells for segments L_6, S_1 and S_2 in the rat, even when dorsal root efferents and sympathetic fibers are excluded. Accordingly it seems that dorsal root axons branch in the ganglion or the dorsal root itself, a conclusion that is at variance with textbook descriptions that indicate that each dorsal root ganglion cell sends a single axon into spinal cord through the dorsal root.

Supported by NIH grants NS 10161 and 11255.

1822 MORPHOLOGY OF PHYSIOLOGICALLY IDENTIFIED NEURONS IN THE SPINAL MARGINAL ZONE AND SUBSTANTIA GELATINOSA. A. R. Light, D. L. Trevino and E. R. Perl. Dept. Physiol., Univ. N. Carolina Chapel Hill, NC 27514

Differing physiological properties have been ascribed to neurons presumably indigenous to the dorsal horn marginal zone (lamina I), the substantia gelatinosa (lamina II) and the nucleus proprius on the basis of extracellularly recorded unitary discharges, which may be derived from a neuron's axon, soma or dendrites. Conventional microelectrodes do not reliably differentiate between these in a dense neuropil. Therefore, pipette electrodes containing horseradish peroxidase (HRP) were used to record unitary activity in lower sacral and coccygeal spinal cords of cats, unanesthetized after decerebration and spinal transection. After functional characterization, HRP was deposited intracellularly by iontophoresis: HRP staining provides a Golgi-like picture of the cell. No cells with somata in lamina I or II responded to afferent volleys confined to fibers conducting over 45 m/sec. Three main classes of neurons with perikarya in lamina I and lamina II responding to afferent input have been identified: 1) Neurons with dominant input from Aδ mechanical nociceptors had small to medium (10-30 μ diam.) somata in lamina I or the immediately subjacent part of lamina II: their predominant dendritic projection was in lamina I. 2) Neurons excited by innocuous thermal changes responded only to volleys containing C fibers: they had somata located in the marginal zone with dendritic projections mainly in the immediately subjacent (dorsal part) lamina II. The thermoreceptive neurons were the only group that consistently had significant spontaneous activity. 3) Two kinds of neurons had a dominant excitatory input from low threshold mechanoreceptors. One type was excited principally by input from the Aδ, "D" hair follicle receptors and had small somata located in the deeper portion of lamina II or the most dorsal part of nucleus proprius (lamina III) with dendrites which ramified extensively rostrocaudally in the same laminae. The second type appeared to receive predominant excitation from the low threshold C mechanoreceptors and had somata located within lamina II: their dendritic extensions were principally within the central part of lamina II. Units dominated by cutaneous receptors with rapidly conducting myelinated fibers had somata located deep to lamina II, however, some were found to have dendrites extending into lamina II and evidenced an excitatory input from low threshold C mechanoreceptors. Units responding inconsistently to primary afferent input or only showing inhibitory postsynaptic potentials were also observed superficially in the dorsal horn. Supported by USPHS, NINCDS grants 10321 & 11132 and a fellowship (NS05526) to ARL.

1823 EPSPs ELICITED BY SINGLE IMPULSES IN LARGE POPULATIONS OF MOTONEURONS. Hans-R. Lüscher*[1], Eberhard Fetz, Paul Ruenzel* and Elwood Henneman. Dept. of Physiology, Harvard Medical School, Boston.

The number of terminals given off by an axon of a motoneuron is a function of its diameter (McPhedran et al. J. Neurophysiol. 28:71, 1965). Furthermore, evidence has been presented that the amplitude of an individual stretch evoked EPSP in a motoneuron is related to the diameter of the I_A fiber conducting the afferent impulses (Mendell et al. J. Neurophysiol. 34:171, 1971). This suggests that the number of terminals from a I_A fiber to a motoneuron is also a function of its diameter. It is uncertain whether this inference is correct, however, because the amplitude of an individual EPSP is also strongly influenced by the input resistance of the motoneuron and the location of the synaptic terminals. If the influence of such variables could be minimized by averaging the EPSPs caused by single I_A impulses in a large number of motoneurons, the total synaptic effect of a I_A impulse could be determined and related to the number of its terminals.

By infiltrating a ventral root with isotonic sucrose solution so that its extracellular resistance is extremely high, the interiors of its axons can be used as wick electrodes to record voltage changes in all the motoneurons of that spinal segment. With an averaging computer triggered by the action potentials from a single, functionally isolated but intact afferent, the synaptic effect of a I_A impulse upon the whole population of motoneurons can be extracted from the physiological and electrical "noise" in the recording system. Potentials with the general waveform of EPSPs but of somewhat longer duration can be recorded under these conditions. They may be referred to as individual "post-synaptic population potentials" (PSPPs).

Individual PSPPs were recorded from S_1 ventral roots of cats under chloralose anaesthesia. The conduction velocity of the afferent impulses, all originating in the triceps surae muscles, covered the range for group I_A and group II afferents. The amplitude of these PSPPs showed a consistent and significant relationship to the conduction velocity of the afferent fibers. Impulses in slowly conducting I_A fibers yielded small PSPPs, while fast fibers were associated with larger PSPPs. Group II impulses elicited very small PSPPs.

These findings confirm the hypothesis that the number of terminals given off by an afferent fiber is a direct function of its diameter and suggest that the size principle applies also to primary sensory neurons in the stretch reflex.

Supported by a grant from the National Institutes of Health.
[1]Supported by the Swiss National Science Foundation.

1824 THE ORGANIZATION OF RETICULOSPINAL PROJECTIONS IN THE NORTH AMERICAN OPOSSUM. George F. Martin, Michael Panneton and Irene Tschismadia. Dept. Anat., Sch. Med., The Ohio State Univ., Columbus, Ohio, 43210.

Injections of ^{3}H-leucine were placed into each of the cell groups revealed by the HRP technique to be the origin of reticulospinal projections. The developed autoradiograms reveal that the nucleus cuneiformis projects by the ipsilateral sulcomarginal and ventral funiculi to laminae VII and VIII of the cord and that medially situated neurons throughout the nucleus retilaris pontis (RP) relay through the same routes to comparable targets. The rostral nucleus magnus raphe (RaM) distributes bilaterally through dorsolateral tracts to lamina I. When injections involve specific neurons in the ventrolateral part of the rostral RP there is additional labelling of axons which interdigitate with rubrospinal fibers within the contralateral cord and which also distribute to lateral parts of laminae IV through VI. Deposits of ^{3}H-leucine which cover the nucleus locus coeruleus and/or subjacent areas produce labelling within the ventral, lateral and dorsolateral white matter, ipsilaterally, as well as labelling of axons in the dorsolateral white matter contralaterally. Many of the contralateral axons course among rubral axons. Some of the cases with injections of the dorsolateral pons show light label within lamina I and lateral parts of laminae V and VI, bilaterally, as well as within laminae VII and VIII (mainly ipsilaterally) and lamina X. Injections of the nucleus reticularis gigantocellularis (RG) and/or the nucleus reticularis gigantocellularis, pars ventralis (RGcv) produce labelling within the ventral, lateral and dorsolateral white matter, bilaterally, as well as terminal label within lateral parts of lamina V and VI, ipsilaterally, and over laminae VII and VIII, mainly ipsilaterally. If serotonin containing neurons of the RaM and the RGcv are included in the injection there is evidence of a strong projection to laminae I and X, bilaterally. A somewhat different pattern of spinal label is produced by injections lateral to RG. Injections which include paramedian areas of the medulla, as well as the nucleus obscurus of the raphe, produce labelling of axons within both the ventral and lateral funiculi and terminal label within laminae VII through X. Label is especially heavy within laminae IX and X. Injections limited to more lateral areas of the medulla also result in spinal labelling and those which cover the rostral lateral reticular nucleus elicit labelling of terminals, bilaterally, within the intermediolateral cell column at thoracic levels. The nucleus retroambiguus projects mainly via the contralateral ventral funiculus to laminae VIII and VII.

Supported by U.S.P.H.S. Grants NS-07410 and NS-10165.

1825 SPINAL CORD TRANSECTION: A QUANTITATIVE ANALYSIS OF REACTIVE EVENTS WITHIN THE SITE OF LESION FOLLOWING ADMINISTRATION OF PIROMEN, CYTOXAN OR TRYPSIN. M. A. Matthews, M. F. St. Onge, C. L. Faciane, J. B. Gelderd. Dept. Anat., LSU Med. Ctr., New Orleans, LA 70119.

Long-Evans hooded rats were cordotomized at the T-5 level and given: 1) cyclophosphamide, an immunosuppressive agent; 2) Piromen, a bacterial polysaccharide-nucleic acid complex; 3) topical and systemic trypsin; 4) no further specific treatment. Because of past and present controversy surrounding the proposed ability of these agents to promote spinal cord regeneration, a systematic study, employing light and electron microscopy, and quantitative methodology in a single animal model, was done in order to re-evaluate the effects of such treatment.

After an initial inflammatory reaction during the first week following surgery, the lesion zone is characterized either by areas of dense collagenous connective tissue with occasional fibroblasts and macrophages, or a loose, areolar tissue with numerous sheets and cords of mesodermal cellular elements but minimal collagen. By 45 days postoperative (dpo), axons supported by Schwann cells, invade and become entangled in the loose connective tissue matrix. With longer postoperative survival, cysts appear cranial and caudal to the lesion and erode much of the scar together with viable neural tissue. Administration of Cytoxan and Piromen did not result in any qualitative alteration of the scar matrix as evidenced by electron microscopy. Quantitative analysis revealed a slight reduction in the fibrous connective tissue component of the scar at 45-90 dpo associated with an elevated incidence of axons in this zone but this proved to be a transient occurrence as longer postoperative periods were studied. The use of trypsin resulted in significant reduction of the amount of fibrous connective tissue with a concomitant increase in loose connective tissue and the appearance of a few distinctive, compact bundles of unmyelinated axons lacking Schwann cells.

Consistent behavioral modifications were not observed in any of the treatment groups which would significantly distinguish them from controls. Our results appear to contradict the findings of Matinian and Andreasian (1976) who reported return of normal sensori-motor function in 80% of their animals treated with topical and systemic trypsin.

It is concluded that a major impediment to whatever long term regenerative potential exists within the spinal cord is the lack of axonal guiding elements within the scar, but more importantly, the severe erosion of the remaining spinal cord due to cyst enlargement.

Supported by a grant from the Edward G. Schlieder Foundation.

1826 EVALUATION OF ETHACRYNIC ACID AS A THERAPEUTIC AGENT IN EXPERIMENTAL SPINAL CORD TRAUMA. J.T. Molt, D.A. Poulos, H.K. Kimelberg*, and R.S. Bourke. Div. of Neurosurgery and Dept. of Physiology, Albany Medical College, Albany, N.Y. 12208

Hemorrhagic necrosis resulting from blunt trauma to the spinal cord is a progressive destructive process which radiates from gray matter into surrounding white matter. Recent evidence has shown that an early event following traumatic injury to the cord is astroglial swelling. Such swelling may be important in the spread of the hemorrhagic lesion due to the following mechanism. It is known that during trauma-induced hypoxia and ischemia, intracellular K^+ is released into the extracellular compartment of the spinal cord reaching concentrations of up to 70 mM. Swelling may be due to 1) uptake of K^+ plus Cl^- in accordance with Donnan equilibrium and 2) an extra component of HCO_3^--stimulated swelling involving the uptake of Na^+ and Cl^- into glia and possibly other cells. When swelling occurs in perivascular astroglia it may compress or occlude the microvasculature. This may reduce blood flow and increase intercapillary distances which will increase the diffusion path for oxygen resulting in a hypoxic insult to intact neurons. We have suggested that swelling by mechanism 2) above involves mediated Cl^- transport which can be inhibited by an unsaturated ketone derivative of aryloxyacetic acid (ethacrynic acid) among other agents. We tested the hypothesis that the K^+-dependent, HCO_3^--stimulated component of astroglial swelling is an important factor in the evolution of spinal cord injury and that its inhibition by ethacrynic acid might improve recovery from such an injury. Anesthetized cats were injured by dropping a 25 gm weight 20 cm onto an impounder and strain gauge centered on the exposed dural surface of the cord at the L2 segment. The impulse of the blow was recorded. Animals were treated with saline or ethacrynic acid 5 min following injury. Cats were evaluated 48 hrs post injury using a clinical examination and an electrophysiological test. The extent of the cord injury was then assessed histologically. All three methods of assessment revealed a significant improvement in animals treated with ethacrynic acid when compared with saline-treated control cats. Although unable to walk, the treated animals showed significantly better leg movement than untreated cats. Electrophysiologically, the descending tracts which modulate spontaneous spinal cord activity were shown to be more viable in treated animals than in controls. Histologically an average cross-sectional area of 85% of the untreated cords appeared injured compared to an average of 60% of the area in the cords of treated animals. These results indicate that inhibition of astroglial swelling by ethacrynic acid may be useful as a treatment modality for spinal cord injuries due to blunt trauma. (Supported by Grant NS13042 from NINCDS).

1827 PROPERTIES OF CENTRAL Ia AFFERENT FIBERS PROJECTING TO MOTONEURONS. J.B. Munson and G.W. Sypert, Univ. of Fla. Coll. of Med. and VA Hospital, Gainesville, FL 32610

Physiological studies of single medial gastrocnemius (MG) Ia afferents were made to determine their rostro-caudal distributions, branching patterns, terminal distributions, conduction velocities and EPSP generating properties in the cat spinal cord. Through the use of signal averaging techniques, field potentials ("branch potentials") and EPSPs occurring synchronously with action potentials in single MG Ia dorsal root afferents were studied in the region of the triceps surae motoneuron pool.

MG Ia afferents bifurcate upon entering the spinal cord and conduct both caudally (at 22 M/S) and rostrally (at 38 M/S). At intervals of 200μ to 1800μ ($\bar{x}$ = 1267μ), 6-8 major collateral branches conduct ventrally at 8-19 M/S. Prominent branch potentials and signs of synaptic activity can be distinguished in the regions of lamina VI (intermediate region) and lamina IX (motoneuronal pool). Mean amplitude of EPSPs generated by some major collateral branches is significantly larger or smaller than the mean for all EPSPs generated by that afferent. Branch potentials in the motoneuronal pool were recorded over a 8600μ maximum rostro-caudal extent.

These physiological results are integrated with previous anatomical studies to resolve inconsistencies and depict clearly the morphology of central MG Ia afferents projecting to motoneurons. (Supported by EY 01264 and VA Hospital Medical Research Service.)

1828 ELECTROPHYSIOLOGY OF ACUTE SPINAL TRAUMA. A.C. NACIMIENTO, M. BARTELS and H.-D. HERRMANN, Dept. Physiology I, and Dept. Neurosurgery, Saar University, School of Medicine, 6650 Homburg (Saar) G.F.R.

An exposed, otherwise intact spinal L7 segment in the cat was subjected to a sudden, short-lasting compression by an electromagnetically driven metal rod. Pathophysiological events at different degrees of compression were highly reproducible, allowing quantification. The following functions were monitored before, during and up to 6 hr after injury; a) polysynaptic reflex discharges (PRD); b) cortical evoked potentials (CEP) in response to the same afferent volley triggering PRD; c) conduction through the injured segment by recording from a S3 dorsal rootlet antidromic impulses set up by dorsal column stimulation at L1. With a compression of 1 mm there was initially little change in PRD and CEP; conduction was reversibly diminished for a few min. At 2 hr a decrease of PRD ensued, with no changes in CEP or conduction. PRD decreased further with time and disappeared 4-5 hr after injury. A compression of 2 mm caused an immediate loss of both PRD and conduction, leaving CEP unimpaired. At 30 min there was some degree of recovery of PRD and conduction, with a subsequent decline setting in after 60-90 min, and disappearance in 3 hr. At 3 mm there was immediate loss of PRD and conduction, delayed (after 60-120 min) signs of recovery, and final abolition. CEP were decreased by about 40% 10 min after injury followed by complete recovery. At 4 mm all responses were lost, with CEP recovering slowly after 10 min, to reach pre-lesion levels in 3-4 hr. No recovery of PRD or conduction was observed. Light microscopy of the injured segment displayed hemorrhages in the central grey matter, edema and axonal swelling in the white matter, well correlated with the degree of compression. These results show: 1) a differential sensitivity of spinal functions to injury, reflex activity being most susceptible; 2) a relative resistance and clear recovery tendency of CEP; 3) that post-traumatic changes in reflex activity and conduction set in immediately following injury, in the face of an essentially normal morphology.
(Supported by Grant Na 115/1, Deutsche Forschungsgemeinschaft).

1829 EFFECT OF DORSAL RHIZOTOMY ON THE SYNAPTIC POPULATION IN THE SACRAL SECONDARY VISCERAL GRAY. Michael F. Nolan and H. Keith Brown. Dept. of Anat., University of South Florida, Tampa, FL 33612.

Afferent impulses from pelvic visceral structures are relayed rostrally to brainstem levels via the sacrobulbar pathway. The cellular origin of this fiber tract is the secondary visceral gray located in the lateral funiculus at mid-sacral levels immediately dorsolateral to the sacral parasympathetic nucleus. This study was undertaken to identify the types and characteristics of synapses in this neuropil arising from ipsilateral dorsal root fibers. Adult male and female cats were anesthetized with pentobarbital sodium and subjected to unilateral intradural section of the S1, S2 and S3 dorsal roots. On the fifth postoperative day, the animals were reanesthetized and killed by transcardiac perfusion with buffered saline followed by 2% paraformaldehyde - 3% glutaraldehyde in 0.1M cacodylate buffer, pH 7.3. Sacral spinal segments were removed, osmicated and processed for transmission electron microscopy. The sacral secondary visceral gray was identified in 1μm toluidine blue cross sections. At the E.M. level, a heavy concentration of dense core vesicles both in axons and in terminal boutons clearly distinguishes this nuclear region from the nearby substantia gelatinosa. Both myelinated and unmyelinated degenerating fibers were observed throughout the extent of the sacral secondary visceral gray. In addition, degeneration was noted in the ipsilateral dorsal funiculus, Lissauer's tract and superficially in the dorsal part of the lateral funiculus. Synaptic terminals undergoing degenerative changes were found at all levels of this nucleus. The majority of degenerating boutons contained clear spherical vesicles and were found in association with dendrites of various sizes. These terminals were sometimes surrounded by glial elements and characteristically contained a tight clump of electron dense material consisting of synaptic vesicles and mitochondria. Boutons containing predominantly dense core vesicles did not appear to be affected by dorsal rhizotomy. These results suggest that dorsal root influence over neurons in the sacral secondary visceral gray is mediated by terminals containing clear spherical vesicles. Boutons containing dense core vesicles appear to originate from nerve cells other than those in sacral dorsal root ganglia. Possibly, these latter terminals are part of a descending bulbospinal tract system. This work was supported in part by NIH/BRSG RR5749-05.

1830 CENTRAL TERMINAL EXCITABILITY CHANGES OF SINGLE TYPE I AFFERENT FIBERS. Ronald E. Paul* and Daniel N. Tapper. Sch. of Elec. Eng.; Sect. of Physiol., N.Y. State Coll. Vet. Med.; Cornell U., Ithaca, N.Y. 14853.

Changes in activation threshold of central terminals of single Type I afferent fibers of hairy skin of cat, induced by small input conditioning stimuli, were studied in the sacral dorsal spinal gray matter. Conditioning stimuli, consisting of either single or short trains of action potentials, produced by electrical stimulation of the Type I terminal on the skin (the touch dome), were induced in either the fiber whose central terminal was to be tested or in the afferent of another adjacent Type I terminal. Central excitability of the test fiber was determined by Wall's technique of antidromic activation of the intraspinal terminals, using a tungsten microelectrode inserted in the spinal cord in proximity to the central terminal in upper lamina IV.

When a conditioning stimulus consisting of a single impulse was applied to the test fiber, a bimodal change in central excitability lasting up to 50 msec was observed. It consisted of a depolarization with a major peak at 8 msec and a second peak at 25 msec. The central delay for this effect was found to be less than 2.5 msec. When such a conditioning stimulus was applied to a Type I fiber other than the test fiber (spatial interaction), in some cases a similar change in central excitability of the test fiber was observed. In some other cases, a train of impulses (5 action potentials, 100 Hz) was required to produce the effect. The excitability change in the test fiber produced by such spatial interaction was observed to have a duration of up to 50 msec and a central delay of approximately 4-5 msec.

Work currently in progress using a GABA antagonist, bicuculline (dose range: 1-2 mg/kg) indicates that the reduction in activation threshold of the central terminals is due in large part to GABA activity. These results suggest that in addition to a possible intrinsic component to the post-conditioning threshold reduction (e.g. afterdepolarization), a presynaptic inhibitory process, such as primary afferent depolarization (PAD), is evoked by small scale input, both in the case where the conditioning impulse is applied directly to the test fiber and that where the conditioning pulse is applied to an adjacent fiber (spatial interaction).

By inserting this data into models of the transfer function between presynaptic membrane depolarization and transmitter release, it is possible to predict the time course of PSP size modulation caused by the calculated presynaptic potential changes. These results are important for understanding the role of PAD in information processing in central neural networks. (Supported by USPHS Grant 07505).

1831 THE COERULEOSPINAL PATHWAY: AN ANATOMICAL, NEUROCHEMICAL AND ELECTROPHYSIOLOGICAL STUDY. J.A. Pearson. Dept. of Physiology, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Large bilateral injections (2.0 µl) of a 30% solution of horseradish peroxidase (HRP) were placed in the lumbar enlargement of the spinal cord of Nembutal-anaesthetized rats. After a survival period of 48 hrs retrograde transport of HRP to neuronal cell bodies in the brainstem was determined using the procedure described by Mesulam. Labelled neurones were present in the ventral division of the caudal region of the locus coeruleus (LC) and the subcoeruleus area. Following hemisection of the cord, at the mid-thoracic level, HRP-positive cells were situated only in the LC contralateral to the lesion. Labelled cells were also found in the nucleus reticularis gigantocellularis, pontis caudalis and oralis, raphe magnus, pallidus and obscurus.

To confirm that the axonal transport of HRP to the LC had occurred in catecholamine containing neurones, the effect of 6-OHDA (12 µg in 4.0 µl) injected bilaterally into the caudal brainstem (5.5 mm posterior to lambda, 1.5 mm lateral, 7.5 mm from dura) was investigated. After 14 days, HRP was injected into the cord as described above. The number of labelled cells in the LC was markedly reduced (less than 10%) in comparison to the untreated controls. The extent to which cells in the adjacent reticular formation and in the raphe nuclei were labelled was unchanged.

Noradrenaline levels in the lumbar cord were determined by a radioenzymatic assay based upon the technique of Coyle and Henry. 6-OHDA treatment resulted in an almost complete depletion of noradrenaline (0.292±SE 0.010 µg/g in control animals, 0.030±SE 0.015 µg/g after 6-OHDA).

The effect of stimulation of LC upon the activity of spinal interneurones was investigated in urethane-anaesthetized rats. The type of neurone which was of particular interest responded to both non-noxious and noxious levels of cutaneous stimulation. Both types of response were inhibited by trains of stimuli applied to the LC. This effect could not be demonstrated in rats pretreated with 6-OHDA.

These results indicate that the LC gives rise to an ipsilateral, noradrenergic, inhibitory pathway to the lumbar spinal cord. The possibility exists that this system might be involved in modulation of nociception.

Supported by the Medical Research Council of Canada.

1832 ELECTRON MICROSCOPIC OBSERVATIONS ON THE SPINAL TERMINATION OF PHYSIOLOGICALLY IDENTIFIED DORSAL ROOT AXONS. Henry J. Ralston, III, Alan R. Light, and Edward R. Perl. Dept. Anat., Univ. of Calif. San Francisco, CA 94143 and Univ. N. Carolina, Chapel Hill, NC 27514.

Single primary afferent fibers in sacral and coccygeal segments were characterized functionally by electrophysiological recording with microelectrodes; subsequently they were stained by the iontophoretic, intracellular injection of horseradish peroxidase (HRP) from a solution contained within the micropipette. The HRP is transported, from the point of injection in the dorsal root, centrally for several millimeters and after fixation and reaction provided a Golgi-like picture of the central distributions. Myelinated fiber receptive units (Type I cutaneous and G-2 hair follicle) were selected for study and were identified on the basis of conduction velocity and their response to a variety of graded mechanical stimuli applied to the skin. After reaction to develop the HRP product, thick serial sections containing the stained axon were treated with osmium and imbedded in epoxy resins. The arborization of the axon was reconstructed with camera lucida. Selected axonal branches were prepared for serial thin sections for electron microscopy. The extensive branches of a single dorsal root axon from a Type I receptor made hundreds of synaptic contacts as they distributed in the dorsal horn. All synaptic contacts of this type of receptor were deep to the substantia gelatinosa (lamina II). All of the observed synaptic profiles contained round vesicles. The various synaptic profiles from a single Type I axon have differing morphologies: simple axodendritic contacts as well as complex "central" terminals which had multiple axodendritic and axoaxonal synapses. Other axoaxonal synapses were also observed; in all axoaxonal synapses the labeled primary afferent axon was post-synaptic to other profiles. G-2 hair receptor axons in cat distribute throughout the nucleus proprius (lamina III and IV) in a fashion generally similar to that for the Type I receptor. The fiber from a cat G-2 hair follicle receptor also showed myriads of synaptic contacts; to date simple axodendritic and the "central" terminal with multiple axodendritic synaptic contacts have been identified. (Supported by USPHS, NINCDS research grants NS11614, NS10321, and NS11132 and a fellowship NS05526 to A.R.L.)

1833 LYSOSOMAL AUTOLYSIS OF CONTUSED SPINAL CORDS. D. Rigamonti J. Wrathall* and C.C. Kao* Department of Anatomy, Georgetown Univ. Schools of Medicine and Dentistry, Washington, D.C.20007.

The time course change of the spinal cord after contusion can be dramatically demonstrated by the following model. Eighteen adult female dogs were used. The 7mm diameter spinal cord was compressed to 1mm for 2 seconds between the blades of a pair of hemostatic forceps at T10 cord level. Immediate findings after this mode of injury were a few petechial hemorrhages with no disruption of the spinal cord. Nevertheless, at one week after the contusion, a cavity extending throughout the entire cross-sectional area of the spinal cord was found. Apparently, this spinal cord cavitation was not due to the contusion, but was the result of "secondary destructive processes" which develope after the contusion.

We have previously reported massive mobilization and accumulation of neuronal lysosomes and release of lysosomal hydrolases near the plane of spinal cord transection which was associated with autolysis of the spinal cord tissue bordering the cut ends of the spinal cord stumps (J.Neurosurg. 46: 197, 1977).

We now report a correlated histological, histochemical and electron microscopic study in the contusion model, and identify that lysosome mediated spinal cord autolysis is one of the "secondary destructive processes" which cause spinal cord cavitation after spinal cord contusion.

Acid phosphatase (APase), used as the marker for lysosomes, was demonstrated by Gomori's and azo-dye methods and by electron microscopy. In a normal spinal cord, APase is positive in the meninges, in the blood vessel wall, and in nerve cell bodies of the grey matter, but not in the white matter tracts. At 3 to 6 hours after contusion, large quantities of lysosomes accumulate within short segments of axons in otherwise normal-appearing white matter about 2mm from the area of petechial hemorrhage proximal and distal to the injury. At 12 hours, the lysosome-filled axons form club-shaped axonal terminals (terminal clubs) which always pointed to the area of injury. At one day, the terminal clubs may reach 100µ in diameter and begin to leak APase into the surrounding tissue. Electron microscopic study revealed rupture of the terminal club with subsequent release of lysosomes into the interstitial space. The injured area subsequently became necrotic and detached from the spinal cord at the level where the lysosomes were released.

Since electron microscopy revealed the localization of the lysosomes within the axons and terminal clubs, it is unlikely that the APase originated from the circulation or from macrophages. We therefore propose that the lysosomes that accumulated in the spinal cord following spinal cord contusion originated within the nervous system. (Supported by:NIH NS14413-01)

1834 DENDRITES IN WHITE MATTER OF THE UPPER CERVICAL CORD OF THE ADULT CAT. P.K. Rose and F.J.R. Richmond.* (SPON: J.V. Milligan). Department of Physiology, Queen's University, Kingston, Ontario. Canada. K7L 3N6.

Both anatomical and electrophysiological studies have shown that descending spinal and propriospinal systems terminate on neurons at several spinal levels via short axon collaterals arising from parent axons in white matter. Yet some cells in the lumbrosacral cord have dendrites which extend into white matter raising the possibility that descending and propriospinal systems could synapse on spinal neurons without sending collaterals to the grey matter. The dendritic arrangement of cells in the upper cervical ventral horn of adult cats have therefore been examined with particular emphasis on their extensions into the surrounding white matter. Using either Golgi staining methods or intracellular injection of horseradish peroxidase (HRP), dendrites of ventral horn neurons were found to extend into all funiculi surrounding the ventral horn. Cells located close to the interface between grey and white matter often had a substantial part of their dendritic trees in the white matter. These dendrites typically had smooth surfaces even when they originated from cells whose dendrites in grey matter were covered by spines and intricate surface specializations.

Using intracellular injections of HRP we found that some white matter dendrites originated from neck muscle motoneurons. These HRP-filled dendrites, as well as unlabelled white matter dendrites, have been further examined at the electron microscopic level. In the ventrolateral and ventromedial funiculi dendrites formed horizontal bands which extended, at right angles, between the long myelinated fibre tracts running rostrally and caudally. Within these bands axodendritic synapses were found which may have been formed by the many small myelinated and unmyelinated axons running parallel to the dendrites. These results indicate that the white matter surrounding the upper cervical ventral horn must be considered as another site of synaptic integration.

Supported by M.R.C. of Canada

1835 PRESYNAPTIC DEPOLARIZATION OF CORTICO-SPINAL AND RUBRO-SPINAL TERMINAL ARBORIZATIONS PRODUCED BY CONDITIONING VOLLEYS TO SENSORY NERVES. P. Rudomín, E. Jankowska+ and J. Madrid+. Department of Physiology and Biophysics, Centro de Investigación del IPN. México 14, D.F. and Department of Physiology, University of Göteborg, Sweden.

Studies on presynaptic depolarization (PD) in the cat spinal cord have been limited to the terminal arborizations of primary afferents. However, there is indirect evidence suggesting that vestibulo-spinal (VS) fibers synapsing with motoneurons are not subjected to PD. This lack of PD could be a general feature of descending fibers in the spinal cord. On the other hand, it is known that stimulation of sensory nerves increases the extracellular K^+ concentration mainly in the dorsal horn (DH) and intermediate nucleus (IN). If (K^+) were the main factor in evoking PD, the fact that VS fibers do not end or course through the DH and IN could explain why the VS fibers are not depolarized by conditioning volleys to sensory nerves. Present experiments were made to analyze PD of other descending fibers and in particular the specificity of its mechanism. We found that the excitability of cortico-spinal (CS) fiber terminals was significantly increased by conditioning volleys to cutaneous nerves, although practically unaffected by volleys to group I muscle afferents. Changes in the excitability of rubro-spinal (RS) fiber terminals showed the same pattern. The excitability of group Ia muscle afferents of extensor muscles terminating in that same neighborhood was markedly increased by conditioning volleys to group I afferents of flexor muscles but not so much by cutaneous volleys, as previously described. Such a differential action speaks of a highly specific organization of the mechanisms leading to PD of RS and Ia axon terminals within the IN. It is more consistent with the idea of a specific transmitter being responsible for the PD rather than electrical field interactions or K^+ accumulation. The latency of the excitability increase of the CS fibers in the DH could be as short as 2.7 ms after cutaneous conditioning and lasted up to 100 ms. The PD of RS terminals in the IN appeared to have a longer latency (7 to 9 ms), suggesting mediation by a greater number of interposed interneurons. The PD of CS fiber terminals in the DH may be explained by a dual mechanism: an early depolarization resulting either from electric field interactions (or K^+ accumulation) and a delayed PD probably resulting from transmitter action.

Partly supported by NIH Grant NS 09196-07.

1836 THE SPECIAL RELATIONSHIPS OF CHICKEN EPENDYMAL CELLS IN THE SPINAL CORD AND CERTAIN BRAINSTEM AREAS. Frances M. Sansone, Dept. of Anat. Sci., Sch. Med., State University of New York at Buffalo, Buffalo, N.Y. 14214

An earlier investigation has shown that glycogen-rich cells surround ependymal cells throughout the entire chicken spinal cord and lower medulla. When the canal opens into the fourth ventricle, these cells assume a ventral midline position immediately deep to the ependymal cells and persist up to the level of the oculomotor nucleus (Sansone, J. Morph. 153:87, 1977). It was observed in that study, that the ventricular ependyma (VE) dorsal to the glycogen area retained the columnar appearance of spinal cord ependyma (SCE). Additional studies were therefore undertaken on the histology, histochemistry and ultrastructure of VE and SCE in young (8-14 day) and adult (1.5 yr.) chickens. SCE are tall columnar cells with basally placed nuclei. These nuclei form two to three layers giving the appearance of a subventricular or stratified epithelium. They are bounded by small basophilic neurons, so one is indeed struck by the similarity to the developing spinal cord. However, no mitotic figures are seen. Toluidine blue semithin sections and alkaline phosphatase studies show capillaries to be present just beneath the ependymal cells. At the ultrastructural level both the VE and SCE cells display basally located spherical or ovoid nuclei and the apical portions are filled with an elaborate Golgi apparatus, many smooth vesicles and large numbers of lysosomes. Rough endoplasmic reticulum and gliofilaments are sparse. A few polysomes are scattered about. Some of the SCE also contain rather large amounts of beta glycogen particles. Rare cilia and irregular margins characterize the luminal surfaces. Below the apical junctional complexes, their lateral borders are replete with complex invaginations very similar to the pattern seen in mammalian tanycytes (Millhouse, Brain-Endocrine Interaction II:3, 1974). A Golgi-Cox impregnation of some of the ependymal cells show them to have a radially oriented tail process which ends around blood vessels in the glycogen areas. These cells seem to fit the classification of ependymal tanycytes (Tennyson and Pappas, J. Comp. Neurol. 123: 379, 1964). Because of the unique arrangement of chicken ependymal cells, it seems plausible to postulate that they either release a substance into the cerebrospinal fluid or transport substances from the fluid to the blood.

Neural processes containing clear synaptic vesicles have been found between ependymal cells of the sacral cord region, but no definitive type synapses have been found. It is assumed that these axons arise from the contiguous small neurons. (Supported by the University of Buffalo Foundation).

1837 RESPONSE OF PRIMARY AFFERENTS TO CHANGES IN EXTRACELLULAR POTASSIUM CONCENTRATION IN THE BULLFROG SPINAL CORD. Sarah A. Shefner and Richard A. Levy. Dept. Pharmacol., Univ. Ill. Med. Ctr., Chicago, IL 60612.

Upon afferent stimulation, K^+ accumulates specifically around primary afferent terminals. This increase in extracellular K^+ concentration ($[K^+]_o$) has been proposed as a mechanism for generation of the dorsal root potential (DRP). This hypothesis assumes that increases in $[K^+]_o$ will substantially depolarize primary afferent terminals. However, measurements on other neuronal preparations show their membrane potentials to be rather insensitive to changes in $[K^+]_o$ comparable to those occurring around primary afferent terminals upon afferent stimulation. It was of interest, therefore, to measure the response of primary afferent terminals to known changes in $[K^+]_o$.

K^+-rich Ringer solutions were superfused over an isolated, hemisected portion of bullfrog spinal cord. Each K^+ concentration was applied until the resulting depolarization of primary afferents reached steady state. This depolarization was recorded with Ag-AgCl hook electrodes on a lumbar dorsal root. The same electrodes were used to record DRP's evoked by stimulation of an adjacent dorsal root.

K^+ dose-response curves showed that only at $[K^+]_o$ values greater than 25 mM did depolarizations approach those predicted by the Nernst equation. At $[K^+]_o$ values below 25 mM, depolarizations were smaller than those predicted by the Nernst equation, with changes in $[K^+]_o$ near rest levels causing only very small depolarizations. Addition of 20 mM Mg^{++} to the superfusate to block any K^+-induced transmitter release, decreased the K^+-induced depolarizations. This suggests that the direct depolarizing effect of physiologically occurring K^+ accumulation around primary afferents may be amplified by K^+-induced transmitter release from nearby neurons. Even in the Mg^{++}-free condition, where both direct and indirect effects of K^+ were present, we found the $[K^+]_o$ required to cause a depolarization equal to the DRP height was significantly larger than the amount of K^+ found (Sykova et al. Brain Res. 106:413, 1976) to accumulate around frog afferent terminals, upon a single afferent volley. However, these workers found high-frequency afferent stimulation could cause K^+ accumulations of up to 9 mM. In our preparation, a change in $[K^+]_o$ of this magnitude causes a large depolarization of the primary afferents, similar in amplitude to the DRP. Taken together, these data indicate that K^+ accumulation is not the sole mechanism for generation of the DRP, but may supplement PAD induced by GABA released at axo-axonic synapses onto the afferent terminal. The contribution of K^+-mediated PAD would be expected to be small for single afferent volleys, but much more significant upon high-frequency afferent activity.

1838 CORRELATIVE STUDIES OF INDIVIDUAL Ia-MOTONEURON PRESYNAPTIC TERMINAL SPIKES AND EPSPs. G.W. Sypert and J.B. Munson. Univ. of Fla. Coll. of Med. and VA Hospital, Gainesville, FL 32610

Electrical potentials in the triceps surae motoneuron pool generated synchronously with action potentials in single medial gastrocnemius (MG) Ia dorsal root afferents were studied in cats, using low impedence bevelled microelectrodes and signal averaging techniques. These potentials were of several types, including 1) presynaptic terminal spikes and 2) EPSPs. Presynaptic terminal spikes were usually a positive-negative sinusoid less than 20 μV peak-to-peak amplitude and about 400 μsec total duration. They were readily recorded in extracellular space and within motoneurons. EPSPs were recorded in 85% of 116 MG motoneurons and in 74% of 65 LG/S motoneurons.

Simultaneous recording of presynaptic terminal spikes and EPSPs permitted correlative studies of these two events. We have been particularly interested in correlates of EPSP latency from the presynaptic terminal spike. This latency is composed of synaptic delay plus electrotonic conduction time from synaptic site to recording site in the motoneuron soma, and thus should relate directly to electrotonic distance from generation to recording site. Also related to this electrotonic distance are dimensions of the EPSP: half width (HW), rise time (RT), amplitude and slope.

Latency was measured from the positive peak of the presynaptic terminal spike to the onset of the EPSP. Mean homonymous latency was 448 μsec (range 260-840); mean heteronymous latency was 463 μsec (range 320-640). Significant linear correlations were found between latency and RT and between latency and HW. Significant logarithmic correlations were found between latency and amplitude and between latency and slope (defined as 10-50% dv/dt). The latency-slope correlation was most powerful (homonymous $r=.65$ $p<.001$); heteronymous $r=.87$ ($p<.0001$). Thus, EPSP latency and slope should provide useful approximations of synaptic location on the some-dendritic membrane, in addition to the widely used parameters of RT, HW and amplitude. These data are also relevant to the possibility of electrical transmission at the Ia-motoneuron synapse. (Supported by VA Hospital Medical Research Service and EY 01264).

1839 PROJECTIONS OF AREA 4 TO THE SPINAL CORD IN SQUIRREL MONKEY (SAIMIRI). Johannes Tigges, Shiro Nakagawa* and Margarete Tigges. Yerkes Regional Primate Res. Ctr. and Dept. Anat., Emory Univ., Atlanta, GA 30322; and Dept. Anat., Wakayama Medical College, Wakayama-shi, Japan.

A single injection of either tritiated proline or a mixture of proline and leucine was made in area 4 of 9 squirrel monkeys. The locus of injections was systematically varied from medial to lateral among animals. The injected volume was 0.2-0.9 µl with a constant ^{3}H concentration of 100 µCi/µl. Following survival times of 2 days, the monkeys were perfused with 10% formalin. Transverse sections of the spinal cords were processed according to routine autoradiographic procedures (Cowan et al., '72). Counts of developed silver grains were made over segments of the lower cervical, lower lumbar and upper sacral cord at a magnification of 1000 X with the aid of a reticule (128 x 128 µm). The majority of silver grains was found over the contralateral side of the spinal cord; however, in sacral segments, the number of grains ipsilaterally to the injected side was also considerably above background level. On the contralateral side, the grains were concentrated over Rexed's laminae V, VI and VII; fewer grains were located over laminae IV and VIII. The number of grains over layer IX was slightly above background. No grains above background level were seen over laminae I, II and III. In cervical and lumbar segments, the ipsilateral projection was restricted to lamina VIII, whereas the termination in ipsilateral sacral segments was more widespread. There were only few direct corticospinal projections to lamina IX, which contained large α-motoneurons. This sparseness of contacts in lamina IX may be indicative of a relatively low degree of skilled manipulative capacities of hands and feet in squirrel monkey. Based on the distribution of silver grains over the white matter of the cord, the efferents of area 4 coursed mainly within the contralateral corticospinal tract; some fibers of this tract apparently recrossed at the level of their termination. The number of radioactively labelled fibers in the ipsilateral tract was small; these fibers seemed to terminate ipsilaterally. In one animal, there was evidence of an anterior tract ipsilateral to the injection. (Supported by USPHS Grant RR-00165 to the Yerkes Regional Primate Research Center.)

1840 TEMPORAL ANALYSIS OF POST TETANIC DEPRESSION OF ANTIDROMICALLY ACTIVATED RENSHAW CELLS: William G. Van Meter. Department of Veterinary Anatomy, Pharmacology & Physiology, Iowa State University, Ames, Iowa 50011

Adult mongrel cats of both sexes were anaesthetised with Dial; a laminectomy performed (S-1 to L-13); the spinal cord transected at L-1/T-13 and preparation made for extracellular recording of Renshaw cell bursts evoked by supramaximal antidromic stimulation of isolated ventral roots (L-7, primarily).

On-line computer analyses of Renshaw cell discharges (1st and Oth order PST histograms) were made. Stimulation rates varied from 10Hz to 50 Hz for durations of 30, 60 and 120 seconds. Post tetanic responses were recorded during the first 60 seconds following tetanic stimulation (recovery stimulation rate of 2Hz).

Post-tetanic potentiation was not seen during these early recovery periods. Depression of bursts was found to be directly related to the duration of tetany at a given frequency, for example at 20Hz for 30 seconds a slight depression of a few seconds was noted while 20 Hz for 60 seconds resulted in depressions of 15-30 seconds. Depression of the post tetanic discharges was also found to be directly related to the degree of inhibition of Renshaw cells during the tetanic stimulation. Recovery of depressed Renshaw cell discharges subsequent to tetany began after a delay of 20-30 seconds and responses to individual stimuli (at 2Hz) began after delays of 50 msec. or greater, again directly dependent on the duration and frequency of stimulation.

1841 STRUCTURE OF THE MICROVASCULATURE IN THE DORSAL ROOT GANGLION OF THE CAT. L.E. Wittmers, Jr.*, R.S. Pozos, and K. Stocker* (SPON: D.J. Forbes). Dept. of Physiology, UMD Med. Sch. & Dept. of Biol., College of St. Scholastica, Duluth, MN 55811.

The rate at which substances reach a given group of cells in a multicellular organism depends upon a multiplicity of factors, a specialized circulation, membrane permeability, circulation flow rates and geometric arrangement of the capillary vessels. The mammalian nervous system is highly sensitive to changes in its supply-removal system by way of the circulation. The present studies are aimed at quantitating the vascular geometry of the dorsal root ganglion (DRG) in an attempt to assist in the estimates of transport to and from this tissue. Colony raised mongrel cats were anesthetized and perfused with Microfil (Canton Biomedical Products) via aortic cannulation. The systemic arterial pressure was maintained within physiologic limits during the Microfil perfusion. The entire spinal cord and associated dorsal root ganglion were removed in block and prepared for light microscopy (formalin fixed, paraffin embedded) and three dimensional visualization (cleared in ethanol-methyl salicylate). Cells and Microfilled vessels were counted on paraffin sections employing an eyepiece grid. Three dimensional vascular casts were photographed through a dissecting microscope. Number of cells and vessels per unit field in the DRG were obtained for cervical (C), thoracic (T), lumbar (L) and sacral (S) cord levels. The data is presented in the following table.

	C (51)#	T (63)	L (300)	S (250)
C/F	73 ± 3 ##	94 ± 4	95 ± 2	51 ± 1
V/F	30 ± 1	22 ± 1	26 ± 1	29 ± 0.5
C/V	2.5 ± 0.1	4.7 ± 0.3	3.0 ± 1	1.8 ± .04

C/F = number of cell per field, V/F = number of vessels per field and C/V = cell to vessel ratio.

\# number of (n) indicates number of fields counted; entries are M ± SEM

± number indicates standard error of the mean

Idealized models for capillary-cell density and arrangement compatible with the above data will be presented along with three dimensional views of the DRG circulation.

1842 ULTRASTRUCTURAL CHANGES CAUSED BY SPINAL CORD ISCHEMIA. Shokei Yamada*, Robert L. Schultz*, (SPON: G.M. Austin) Sect. of Neuros., & Dept. of Anatomy, Loma Linda Univ., Loma Linda, CA., 92350

It is well known that ischemia of the spinal cord results in paraplegia in humans. Experimental paraplegia in animals has been produced by occlusion of the descending aorta by, Yamada, et al.* They reported changes in cytochrome a,a_3 and spinal cord potentials in response to posterior root stimulation in experimental cat spinal cord ischemia. The authors conducted electron microscopic studies on the spinal cord which had undergone ischemia produced by occlusion of the descending aorta for periods of 3, 5, 10, and 15 minutes. The spinal cord was perfused with a cacodylate-buffered mixture of glutaraldehyde, formaldehyde, and acrolein, through the descending aorta 30 minutes after the completion of the occlusion. There were minimum changes in the mitochondria and dendrites in the cord which underwent 10 minute occlusion. However, after the 15 minute occlusion there were marked changes in mitochondria, neurotubules, and endoplasmic reticulum. These changes involved the findings of abnormally shaped mitochondria and myelin figure formation from mitochondria. Neurotubules were decreased in amount and this was most obvious in the larger dendrites. The cisternae of the endoplasmic reticulum were obliterated in some areas, and the membranes close together. However, 3 and 5 minute ischemic cords showed little change in ultra structure. After 10 minute occlusion detectable changes were sometimes observed in mitochondrial structure and in the quantity of neurotubules present. It is noteworthy that no changes were seen in endothelial tight junctions at any time. These changes are confirmative of *in vivo* experiments involving marked changes in redox level of cytochrome a,a_3 and in interneuron activity of the spinal cord produced by 15 minute occlusion of the descending aorta.

* S. Yamada, et al.: Metabolic and functional changes of spinal cord in experimental ischemia. Abstract # 1635, Society for Neuroscience, 7th Annual Meeting, Anaheim, CA., Nov. 6-10, 1977.

1843 Online, real-time computer monitoring of single unit activity in the mammalian spinal cord. Robert P. Yezierski, Richard T. Gumbel* and Paul B. Brown. Dept. of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, WV 26506.

Monitoring the activity of small neurons in the mammalian central nervous system is a difficult task due to the small amplitude spikes produced by these cells. Conventional recording techniques used in combination with averagers, special electrodes and off-line data analysis have been used with some success in characterizing the functional importance of small neurons in the spinal cord. In an attempt to simplify the process of characterizing peripheral input influencing small neuronal elements in the dorsal horn an online, real-time, computer aided wave form recognition technique has been developed. This technique uses a FORTRAN program running on a PDP-12 computer to automatically select template wave forms from digitized multi-unit data. Values representing sequential analog voltages of a single template are then used by an assembler program for real time wave form matching with incoming data. The initial application of this technique has been the tracking of spike amplitudes as a function of depth along an electrode track. In this process templates representing spikes of single units are obtained at different dorsoventral levels. Templates at adjacent electrode depths which possess similar shapes and similar physiological characteristics, e.g. receptive field and afferent input, are compared to determine the level at which the amplitude reaches a maximum. The depth at which a template attains maximum amplitude is marked by the deposition of ferric ions. Amplitude profiles have been obtained in the lumbar cord for neurons in laminae I-V. Results indicate that neurons in deeper laminae can be reliably tracked for greater distances than neurons in more superficial laminae, suggesting that the amplitude profile for a given unit provides an indication of cell size.

This work was supported by USPHS grant #NS12076 awarded to P. B. Brown.

1844 EVALUATION OF DORSAL COLUMN STIMULATION IN THE TREATMENT OF CHRONIC PAIN. Ronald F. Young, Division of Neurosurgery, UCLA School of Medicine, Harbor General Hospital Campus, Torrance, CA 90509.

The proposal of the gate theory as a neurophysiological mechanism of pain perception stirred considerable enthusiasm for the clinical treatment of patients with chronic pain. Further study indicated inhibition of some cells in laminae I, IV and V of the dorsal horn, thought to be involved in nociception, by dorsal spinal cord stimulation. The idea that electrical stimulation of inhibitory portions of the nervous system might abolish pain without altering normal sensory function led to the use of dorsal spinal cord stimulation (DCS) in humans for the treatment of chronic intractable pain.

This report reviews fifty-one patients with chronic pain who underwent dorsal spinal cord stimulation between 1972-1977 and have been followed for a mean of 38 months (range 12-67 months). Chronic pain in these patients was related to lumbar disc disease, previous surgical procedures, multiple sclerosis, spinal cord injury, cancer, and peripheral vascular disease. Thorough neurological evaluation, psychological testing, transcutaneous electrical stimulation and in some cases temporary dorsal spinal cord stimulation via percutaneously implanted leads were employed in selection of these patients from a much larger group with chronic pain.

Immediately after DCS implantation 47% of patients reported that they had essentially complete pain relief but by three years this had decreased to 8%. No patient followed four years or longer reported complete pain relief. Thirty-three% of patients were able to discontinue regular narcotics usage for pain relief after DCS. Only 16% of patients were able to return to gainful employment or full physical activity after DCS. Although no major complications occurred a total of thirty-three operative procedures were required in 21 patients to correct relatively minor problems related to stimulator implants. The conclusion is proposed that dorsal spinal cord stimulation is a relatively ineffective method for treatment of chronic pain. The relationship of this low success rate to the treatment of chronic pain in general and to the theories on which it was originally based will be discussed.

SYNAPTIC TRANSMISSION

1845 EFFECT OF AMMONIUM IONS ON IPSPS IN THE HIPPOCAMPAL SLICE
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Ammonium (NH_4^+) acetate and chloride have been shown to reduce or eliminate hyperpolarizing IPSPs in several systems, including spinal motoneurons (MNs) (Lux 1972), trochlear MNs (Llinas, et al. 1974), neocortical cells (Raabe and Gumnit, 1975) and crayfish stretch receptor neurons (Meyer and Lux, 1974). However, doses of NH_4^+ effective on these neurons (i. e., 1-4mM/Kg) have little effect on hippocampal IPSPs in vivo (Eccles, et al. 1977). We have reexamined the action of NH_4^+ on hippocampal pyramidal cell IPSPs using the in vitro hippocampal slice preparation. Direct measurements demonstrated turnover times in the slice chamber are 95% complete in about 2 min and equilibration within the slice itself occurs at about 3 min. After impaling a CA1 neuron with a potassium methysulfate filled electrode, NH_4^+ was applied by perfusion at concentrations from 4-8mM. E_{IPSP} was determined by applying hyperpolarizing current pulses through the electrode. Because the presence of EPSPs can complicate measurements of E_{IPSP}, pentobarbital (10^{-4}mM) was used to prolong the IPSP and permit IPSP measurements to be made at times when EPSPs would be minimal. Pentobarbital also reduced the seizure activity occasionally seen in NH_4^+. All doses of NH_4^+ had a generalized depressant action on excitability in pentobarbital, resulting in abolition of the field potential population spike within 8 min at 8mM. Perfusion with 4mM NH_4^+ for at least 10 min had little effect on E_{IPSP}, while perfusion for 20 min caused a slight and variable reduction (ca.10-20%). 8mM NH_4^+ caused a rapid (<5min) and reversible depolarization (ca. 5 mV) and a reduction of about 50% in E_{IPSP}. A similiar depression of double shock inhibition of field potentials, which has the same time course as the IPSP recorded intracellularly, was not seen with 8mM NH_4^+ at times when NH_4^+ had reduced the original population spike amplitude by 75%. In conclusion these results indicate that doses of NH_4^+ which virtually abolish hyperpolarizing IPSPs in other systems (e.g. 5mM in stretch receptor causes a 90% block) have little effect on hippocampal IPSPs. However, considerably higher doses can reversibly reduce E_{IPSP} in this system. The failure of NH_4^+ to alter double shock inhibition of the field potentials suggests that either the conductance increase during the IPSP, rather than the hyperpolarization, is responsible for the inhibition, or that IPSPs in unimpaled cells are not hyperpolarizing.

1846 BIOCHEMICAL CHARACTERIZATION OF CYTOCHEMICALLY LOCALIZED CYCLIC NUCLEOTIDE PHOSPHODIESTERASE.
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Cyclic nucleotide phosphodiesterase (PDE) activity has been cytochemically visualized at postsynaptic sites of asymmetrical terminals within the neuropil of rat corpus striatum and cerebral cortex. Formation of an electron-dense PDE reaction product occurs following incubation of 100 µM tissue slices of aldehyde-fixed brain with cyclic 3',5'-guanosine monophosphate (cyclic GMP) or cyclic 3',5'-adenosine monophosphate (cyclic AMP) as substrates. Visualization of the cyclic GMP PDE activity requires the addition of calcium and a heat-stable protein activator factor to the incubation medium.

PDE has been shown to exist in multiple forms which have different substrate specificities and kinetic properties, and can be differentially modulated. DEAE cellulose chromatography of extracts of both cortex and caudate, prepared by homogenization, sonication, and centrifugation shows the presence of three discrete active fractions. The first active fraction is a cyclic GMP-specific phosphodiesterase, requiring calcium and a protein activator for full activity. This enzyme is selectively inhibited by the phenothiazine, trifluoperazine, but the inhibition can be overcome by increasing the amount of protein activator. The second active DEAE fraction is a cyclic GMP-stimulated cyclic AMP PDE and the third is cyclic AMP-specific. Neither the second nor the third PDE fractions are activated by calcium and protein activator or significantly inhibited by trifluoperazine.

Kinetic analysis of tissues processed for electron cytochemistry demonstrates the presence of only one PDE form, with properties similar to the first DEAE fraction of fresh cortical or striatal homogenates. The surviving PDE form is stimulated by protein activator and calcium, and is inhibited by trifluoperazine.

Localization of phosphodiesterase activity at synaptic terminals supports the contention that cyclic nucleotides are involved in neurotransmission. Biochemical demonstration that this enzyme preferentially hydrolyzes cyclic GMP suggests that the guanosine nucleotide may have an important function in this neural process.

Kroc Foundation and USPHS grants AM16367 & K4-AM70518.

1847 FACILITATION OF TRANSMITTER RELEASE AT CRUSTACEAN SYNAPSES
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We find that restricted sets of synaptic terminals made by the excitor axon on crayfish (Procambarus clarkii) opener muscles of the cheliped show very different amounts of facilitation for different stimulus rates and for different stimulus patterns at a given rate. Furthermore, the amount of facilitation at a given set of synapses varies according to the way in which "facilitation" is measured. For example, very different values for "facilitation" may be obtained if one measures

(1) the amplitude of the nth pulse with respect to the amplitude of the first pulse in a train; or
(2) the mean amount of transmitter released per impulse in a train of pulses compared to the amount released by 1Hz stimulation; or,
(3) the peak depolarization produced by a given patterned train compared to the peak produced by 1Hz stimulation or by a constant interval train at any given frequency.

Finally, a single inhibitor axon may greatly reduce transmitter release and facilitation of the excitor axon via its pre-synaptic effect on the excitor axon. However, if the inhibitor and excitor axons are stimulated together at an optimum timing for pre-synaptic inhibition and the inhibitor stimulation is suddenly stopped, the excitor axon at the next pulse in the train often releases the same amount of transmitter it would have released if only the excitor alone had previously been stimulated. This result implies that facilitation process is much more dependent upon the pattern than the amplitude of the pre-synaptic stimulation. This result is in agreement with facilitation at squid synapses (Charlton and Bittner, J. Gen. Physiol., in press).

1848 DEPOLARIZATION-RELEASE COUPLING AT A SYNAPSE LACKING REGENERATIVE SPIKES.
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An investigation was made of the interaction between the "non-spiking" stretch receptor neurons of the crab (Callinectes) and the coxal promotor motoneurons which are activated in the stretch reflex. A parallel ultrastructural study demonstrated that the sensory fiber (T fiber) establishes synaptic junctions with the motoneuron dendrites within the thoracic ganglion, the presynaptic region being a simple cylinder 40-60µ in diameter and c. 200µ in length, with large stores of synaptic vesicles at its periphery, opposite the postsynaptic complex of motoneuron dendrites. These synapses are capable of continuous transmission over many seconds without depletion or desensitization, thus allowing the study of the functional linkage between the stretch reflex and the depolarization-release coupling at the junction. Analysis of transmission characteristics with simultaneous intracellular pre- and postsynaptic recording and presynaptic current-injection showed the depolarization-release coupling to be similar to that in the squid giant synapse. The relationship between pre- and postsynaptic levels of depolarization (with or without TTX poisoning of the voltage-dependent sodium conductance) follows the same bell-shaped curve, with quantitatively similar levels to those in the squid of onset, peak-release, and suppression-potential (Llinás et al., Proc. Natl. Acad. Sci. USA 73: 2918-2922, 1976). Also, in agreement with these findings, a maintained, low-level, presynaptic depolarization was found to give a linearly rising post-response. Peripheral conduction in the sensory fiber has been thought to occur with simple decremental conduction, but a graded sodium-dependent voltage transient in the fiber to step-depolarization may serve to compensate the capacitative distortion of the signal which arrives at the synapse. A voltage-dependent calcium conductance at the synaptic terminal was revealed by the intracellular injection of tetraethylammonium which allowed the development of regenerative calcium spikes with large depolarizing current-pulses. (Supported by USPHS Research Grant NS-13742 from NINCDS and by a NATO fellowship from the Science Research Council).

1849 MODIFICATION OF SYNAPTIC TRANSMISSION AND BURSTING PATTERNS IN APLYSIA CALIFORNICA BY AIR PRESSURE TO 10 ATMOSPHERES. Howard J. Bryant and James E. Blankenship. Marine Biomedical Institute and Department of Physiology and Biophysics. University of Texas Medical Branch, Galveston, TX 77550.

The onset of the symptoms of neurological dysfunction in man can occur at hyperbaric air pressures from 2 to 6.4 atmospheres absolute (ATA). These readily observable symptoms, such as those of nitrogen narcosis, may be ultimately related to alteration in the passive or active properties of the excitable membrane.

To determine if 10 ATA of air pressure can alter behavior at the cellular level we examined the unitary excitatory post synaptic potential (EPSP) produced in cell R15 of Aplysia californica when the right pleurovisceral connective is stimulated. The cell was hyperpolarized to -100 mV by passing current through one of two intracellular microelectrodes to supress its characteristic bursting pattern. Trains of 100 stimuli at 2 Hz were presented to the right connective and EPSP amplitudes were measured in the soma. Facilitation ratios were calculated as the ratio of the amplitude of the 2nd EPSP to that of the first ($f_2 = EPSP_2/EPSP_1$) and the 100th to that of the first ($f_{100} = EPSP_{100}/EPSP_1$). These experiments were repeated at 10 ATA of air pressure, in a 2% oxygen 98% nitrogen gas mixture, and in a 1/3 Ca^{++} solution. Application of pressure had no effect on the initial facilitation f_2. However, the facilitation of the 100th EPSP, f_{100}, was increased by 17 ± 5% ($\bar{X}$ + SE). Similar results were obtained with pressure in 1/3 Ca^{++} solutions and with 2% oxygen 98% nitrogen as the pressure medium. Time to peak of all the EPSP's was unaltered by pressure.

The bursting behavior of R15 was also altered by the application of pressure. The cell was penetrated with one microelectrode and allowed to produce its characteristic bursting pattern without stimulation or artificial polarization. The number of action potentials per burst was reduced by 25 ± 4% ($\bar{X}$ ± SE) at 7.8 ATA of air. Concurrently, the duration of the burst was reduced 31 ± 4%. The interval between bursts was also reduced 29 ± 4%. These parameters returned to control values upon lowering the pressure to 1 ATA.

This work was supported by ONR contract N00014-75-C-0547 by NIH grant NS 11255 and NIH award NS 70613 to JEB.

1850 INCREASE IN CYCLIC AMP IN RAT HYPOTHALAMUS FOLLOWING THE ADMINISTRATION OF 5-HYDROXYTRYPTOPHAN. M. C. Bundman* and R. A. Browning (SPON: R. P. Lehr, Jr.). Southern Illinois University School of Medicine, Carbondale, Ill. 62901.

In recent years considerable evidence has accumulated implicating cyclic AMP in the post-junctional receptor events of several neurotransmitters including norepinephrine and dopamine. In the case of serotonin, however, the evidence is far less conclusive. Most studies have shown little or no stimulation of cyclic AMP production in response to serotonin, although a recent study demonstrated the presence of serotonin sensitive adenylate cyclase activity in some synaptosomes (Pagel et al., Life Sci. 19, 819, 1976). The present study was undertaken to further investigate the potential role of cyclic AMP in the serotonin mediated post-junctional events.

Animals were made supersensitive to serotonin in order to increase the likelihood of detecting changes in cyclic AMP levels. Trulson et al. (J. Pharmacol. Exp. Ther. 198, 28, 1976) demonstrated a marked supersensitivity of central serotonin receptors in response to low doses of 5-hydroxytryptophan (5-HTP) following destruction of serotonergic nerve terminals by 5,7-dihydroxytryptamine (5,7-DHT).

Two hours following pretreatment with the catecholamine uptake inhibitor, protriptyline (20mg/kg, i.p.), male Sprague-Dawley rats (190-200g) were treated intraventricularly with 5,7-DHT (150µg) or vehicle. Ten to twelve days later animals were given an intraperitoneal injection of either 5-HTP (100mg/kg) or saline and sacrificed by focused microwave irradiation to the head. Endogenous cyclic AMP levels were measured by a modification of the ^{125}I radioimmunoassay of Steiner et al. (J. Biol. Chem. 249, 1121, 1972).

When compared with controls, 5,7-DHT treated rats exhibited a 100% increase ($p<0.001$) in hypothalamic cyclic AMP 15 min after the administration of 5-HTP. This increase was associated with a time dependent, concomitant increase in brain serotonin. In contrast, 5-HTP failed to produce an increase in the concentration of cyclic AMP in the caudate nucleus (an area with fewer serotonergic nerve endings). Moreover, rats not treated with 5,7-DHT failed to exhibit an increase in cyclic AMP in either hypothalamus or caudate nucleus. These findings show that 5-HTP increases cyclic AMP in the hypothalamus. However, further studies are needed to establish whether this is due to a direct effect of serotonin on its receptors.

1851 EFFECTS OF HALOTHANE ON SYNAPTIC TRANSMISSION IN A MAMMALIAN SUPERIOR CERVICAL GANGLION. Daryl Christ* (SPON: T.W. Schoultz). Dept. Pharmacology, University of Arkansas for Medical Sciences, Little Rock, AR 72201.

The mechanism of action of halothane on single ganglion cells in the rabbit superior cervical ganglion, in vitro, was explored with microelectrode techniques. Halothane (0.1 - 1 mM) reduced the amplitude of subthreshold excitatory postsynaptic potentials (e.p.s.p.s) with no significant changes in the times of rise or half-decay of the e.p.s.p.s. If the e.p.s.p. was suprathreshold, halothane reduced the e.p.s.p. to subthreshold amplitudes; although the concentration required depended on the safety factor of transmission at that synapse. At the concentration range which reduced the e.p.s.p., halothane had no effect on the amplitude or time-course of the action potential which was evoked by direct stimulation through the recording electrode. Also, there were no changes in the hyperpolarizing electrotonic potential, indicating that halothane had no effect on the time-constant or effective membrane resistance of the postganglionic cell membrane. Changes in the resting membrane potential did occur, but they were small and inconsistent. Even at concentrations of halothane in the range of 1 to 5 mM, there were only small changes in the properties of the ganglion cell membrane.

Acetylcholine, which was added to the physiological solution, produced a depolarization which was concentration dependent. Halothane in concentrations which reduced the e.p.s.p., also reduced the amplitude of the depolarization by ACh, but prolonged the duration of the response. If the depolarization by ACh was large, it was followed by a hyperpolarization. Halothane also reduced the hyperpolarizing phase of the ACh action.

These results indicate that the effects of halothane on the superior cervical ganglion are due to alterations of the processes of synaptic transmission. One effect would appear to be a depression of the postsynaptic sensitivity to the neurotransmitter. (Supported by NIH Grant NS-10393.)

1852 PURIFICATION OF A SYNAPTIC VESICLE-BOUND PROTEIN MEDIATING CALCIUM DEPENDENT VESICLE NEUROTRANSMITTER RELEASE AND PROTEIN PHOSPHORYLATION. Robert J. DeLorenzo, Steven D. Freedman*and Wendy B. Yohe*. Dept. of Neurol., Yale Med. Sch ., New Haven, CT. 06510.

An isolation procedure was developed in this laboratory for obtaining highly enriched, more physiologically active synaptic vesicles that could be maintained in vitro for biochemical or physiologic studies (BBRC 80: 183, 1978). This preparation was employed to demonstrate that calcium simultaneously stimulated synaptic vesicle release of neurotransmitters and phosphorylation of specific vesicle proteins, especially proteins DPH-L and DPH-M (Neurol. 28: 367, 1978; BBRC 80: 183, 1978; Epilepsia 18: 357, 1977). The results suggested that calcium-dependent vesicle protein phosphorylation may play a role in mediating neurotransmitter release and other aspects of vesicle function. This investigation was initiated to study how different preparation conditions could affect calcium's ability to stimulate neurotransmitter release and protein phosphorylation in synaptic vesicle preparations. Washed vesicle fractions were treated under numerous conditions to attempt to dissociate calcium's ability to stimulate release and phosphorylation from the vesicles. Treating the vesicles with EDTA and homogenization and isolating them by centrifugation removed the ability of calcium to stimulate release and phosphorylation without significantly affecting the norepinephrine concentration of the treated vesicles. When the solubilized vesicle fraction was added back to the treated vesicle preparation, the ability of calcium to stimulate release and phosphorylation was restored. A highly purified activator protein was isolated from the solubilized fraction by column chromatography. This protein restored the ability of calcium to stimulate neurotransmitter release and protein phosphorylation when added to the treated vesicle preparation. The binding of this activator protein to the vesicles was dependent upon the presence of physiologic intracellular concentrations of magnesium ions in the isolation media, accounting for the variable degree of removal of this protein from vesicles prepared by conventional techniques. The calcium and activator protein-dependent stimulation of protein phosphorylation was shown to be mediated by the activation of a vesicle-bound protein kinase that had essentially identical substrate requirements for activation as those for initiating norepinephrine release. The results of this investigation are consistent with and expand the initial hypothesis from this laboratory that the calcium and vesicle-bound activator protein dependent phosphorylation of specific vesicle-associated proteins may be the underlying biochemical mechanism mediating some of the physiologic effects of calcium on neurotransmitter release and synaptic vesicle function.

1853 INHIBITORY TRANSMISSION IN RAT CORTICAL NEURONS IN CULTURE. Marc A. Dichter and Bernard Biales*. Department of Neurology, Beth Israel Hospital and Harvard Medical School, Boston, Mass. 02215.

Rat embryo cortical neurons maintained for 3 - 8 weeks in dissociated cell culture form abundant inhibitory (as well as excitatory) synaptic connections with one another. IPSPs are associated with an increased conductance to Cl and can be inverted by neuronal hyperpolarization to beyond approximately -70 to -80 mV. GABA applied by iontophoresis or mini-perfusion also causes an increased Cl conductance in all neurons tested. Threshold GABA concentration appears to be ca. 2 - 4 uM. Near saturation (exceeding the limits of measurement) occurs at ca. 50 uM. Glycine produces a similar effect at 20 - 50 times higher concentrations but with much greater variability. Some neurons are insensitive to even 1 mM, although neurons insensitive to glycine may still exhibit large IPSPs.

IPSPs are inhibited by 1 uM bicuculline or 10 uM picrotoxin, but are also markedly reduced by 10 uM strychnine. Bicuculline blocks GABA effects but not glycine. Strychnine, at a level which blocks IPSPs, blocks GABA effects on these cells.

Bathing the neurons in 10 uM GABA reversibly abolishes synaptic activity, although neurons may have high resting potentials and good action potentials. Bathing the cells in 100 - 1000 uM glycine did not abolish synaptic potentials.

Our physiological and pharmacological data, in conjunction with the autoradiographic and biochemical support for the GABA system (Snodgrass et al, this meeting) indicate that GABA is the predominant inhibitory transmitter utilized by mammalian cortical neurons in vitro.

1854 ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL CHARACTERIZATION OF THE DISYNATIC MEDIATION OF THE SLOW INHIBITORY POSTSYNAPTIC POTENTIAL IN THE SYMPATHETIC GANGLIA OF RABBITS. N. J. Dun and A. G. Karczmar, Dept. Pharmacol., Loyola Univ. Stritch Sch. Med., Maywood, Ill 60153.

Acetylcholine (ACh) was applied electrophoretically to cells of isolated rabbit superior cervical ganglia, and the response was recorded by means of intracellular recording techniques. In the presence of d-tubocurarine (5 μM), ACh applied by repetitive current pulses (10-20 Hz, 10 msec/pulse, 1-2 sec) elicited three distinct membrane potential changes: a slow depolarization, a slow hyperpolarization, or a biphasic response consisting of an initial hyperpolarization followed by a depolarization. Atropine (1 μM) blocked both the depolarizing and hyperpolarizing response to ACh. On the other hand, superfusion with a low Ca/high Mg solution, tetrodotoxin (0.1 μM), haloperidol (0.1 μM) or phenoxybenzamine (1 μM) selectively and reversibly abolished ACh-induced hyperpolarization without appreciably affecting the depolarization. The membrane resistance remained relatively constant during the course of hyperpolarization. Application of steady depolarizing and hyperpolarizing currents decreased and increased, respectively, the amplitude of hyperpolarization. These results demonstrate that hyperpolarization elicited by iontophoretic application of ACh is electrophysiologically and pharmacologically similar to the slow inhibitory postsynaptic potential (slow ipsp) induced by nerve stimulation. Furthermore, the results provide evidence that the slow ipsp is mediated by a cholinoceptive adrenergic interneuron interposed between the preganglionic fibers and principal ganglion cells; when activated muscarinically by cholinergic fibers the interneuron liberates a catecholamine, possibly dopamine, which then acts postsynaptically in the production of slow ipsp.

This study is supported in part by NIH Grant NS 6455.

1855 PURINERGIC MODULATION OF MONOSYNAPTIC RESPONSES IN RAT HIPPOCAMPAL SLICES. T. Dunwiddie, B. Hoffer* and G. Lynch. Dept. Pharm., Univ. Colorado Med. Center, Denver, CO 80262 and Dept. Psychobio., Univ. California, Irvine, CA 92717

The electrophysiological actions of purine nucleotides and nucleosides were studied utilizing the in vitro hippocampal slice preparation. Adenosine and related agonists were found to inhibit both the spontaneous and evoked unit activity of CA1 pyramidal cells. A marked reduction in the amplitude of synaptically evoked responses, evoked via stimulation of the Schaffer collateral-commissural afferents to CA1, was produced by these same drugs. The stereospecificity of these effects corresponds to that observed in biochemical studies, in that the ℓ-enantiomer of N^6-phenylisopropyl adenosine was considerably more potent than the corresponding d-form. Superfusion of methylxanthines, which block adenosine receptors, or adenosine deaminase, which catabolizes extracellular adenosine, profoundly antagonizes depressant adenosine responses. Both methylxanthines and adenosine deaminase augment the amplitude of the evoked field potentials when administered alone. Hexobendine, a putative adenosine uptake blocker, had little effect on synaptically evoked responses. In contrast to the depressant effects of adenosine superfusion, 8-parachlorophenylthio- or 8-bromo-cylic 3'5' guanosine monophosphate reliably and reversibly increased the amplitude of evoked field potentials. None of the agents listed above appeared to alter long-term potentiation induced by tetanization of the Schaffer collateral pathway at 400 Hz.

It is concluded that inhibitory "adenosine" receptors are present in the monosynaptic Shaffer-commissural system, and that endogenous release of adenosine or adenine nucleotides may serve to reduce the efficacy of synaptic transmission along this central pathway. Cyclic guanine nucleotides may mediate another type of modulation which results in increased amplitude of synaptic responses. However, the mechanisms whereby these modulatory influences are brought about remain to be determined.

This work was supported by NS 099199 and 5T01 GM01983.

1856 RESPECTIVE ROLES OF ELECTRICAL AND CHEMICAL FEEDFORWARD INHIBITIONS IN REGULATING RESPONSES OF THE GOLDFISH MAUTHNER CELL TO EXCITATORY AFFERENTS. Donald S. Faber and Henri Korn. N.Y.S. Res. Inst. on Alcoholism and Dept. of Physiology, SUNYAB, Buffalo, N.Y. and INSERM U3, CHU Pitie-Salpetriere, Paris, France.

Field effect and chemical inhibitions of the goldfish Mauthner cell (M-cell) are mediated by impulses in the same interneurons (PHP cells) of its recurrent collateral network (Science, 1976, 194: 1166). The hypothesis that these interneurons also exert a feedforward inhibition of the M-cell has been confirmed by comparing their intracellularly recorded responses to stimulations of the ipsilateral posterior eighth nerve, which has well-established electrotonic and chemically mediated excitatory inputs to the latter. For standardization, stimulus strengths were normalized with respect to that required for M-cell orthodromic activation (1.0T).

Eighth nerve stimuli evoked both electrical and chemical inhibitions of the M-cell: i) the electrical component is a short latency (0.25 msec) extrinsic hyperpolarizing potential (EHP) recorded extracellularly in the M-cell axon cap. Its threshold was at 0.2-0.25T, and it was maximal in amplitude (6-8 mV) with stimulus strengths of 1.0T or less. ii) the later chemically mediated inhibitory postsynaptic potential (IPSP) had a similarly low threshold, but its stimulus-response characteristics were not determined as it was masked by the simultaneously occurring monosynaptic EPSPs. Threshold intensity for the excitatory responses was also comparable to that of the inhibitory ones, but with greater stimulus strengths, EHP amplitude increased more sharply. Since EHP peak time (0.65 msec) is intermediate between those measured for the electrotonic and chemical excitatory components (0.35 and 1.0 msec, respectively), the field effect inhibition truncates M-cell excitation at intensities below 1.0T and may even produce a transient net membrane hyperpolarization. During the same experiments 80% of the PHP cells recorded intracellularly fired at a much lower stimulus (0.3-0.6T) than did the M-cell, and the latency of their activation corresponded to that of the EHP, a finding consistent with the postulate that their orthodromic impulses mediate at least a portion of the afferent inhibitions. Finally, simultaneous intracellular recordings from the M-cell and these presynaptic neurons provide evidence that common afferent fibers excite both: auditory stimuli subthreshold for M-cell activation frequently evoke repetitive firing in the interneurons. In conclusion, these findings suggest a functional role for the feedforward inhibitory network. The M-cell initiates a high threshold and extremely rapid startle response to auditory stimuli. The electrical and chemical afferent inhibitions would both contribute to this high threshold property, with the speed of the first component balancing that of the electrotonic excitation of the M-cell

(Supported in part by NIH Grant No. NS-12132).

1857 IONIC BASIS OF PRESYNAPTIC INHIBITION AT THE CRAYFISH CLAW OPENER. Paul A. Fuchs. Neuroscience Program, Stanford University, Stanford, California 94305.

The opener muscle of the crayfish claw is innervated by only two axons, an excitor and an inhibitor. The inhibitor axon innervates not only the opener muscle fiber, but the terminals of the excitor axon as well; thus providing both post- and presynaptic inhibition. Intracellular recording from the excitor axon on the surface of the opener muscle reveals hyperpolarizing IPSPs of 100 μV amplitude and 80 msec duration in response to action potentials in the inhibitor. The membrane potential of the axon can be varied by passing current through a suction electrode placed over the opener nerve near the intracellular electrode. Using this technique, it is possible to obtain a reversal potential for the IPSP in the excitor axon. For 20 cells the reversal potential was 5.37 ± 1.68 mV (mean ± s.d.) hyperpolarized to the 80 mV rest potential. To learn the ionic basis of the IPSP, the reversal potential was determined while bathing the claw in saline containing varied concentrations of potassium and chloride. Reducing external chloride from 240 to 10 mM caused a 10 mV depolarizing shift in the reversal potential, bringing it above rest. A 10-fold change in extracellular potassium (from 1 to 10 mM) produced a 25 mV depolarizing shift in the reversal potential. Thus the reversal potential of the presynaptic IPSP depends on external potassium as well as chloride, suggesting that GABA (the inhibitory transmitter) activates both conductances during inhibition of the excitor. In contrast, the reversal potential of the postsynaptic IPSP shows a 35 mV depolarizing shift for the same chloride concentration change, and little or no dependence on the extracellular potassium concentration. This confirms previous descriptions of the postsynaptic IPSP as primarily a chloride conductance increase. It is intriguing that the opener inhibitor produces different synaptic responses on two different cells. There is an interesting correlation between this ionic differentiation and pharmacological differences between pre- and postsynaptic inhibition at this junction. (Dudel and Hatt, Pflügers Arch. 364: 217-222, 1976.; Marder and Paupardin-Tritsch, J. Physiol in press, 1978.)

1858 MEMBRANE POTENTIAL EFFECT ON INHIBITORY POSTSYNAPTIC CURRENTS OF APLYSIA. Daniel Gardner. Dept. of Physiology, Cornell Univ. Medical College, New York, N.Y. 10021.

Gardner and Stevens have previously reported that postsynaptic currents (PSC) underlying a class of inhibitory synapses in the buccal ganglia of Aplysia californica decay as a single exponential which is invariant with membrane potential (V_m) from -150 to -40mV. We ascribed the PSC decay to voltage-independent relaxation of open postsynaptic channels. Adams et al have recently reported smooth logarithmic voltage-dependence of PSC decay from -80 to -10mV in A. juliana and dactylomela. To reconcile these data, I have re-examined PSC decay over the range -150 to 0mV in A. californica. Two-electrode clamp currents were Bessel filtered at 1 kHz and fitted to exponentials to determine PSC decay time constant τ. From -150 to -40mV, τ =19±7msec (n=57) did not vary with V_m. At more depolarized V_m, input resistance decreased dramatically. In addition, many cells showed a) faster PSC decay b) decay faster than exponential, in some cases with undershoot, and/or c) decreased peak conductance. Three sets of experiments were performed to see whether these effects represented either V_m-dependent PSC relaxation, or else an artifact of remote membrane poorly clamped at the low R_{in} of depolarized levels: 1) Current pulses were injected into the cell, causing voltage relaxations which decayed faster (including undershoot), at -15 and -25mV than at -135mV. 2) TEA was injected into the postsynaptic neuron to block delayed rectification which might be activated by a poorly-controlled remote synapse; TEA reduced the decrease in R_{in} with only slight effect on PSC τ. 3) The decay seen at hyperpolarized V_m might represent uncontrolled slow axonal charging from a fast remote synaptic current. To test this, step commands across the reversal potential were given during PSC. Currents reversed direction and relaxed consistent with the new V_m, showing that recorded current decay represents PSC relaxation, rather than charging of intervening membrane. I conclude that the PSC decay seen at hyperpolarized V_m shows the true time course of the synaptic conductance. At depolarized V_m, clamp control is poor due to decreased R_{in}, and the faster non-exponential decay seen includes superimposed non-synaptic inward current, perhaps resulting from IPSP-induced removal of Na-inactivation. Supported by NIH-NINCDS grant NS11555 and RCDA NS00003.

1859 STIMULUS-SECRETION COUPLING: ELECTROPHYSIOLOGICAL AND MICROSCOPIC STUDIES OF GIANT EXOCRINE GLAND CELLS. James M. Goldring* and Stanley B. Kater. Dept. Zool, Univ. of Iowa, Iowa City, Iowa 52242.

We have studied stimulus-secretion coupling in the salivary gland of the slug, Ariolimax californicus. Histological examination of the gland has demonstrated at least four secretory cell types based on cell size and secretory granule size and staining characteristics. Of particular interest is the finding that many of the cells are 80-120 μm in diameter and contain 6-10 μm secretory granules, thus allowing optical and electrophysiological techniques to be applied simultaneously. Cells in the intact gland have been studied with standard electrophysiologic techniques. Many cells have resting potentials of 60-80 mV and generate all or none, over shooting action potentials. Action potentials are maintained in media containing Ca ions but not Na and are abolished in media containing Na but not Ca. In addition, the spike is reversibly abolished when 10 mM Co is added to normal Ringer (both Na and Ca present). These data suggest that during the rising phase of the action potential Ca ions are the predominant current carrier.

We have also isolated single cells by enzymatic digestion of the gland. Isolated cells have been impaled with microelectrodes while being observed through an inverted microscope equipped with Nomarski optics. These cells also maintain deep resting potentials and generate overshooting action potentials. In addition, action potentials cause distinct morphological changes which may be related to the secretory process.

1860 GLUTAMATE AND SYNAPTIC DEPOLARIZATION OF CEREBELLAR PURKINJE CELLS J.T. Hackett, S.M. Hou, and S.L. Cochran. Dept. Physiology, Univ. of Virginia Medical School, Charlottesville, Va. 22901.

Purkinje cells (PC's) have two easily recognizable excitatory inputs, the parallel fibers (PF's) and the climbing fibers (CF's). A third input, presumably from stellate cells (SC's), is inhibitory. Each of these inputs evokes a postsynaptic potentials (PSP's) in PC's, which can be reversed with extrinsic polarization through the recording electrode, suggesting a chemical intermediary at all three synapses. The PC-PSP's evoked by electrical stimulation of PF's usually have a more negative reversal potential than that of PSP's elicited by CF activation. This difference in reversal potentials provides a basis for characterizing agonists of the PC subsynaptic receptors. Accordingly, we have investigated the excitatory action of glutamate (Glu) on frog PC's. The entire cerebellum and adjacent brainstem were quickly removed from frogs *(Rana pipiens)* and maintained in a bath with superfused Ringer solution. Glu added to the bath in concentrations from 0.2 to 2 mM, increased the frequency of PC action potentials within 5 sec. Intracellular recordings, with beveled glass microelectrodes (less than 0.2 μm tip outer diameter; 4 M K citrate), revealed that Glu acts by an increase in ionic conductance which reduces the membrane potential. This action was rapidly blocked by 2 mM GABA. The GABA selectively increased PC ionic conductance that blocked PF-PC transmission with little or no effect upon intracellular recorded CF-PSP's. Three types of synaptic contacts upon the PC were seen at the electronmicroscope level: 1) PF's contacted spines on the spiny branchlet units of the distal dendrites tree; 2) the CF contacted thorns emitted from the soma and proximal dendrites; and 3) presumed SC terminals contacted directly PC axon hillock, soma, and dendrites. The geometric distribution of these contacts suggests that GABA may act upon the PC dendrite where these SC contacts occur. The resulting increase in conductance would tend to shunt distal excitation, and thus restrict PF synaptic current to the molecular layer, while the CF evoked excitation near the PC soma remains relatively unaffected. The PC depolarizations elicited by Glu and PF stimulation had similar reversal potentials while CF-PSP's inverted at a lower membrane potential, though in some PC's the values apparently overlap. The negative value of the reversal potential indicates that there are both sodium and potassium ionic conductance channels opened by the activation of PC subsynaptic receptors. These results are consistent with the hypothesis that Glu and PF transmitter act on the same PC subsynaptic receptor, suggesting in fact that Glu may be the PF neurotransmitter.

Supported by RSDA 5K02 DA 00009 from NIDA and NSF grant BNS 77-155271.

1861 **EFFECTS OF CHRONIC EXPOSURE TO A 60-Hz ELECTRIC FIELD ON SYNAPTIC TRANSMISSION AND PERIPHERAL NERVE FUNCTION IN THE RAT.** Richard A. Jaffe, Richard D. Phillips* and William T. Kaune,* Biology Department, Battelle Memorial Institute, Pacific Northwest Laboratories, Richland, WA 99352.

There is considerable national concern over the possible biological effects of exposure to 60-Hz electromagnetic fields from extra-high-voltage (EHV) transmission lines. Reports in the literature on biological effects of electric fields have suggested that the nervous system can be affected by exposure to electric fields and that these effects may have detrimental health consequences for the exposed organism. The purpose of this study was to investigate the effects of chronic (30-day) exposure of rats to a 60-Hz, 100-kV/m electric field on synaptic transmission and peripheral nerve function.

A system has been built which is capable of simultaneously exposing 144 rats, housed in individual plastic (lexan) cages, to uniform vertical, 60-Hz electric fields. Extensive tests have been made, and document that the exposure systems are free of corona discharge and ozone formation and that the animals do not receive spark discharges or other shocks in the housing system during exposure to electric fields. The systems are free of significant levels of harmonic distortion and vibration. Electric field measurements showed that the electric field strength within a cage varies by about 7%, due to the effects of the lexan cage material, and that the differences among fields in different cages are less than 3%.

Superior cervical sympathetic ganglia, vagus and sciatic nerves were removed from rats anesthetized with urethan, placed in a temperature-controlled chamber (37.5±0.4°C) and continuously superfused with a modified mammalian Ringer's solution equilibrated with 95% O_2 and 5% CO_2. Several parameters and tests were used to characterize synaptic transmission and peripheral nerve function. These included amplitude, area, and configuration of the postsynaptic or whole nerve compound action potential; conduction velocity and synaptic delay; accommodation; refractory period; strength-duration curves; conditioning-test response, frequency response; post-tetanic response; and high-frequency-induced fatigue. The results of all of the neurophysiologic tests and measurements so far completed indicate that synaptic transmission and peripheral nerve function are not significantly affected by chronic (30-day) exposure to a 60-Hz, high-voltage electric field. There are trends in the data, however, that suggest increased neuronal excitability in exposed animals. Replicate studies are currently underway. (This study was supported by the U.S. Department of Energy under contract EY-76-C-06-1830)

1862 RELEASE OF ACh FROM GANGLIONIC NEURON CELL SOMAS. David A.Johnson* and Guillermo Pilar (SPON: W. Wilson). Physiology Section, Biological Sciences Group, Univ. of Connecticut, Storrs, CT 06268.

Acetylcholine (ACh) release from neuronal cell somas was examined using the chick ciliary ganglia. Cell bodies in this ganglion are known to synthesize ACh (Suszkiw et al, 1976). The complications caused by the presence of presynaptic cholinergic elements was eliminated by using denervated preparations.

Ganglia in 3-5 day old chicks were denervated preganglionically by surgical section of the oculomotor nerve at its entrance into the orbit. At various times after nerve section birds were perfused by aortic injection of glutaraldehyde and post-fixed with osmium for electron microscopic examination.

Presynaptic nerve endings underwent progressive degeneration following operation and by 3 days, no ganglion cells could be found which possessed presynaptic terminals. Therefore, birds chosen for biochemical studies had been denervated 3-4 days.

Additional animals were used to measure ACh release in response to various stimulation parameters. Both the denervated ganglion and its contralateral control were incubated in μM ^{3}H-Ch at 37 C. ^{3}H-Ch taken up from the incubation medium is synthesized into ^{3}H-ACh. ^{3}H-ACh released in response to stimulation was separated from ^{3}H-Ch in the effluent by extraction into tetraphenyl boron in acetonitrile: toluene fluor following conversion of ^{3}H-Ch to ^{3}H-PhCh by choline kinase. Labelled ACh remaining in the ganglion at the conclusion of stimulation experiments was determined by high voltage electrophoresis.

In response to a 3 min. exposure to 55 mM K^+, denervated ganglia showed significant release of ^{3}H-ACh in amounts approximately 15% of those of the contralateral control. No measurable release was observed in Ca^{++}-free solutions. Antidromic electrical stimulation of the ciliary nerve for 3 min. at 20 Hz results in increased amounts of ^{3}H-ACh in ganglionic effluents. Control experiments were conducted with acetylcholinesterase added to the collected samples. No ACh was recovered in the extracted phase.

This evidence shows that ^{3}H-Ch taken up from the incubation medium can be synthesized into ^{3}H-ACh by denervated ganglion cell bodies. This ACh can then be released in response to stimulation. That this release is Ca^{++}-denendent and can be evoked by antidromic electrical stimulation suggests that it is not attributable to release of transmitter from glial elements. Therefore, it appears that denervated ciliary ganglion neurons can release ACh from their cell somas. Supported by NIGMS 1-732-GN-07219, NS 10-388 and the Univ. of Connecticut Research Foundation.

1863 **POLYPEPTIDE NEUROTOXINS FROM SCORPION AND SEA ANEMONE ACTIVATE NEURONAL SODIUM CHANNELS BY SIMILAR MECHANISMS.** Bruce K. Krueger and Mordecai P. Blaustein. Dept. Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

Polypeptide toxins from the scorpion Leiurus quinquestriatus (LqTX) and the sea anemones Anthopleura xanthogrammica (AxTX) and Anemonia sulcata (AsTX), as well as the steroid batrachotoxin (BTX), a sodium channel activator, were examined for their effects on sodium channels in pinched-off nerve endings (synaptosomes) from rat brain. Toxin-induced increases in sodium permeability resulted in membrane depolarization which was detected with the fluorescent probe CC_5 (J. Physiol. 247, 589, 1975). None of the polypeptide toxins alone had a significant effect on synaptosome membrane potential; all of the polypeptides potentiated the effect of BTX. Catterall has reported similar results for LqTX in neuroblastoma (J. Biol. Chem. 251, 5528, 1976). BTX alone, at maximally-effective concentrations (30 uM), depolarized the synaptosomes to the same extent as did increasing $[K]_o$ from 5 mM to 150 mM. The concentration of BTX which produced half-maximal depolarization (K_{BTX}) was about 2 uM (see table, below). In the presence of maximally effective concentrations of AxTX and LqTX, K_{BTX} was found to be 80 nM and 30 nM, respectively. In the presence of a just-subthreshold concentration (0.2 uM) of BTX, half-maximal depolarization by polypeptide toxins (K_{TX}) was observed at 40 nM AxTX and 4 nM LqTX. Three A. sulcata toxins ($AsTX_I$, MW 4700; $AsTX_{II}$, MW 4800; $AsTX_{III}$, MW 2700) each depolarized synaptosomes in the presence of 0.2 uM BTX. Preliminary results indicate that LqTX also potentiates the depolarization of synaptosomes by the alkaloid, veratridine. Depolarization by BTX, both in the presence and absence of the polypeptide toxins, was completely abolished by the sodium channel blocker, tetrodotoxin (10 uM), indicating that both BTX and polypeptide toxins interact with sodium channels, possibly at different sites. Recent reports indicate that these polypeptides may have different amino acid compositions and in some cases very different primary structures. This raises the possibility that widely different organisms have different polypeptide toxins all of which bind with high affinity to sodium channels in nerve and muscle, and which act by a common mechanism.

Polypeptide Toxin (TX)	K_{TX} (nM)	K_{BTX} (nM)
None	—	2000
AxTX (MW 5500)	40	80
LqTX (MW 6700)	4	30

[Supported by USPHS grant NS-08442 and fellowship NS-05112]

1864 LOCAL SYNAPTIC CIRCUITS IN RAT HIPPOCAMPAL SLICES: INTERACTIONS BETWEEN PYRAMIDAL CELLS. B.A. MacVicar and F.E. Dudek Department of Zoology and Erindale College, University of Toronto.

Electrophysiological studies of the mammalian brain have begun to emphasize the importance of local synaptic circuits. Direct analysis of neuronal interactions in these systems requires intracellular techniques for stimulating one cell and recording from another. Synaptic physiology of intrinsic hippocampal pathways was studied in vitro with simultaneous intracellular recordings. Concurrent neuronal impalements with KCl and K citrate microelectrodes were obtained from pairs of CA3 pyramidal cells separated by <200 μm. Extracellular stimulation of the mossy fibres (MF) evoked an excitatory postsynaptic potential (EPSP) and/or spikes; these responses were typically followed by a recurrent inhibitory postsynaptic potential (IPSP). Spontaneous depolarizing and hyperpolarizing potentials were not present in some cells; in other cells they ranged from asynchronous to highly synchronous. The amplitude of these spontaneous depolarizations and the MF EPSPs could both be increased with weak hyperpolarizing current injection. Spontaneous hyperpolarizations and recurrent IPSPs both reversed with Cl^- leak from the microelectrode. These spontaneous responses had the properties of synaptic potentials. Since reciprocal current injection experiments showed that most of these cells were not electrotonically coupled to each other, the close synchrony often observed for the spontaneous EPSPs and IPSPs indicates that some pyramidal cells have very similar synaptic input.

In a few pairs of pyramidal cells, spikes from depolarizing current injection in one cell caused EPSPs and spikes in the other cell. The EPSPs had a latency and time course consistent with chemical synaptic transmission and increased amplitude with hyperpolarizing current injection. These results directly demonstrate excitatory synaptic interactions between CA3 pyramidal cells. More often then excitatory interactions, spikes evoked in one pyramidal cell via intracellular current injection caused inhibition in nearby pyramidal cells. Therefore, action potentials in a single pyramidal cell can activate the recurrent inhibitory system, which synapses on many other pyramidal cells. Future analysis of pyramidal cell interactions may allow a precise description of their involvement in potentiation and seizure in the hippocampus.

Supported by grants from the Atkinson, Banting and Connaught Foundations and the National Research Council of Canada.

1865 SYNAPTIC DISSECTION AND THE EFFECTS OF LITHIUM ON SYNAPTIC TRANSMISSION IN THE CENTRAL NERVOUS SYSTEM. Lawrence E. Mallach. Dept. Anat. and Biomath., Sch. Med., UCLA, Los Angeles, CA 90024

In the last twenty-five years, studies of biochemical and pharmacological correlates of affective disorders have yielded and impressive body of data. However, not only are the etiology and pathogenesis of affective disorders unknown, but the mechanisms by which pharmacological agents are able to ameliorate the symptoms remain obscure. Lithium salts are successfully being employed in the treatment of bipolar disease, and the ability of lithium to substitute for sodium is well known. Several hypotheses for the mechanism of action of lithium have been advanced, and include altered resting potential, competition with other important ions (eg. calcium), and involvement with transmitter metabolism (serotonin and noradrenaline). Intra and extra cellular recording is in progress in the invitro hippocampal slice preparation and provides evidence for the relative importance of the several components mentioned above, as well as information regarding the site of activity. Theoretical modeling which treats the synapse formally as a series of coupled chemical reactions has demonstrated the feasibility of partially decoupling the synapse by inducing oscillatory behavior which is characteristic of reaction schemes with simple nonlinearities such as the sigmoidal dependence of neurotransmitter release on calcium influx. This technique, which is currently being developed, may provide information on the reaction rates as well as characterizing the nonlinearities generating the oscillatory behavior. It is also suggested that in combination with pharmacological agents whose site and mode of action is known, may provide an effective technique in dissecting the synapse.

1866 MODULATION OF THE ACTION OF L-GLUTAMATE ON APLYSIA NEURONS. M. J. McCreery* and D. O. Carpenter (SPON: D. E. Evans). Neurobiol. Dept., Armed Forces Radiobiol. Res. Inst., Bethesda MD 20014.

At crustacean neuromuscular junction, the excitatory response of the muscle to glutamate is markedly potentiated by aspartate which has only a small effect when applied alone. We have found a similar modulation of glutamate responses by aspartate on Aplysia neurons. However, these glutamate responses are due to either Cl- or K+ conductance increases rather than a Na+ conductance increase as in the crustacean muscle. Most of our recordings were made from unidentified neurons in the buccal ganglion and some from the abdominal ganglion. Cells were penetrated with two independent microelectrodes for recording and current passing, respectively. Drugs were passed ionophoretically from 3- or 5-barrelled extracellular electrodes or from two single-barrelled electrodes whose tips were placed together microscopically. Most receptors were located in the neuropile.

On different neurons, glutamate may cause no response or specific conductance increases to Na+, Cl-, or K+, and on some cells there are biphasic responses to Cl- and K+. The Na+ responses are rare, and we have not investigated those in detail. For most Cl- and K+ responses, aspartate is very much less effective than glutamate and must be applied at 2-50 times the concentration of glutamate for an equal response. However, when a control ionophoretic pulse of glutamate is preceded by an ionophoretic pulse of aspartate, the glutamate response may be potentiated by as much as fivefold. As the number of preconditioning aspartate pulses is increased, the glutamate responses are first facilitated and then depressed. This suggests that aspartate can interact with and desensitize the glutamate receptor but that the modulatory action is through a different action. The potentiation is similar for Cl- and K+ responses and is also not affected by the membrane potential at which the cell is tested. The potentiation is abolished in Na+ free seawater even though both the Cl- and K+ responses may actually increase in size under these circumstances. In addition to causing an increase in amplitude, Na+ free seawater results in an increase in the time-to-peak of the glutamate response and a depression rather than facilitation when aspartate is applied with glutamate. Although in some experiments cooling depresses the modulation, in most the modulation is still present at temperatures as low as 5^{o}C. Cysteate, a sulfonic acid analogue of aspartate, also causes modulation of the glutamate response and is about equally effective as aspartate. It is of interest that homocysteate, the sulfonic acid analogue of glutamate, blocks the glutamate responses and inhibits the facilitation by aspartate.

Others have ascribed the modulation of the glutamate response by aspartate to a conformational change of the receptor, an inhibition of the glutamate uptake system, or an induced alteration of the rate of onset and recovery of receptor desensitization. Our results are most consistent with an inhibition of Na+ dependent glutamate uptake, although we cannot explain the ineffectiveness of temperature in blocking the modulation by this mechanism.

1867 ACTIONS OF GABA, GLYCINE AND BACLOFEN ON AFFERENT FIBRES AND THEIR CENTRAL SYNAPSES. M. E. Morris, G. Di Costanzo*, S. Fox*, J. F. MacDonald, and R. Werman. Department of Research in Anaesthesia, McGill University, Montreal, Quebec, Canada.

The effects of γ-aminobutyric acid (GABA), glycine, and β-(4-chlorophenyl)-γ-aminobutyric acid (Baclofen) were observed on (i) the excitability of primary afferent fibres, and (ii) the transmission through cuneate synapses of the decerebrate cat. 1 Hz stimulation of a forelimb nerve or micro-electrode excitation of presynaptic terminals evoked antidromic and orthodromic potentials which were respectively recorded for estimate of synaptic input and output from the nerve and medial lemniscus. Transmission efficiency was assessed from input-output curves, constructed from integrals of the simultaneously evoked responses to regularly repeating cycles of varying intensities of either central or peripheral stimulation (Krnjevic & Morris 1976, J. Physiol. 257: 791; Morris 1978, J. Physiol. *IN PRESS*). Glycine (0.1-0.2 g/kg I-V) decreased synaptic transmission and did not alter the resting excitability of the afferent fibres. Baclofen (0.1-2.0 mg/kg I-V) produced a more marked depression of transmission, and a small but distinct decrease in both presynaptic and peripheral nerve excitability. GABA administration (0.1-0.2 g/kg I-V or 10^{-2}M superfusion of the medulla) caused large, reversible increases in afferent terminal and peripheral fibre excitability; synaptic efficiency either fell or was enhanced. During the GABA-evoked changes corresponding increases in both the extracellular K^{+} level (to <4mM) and the negative tissue potential were measured in the cuneate with ion-selective microelectrodes. GABA's direct action on peripheral fibres was confirmed in experiments with the isolated, desheathed frog sciatic nerve, by observing changes in compound action potentials, which were evoked by 1 Hz stimulus intensities which were submaximal for A fibres. Graded, reversible increases in excitability occurred during applications of 10^{-7}-10^{-2}M GABA to the site of stimulation; they could be blocked by picrotoxin. The depression of cuneate transmission which glycine causes appears to be entirely post-synaptic. A component of Baclofen's mainly presynaptic action (Fox *et al.* 1978, Neuroscience *IN PRESS*) may be a decrease in afferent activity reaching the CNS. In contrast are GABA's more complex excitatory and inhibitory effects - the depolarization of afferent fibres, and both depression and facilitation of synaptic efficacy - which suggest extra-terminal receptor distributions, and may be due in part to extracellular K^{+} accumulation, arising either from a direct membrane action and/or glial uptake of GABA.

(Supported by The Medical Research Council of Canada).

1868 THE "CALCIUM BLOCKERS", VERAPAMIL AND D-600, BLOCK BOTH SODIUM AND CALCIUM CHANNELS IN VERTEBRATE NEURONS. Daniel A. Nachshen* and Mordecai P. Blaustein, Dept. Physiol. and Biophys., Washington U. Med. Sch., St. Louis, MO 63110.

The effects of the putative calcium channel blockers, verapamil (VPL) and D-600, were tested on pinched off presynaptic nerve terminals from rat brain and on the frog sartorius neuromuscular junction (n.m.j). ^{45}Ca uptake (J. Physiol. 247: 617, 1975) was measured in synaptosomes which were depolarized either with veratridine, an alkaloid that opens sodium channels, or with high external potassium concentrations. The extent of synaptosome depolarization was determined indirectly with the voltage-sensitive fluorescent dye, di-pentyl oxacarbocyanine (J. Physiol. 247: 589, 1975). VPL or D-600 (40-60 μM) inhibited the potassium-induced ^{45}Ca uptake by about 40%, but had no effect on the potassium-induced depolarization of the synaptosomes; this inhibition of Ca uptake by VPL and D-600 is, presumably, due to blockage of Ca channels. Veratridine-induced ^{45}Ca influx was inhibited by about 70% by 40-60 μM VPL or D-600, and veratridine-induced depolarization of the synaptosomes was reduced by about 50%; the latter observation indicates that Na channels are also blocked by VPL and D-600.

Microelectrode recordings of end-plate potentials (epp's) and minature end-plate potentials (mepp's) were used to evaluate the actions of VPL and D-600 at the frog n.m.j. Low concentrations of VPL (40-50 μM) or D-600 (10 μM) had no effect on mepp amplitude, and either slightly decreased (<25% change) or had no effect on the epp amplitude. These concentrations of drug did, however, increase the threshold for nerve stimulation. These findings suggest that the VPL and D-600 can block sodium channels in the nerve. In preparations where mepp frequency had been made sensitive to changes in the bathing calcium concentration by raising the external potassium, VPL (40-50 μM) and D-600 (10 μM) did not depress mepp frequency. Although VPL and D-600 appear to be highly specific blockers of inward calcium currents in mammalian myocardum (Kohlhardt et al., Pflugers Arch. 335: 309, 1972) our data indicate that these drugs block both sodium and calcium channels in vertebrate neurons. Moreover, calcium channels in vertebrate neurons appear to be much less sensitive to VPL and D-600, than are calcium channels in mammalian myocardial cells. [Supported by USPHS grant NS-08442.]

1869 AMINE MODULATION OF RATE OF DECAY OF POST-TETANIC POTENTIATION IS MEDIATED BY A CYCLIC NUCLEOTIDE. S.A. Newlin, W.T. Schiapfer, and S.H. Barondes. Div. of Physiology and Pharmacology, Dept. of Medicine, and Dept. of Psychiatry, UCSD, La Jolla, CA 92093; and Psychiatry Research, VAH, San Diego, CA 92161.

We have previously presented evidence for serotonergic and dopaminergic heterosynaptic modulation of the rate of decay of post-tetanic potentiation (PTP) at an identified cholinergic synapse (RC1-R15) in the abdominal ganglion of Aplysia californica (i.e. amines speed the rate at which the potentiated epsp falls to its resting value following repetitive stimulation) (Newlin et al., Soc. Neurosci. Abstracts 3, #1654, 1977). Serotonin antagonists SQ10,631 (10^{-4}M) antagonizes the effects of perfused serotonin (10^{-7}M) but not the effects of perfused dopamine; and, conversely, dopaminergic antagonist d-Butaclamol (10^{-5}M) antagonizes the effect on PTP decay rate of perfused dopamine (5x 10^{-7}M) without antagonizing the serotonin effect. Thus, we can conclude that the serotonergic and dopaminergic modulation of PTP decay occur through parallel channels, and not in tandem. Stimulation of neural pathways on the right parieto-visceral connective and the branchial nerve mimic the effects of perfused amines.

Amine effects on PTP decay rate are potentiated by phosphodiesterase inhibitors, isobutylmethylxanthine (5x10^{-4}M) and RO 20-1724 (10^{-4}M). Perfused 8-benzylthioadenosine 3',5'-cyclic phosphate, a cyclic AMP analogue shown to be effective in Aplysia preparations (Treistman and Levitan, Nature 261: 62, 1976), mimics the effect on PTP decay of perfused amines or neuronal activation. (A large, i.e. twofold, increase in post synaptic (R15) input resistance can also be observed in the presence of the cAMP analogue. This effect is distinguishable from the presynaptic effect on PTP decay rate). Effects of the amines as well as the cAMP analogue are completely reversible within one-half hour of washing.

These data are consistent with the hypothesis that presynaptic amine modulation of the decay rate of PTP resembles other effects of biogenic amines in that a cyclic nucleotide appears to be involved. It is notable that two different amines may use the same final common mechanism. What is novel is that the object of modulation is the duration of a long-lasting synaptic plasticity, PTP.

Supported by the VAH, San Diego, and a grant from the NIAAA.

1870 COLICIN-LIKE EFFECTS OF β-BUNGAROTOXIN. Ronald H. Ng, Keith Terasaki* and Bruce D. Howard. Dept. of Biological Chemistry, UCLA School of Medicine, Los Angeles, Calif. 90024.

β-Bungarotoxin (M.W. 22,000) is a presynaptically acting, neurotoxic, protein from snake vemon. It causes neuromuscular blockade and exhibits phospholipase A activity. Colicins K and E_1 are protein toxins made by Escherichia coli. These colicins are known to interfere with several bacterial transport processes, decrease membrane potential and lower ATP levels (1,2). It is believed that the primary effect of the colicins is a de-energization (in the form of depolarization) of the bacterial membrane. Secondarily, ATP is utilized in an attempt by the cell to increase its membrane potential. Under conditions in which the membrane Ca^{2+}/Mg^{2+}-ATPase is made inactive, the membranes of the colicin-treated cells become deenergized without an accompanying decrease in ATP levels (1,3). We have obtained extensive evidence that β-bungarotoxin acts on synaptosomes in a manner similar to that of colicins K and E_1 on bacterial cells. β-Bungarotoxin interferes with synaptosomal transport of several neurotransmitters and nontransmitter compounds. It also decreases the membrane potential and the ATP level of synaptosomes. Our studies indicate that the β-bungarotoxin-induced interference with synaptosomal transport processes and reduction in ATP levels are secondary to membrane depolarization by the toxin. We suggest that the toxin inhibits transmitter release by a combination of depolarization of axonal terminals and a depletion of ATP stores in the terminals. (Supported by NIH Grant NS 12873 and NIH postdoctoral fellowship NS 05706.)

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3. J. Biol. Chem. 249 (1974) 6138-6143.

1871 ELECTROPHYSIOLOGICAL STUDIES IN THE ISOLATED TURTLE BRAIN. M.C. Nowycky*, U. Waldow* and G.M. Shepherd. Dept. Physiol., Yale Univ. Sch. Med., New Haven, Ct. 06510

Isolated preparations of several regions of the vertebrate nervous system have been introduced in recent years to aid in electrophysiological analysis of neuronal properties under more controlled conditions than are found in vivo. We report here preliminary results from a study of the isolated brain of the turtle, Pseudemys scripta. The animals were decapitated, and the entire brain, from medulla to olfactory nerves, was carefully removed, placed in a chamber, and superfused with oxygenated Ringer at room temperature. The parts of the brain of interest were dissected from the rest. Our initial focus has been on the olfactory bulb. The olfactory nerves were stimulated with single or paired shocks, and the responses in the olfactory bulb were recorded with a penetrating micropipette. The field potential responses to orthodromic volleys consist of three successive periods of activity, related to the impulse volley in the olfactory nerves, synaptic responses of the mitral cells, and synaptic responses of the granule cells. The laminar localization of the field potentials was in accord with previous in vivo studies. In paired volley experiments, the olfactory nerve potentials were found to undergo periods of refractoriness and supernormality similar to those previously reported in the in vitro olfactory nerve of tortoise (Bliss, T.V.P. and M.E. Rosenberg, J. Physiol. 239:60-61P, 1974), and the in vivo rabbit olfactory bulb (Getchell, T.V. and G.M. Shepherd, J. Physiol. 251: 523-548, 1975). The synaptically-evoked potentials typically undergo long-lasting suppression, for periods of up to several seconds. Extracellular single unit recordings also reveal long-lasting suppression of test responses. These results are similar to those obtained in the in vivo rabbit bulb (Getchell and Shepherd, 1975), where it has been postulated that the suppression is due to dendrodendritic synaptic interactions between mitral cells and interneurons. The present results suggest that these interneuronal synaptic systems are powerfully active in the isolated preparation. This is an important criterion for the viability of the preparation, and its validity for the analysis of local circuit properties. Intracellular responses have also been obtained, showing impulse generation in presumed mitral cells in response to synaptic input to their dendritic tufts in the olfactory glomeruli. Other parts of the brain appear to retain their viability, as evidenced by the recording of evoked potentials and unitary spikes in regions of the telencephalon in response to volleys in related fiber tracts.

1872 EXTREME SENSITIVITY OF OLFACTORY CORTICAL NEURONS TO KAINIC ACID TOXICITY. J.W. Olney, T. de Gubareff* and T. Fuller.* Wash. Univ. Sch. Med., St. Louis, MO 63110.

Several years ago we reported the neuron-necrotizing but axon-sparing action of kainic acid (KA) when microinjected directly into the rat diencephalon. Many neurons in the injected area were sensitive to the toxic action of KA but some, e.g., magnocellular neurons of the paraventricular hypothalamic nucleus (PVH) were quite resistant. We have now explored the neurotoxicity of KA following various routes of administration - intradiencephalic, intrastriatal, intraventricular and systemic - and are impressed that, although the pattern of brain damage following each route is different, there are definite repeating features, a potentially important one being that the olfactory cortex (OC) is destroyed by any of the above modes of KA administration.

To explore the apparent differential sensitivity of PVH and OC neurons to KA toxicity, we injected KA (3-4 nmoles in 0.5-1 µl) unilaterally into the diencephalon about 1 mm from PVH but much farther (> 5 mm) from OC. Histopathological analysis of the brains at various post-injection intervals revealed complete sparing of magnocellular PVH neurons but widespread destruction of OC neurons bilaterally. In other experiments, 65% of 52 brains examined after intrastriatal injection of KA (1.5-10 nmoles) revealed extensive OC damage. As will be described elsewhere (Schwb etal. NS. Abs.1978),OC neurons are among the most sensitive in the brain to degeneration following systemic administration of KA (10-12 mg/kg i.p.) and small doses of KA (< 1 nmole) administered intraventricularly sometimes cause OC lesions. The observation that KA induces degeneration of OC neurons following various modes of administration, including systemic or direct injection into brain regions quite distant from OC, implies not only extreme sensitivity of OC neurons to KA toxicity, but a remarkable tendency of KA to penetrate barrier interfaces and travel long distances within brain tissue. This underscores the need for histological controls in KA lesioning experiments and for caution in interpreting such studies.

The extreme sensitivity of OC neurons to KA toxicity is of particular interest because these neurons are thought to be glutamergically innervated, there being evidence that olfactory bulb axons which synapse upon OC neurons use Glu as excitatory transmitter. The relative simplicity of the olfactory system and ready accessibility of its components to experimental manipulation coupled with its possible use of Glu as excitatory transmitter and its extreme sensitivity to KA toxicity makes this system a promising target for future KA studies. Supported by USPH grants NS-09156, DA-00259, ES-07066 and RSD Award MH-38894 (J.W.O).

1873 LEPTINOTARSIN: QUANTIZED RELEASE OF ACETYLCHOLINE AT THE RAT NEUROMUSCULAR JUNCTION. L. S. Satin*, A. Siger*, B. C. Abbott, T. H. Hsiao* and W. O. McClure. (SPON: G. P. Moore). Section of Cellular Biology, University of Southern California, Los Angeles, CA 90007, and the Department of Biology, Utah State University, Logan, UT 84322.

Hsiao and Fraenkel have described a toxic protein, leptinotarsin (LPT), which occurs in the hemolymph of Leptinotarsa haldemani (Hsiao, In: Toxins: Animal, Plant and Microbial, ed., P. Rosenberg, Pergamon Press, 1978). We have improved the original fly lethality assay for the toxin, and have studied a number of the properties of LPT using a phrenic nerve-diaphragm preparation from the rat. Intracellular recordings with conventional 3 megohm microelectrodes filled with KCl show that treatment with partially purified LPT (G-200 fraction; Hsiao and Fraenkel, Toxicon 7, 119, 1969) produces a massive outpouring of miniature end plate potentials (mepps) reminiscent of the action of black widow venom gland homogenates (BWGH). Unlike BWGH, LPT induces a biphasic release: about 10% of the total store of releasable mepps are seen in an initial "pulse" of activity which lasts only about a minute, and is followed by a second release which lasts 10-15 minutes before the frequency of mepps falls to zero. The maximum frequency of mepps is also different in the two phases, reaching $>$800 Hz in the first phase, but only 300-400 Hz in the longer, second phase.

Several experiments have been carried out further to study this phenomenon. Impaling either the usual upper layer of cells or the underlying layer yields the same results, indicating that the biphasic release does not come from these two different cell layers. Removing Ca++ from the bathing solution abolishes the first phase, but has very little effect upon the second phase. Heating the toxin (60°, 5 min) completely destroys both its fly killing activity and its ability to stimulate release from the neuromuscular junction.

The data indicate that purification of the toxin, and further study of the homogeneous protein, is worthwhile. Despite the fact that only partially purified material was used in these experiments, the results suggest the possibility that either (a) two different pools of presynaptic ACh can be released in a quantized manner, or (b) release from a single pool can be stimulated by two different mechanisms, both of which yield quantized release. Studies with the purified toxin should be of value in our understanding of the synaptic mechanisms which control release of ACh. Supported in part by the National Science Foundation (BNS 76-80657) and the Nelson Research and Development Co.

1874 DISTRIBUTION AND PHYSIOLOGICAL PROPERTIES OF IVN THROUGH-FIBER SYNAPSES IN THE STOMATOGASTRIC GANGLION. Karen A. Sigvardt and Brian Mulloney. Dept. of Zoology, University of California at Davis, Davis, CA 95616.

The gastric and pyloric motor patterns generated by the neurons of the stomatogastric ganglion of the spiny lobster are disrupted in a characteristic way by a burst in the IVN through-fibers. The change in the motor pattern results from the particular distribution of synapses of the IVN fibers onto neurons of the ganglion and the characteristics of each of these synapses. Two types of synapses have been characterized: excitatory and biphasic. The PSP in VD is excitatory and results in 1:1 spikes in VD at frequencies of IVN stimulation up to 50 Hz. The PSP in PD is biphasic, consisting of a fast excitatory component and a slower inhibitory component. The excitatory component predominates at low frequencies but is less effective at frequencies above 25 Hz. At higher frequencies the slower inhibitory component dominates. The IPSP involves a mixed Cl^- and K^+ conductance increase. Its reversal potential is -74 mV and is changed in the depolarizing direction by increasing external K^+ concentration or internal Cl^- concentration. Inhibitory synapses have been observed but at this writing have not been characterized. A description of the properties of each of the IVN synapses will provide a basis for understanding the changes in the motor pattern which occur during an IVN burst.

Supported by U.S. P.H.S. Grant NS 12295 and NIH Postdoctoral Fellowship to K.A.S.

1875 CHEMICAL STUDIES OF GABA NEURONS IN TISSUE CULTURE. S.R. Snodgrass, Marc A. Dichter, W.F. White and Bernard Biales*. Dept. Neurology & Neuroscience, Children's Hospital and Harvard Medical School, Boston, Mass. 02115.

Biochemical characteristics of GABA neuronal function were studied in low density dissociated rat cortical cell cultures (Dichter, Brain Res., 1978). Physiological and pharmacological data suggest that GABA neurons are present and that GABA is the major inhibitory transmitter, as is thought to be true in vivo. GABA and GAD activity were detected in all cultures and increased in parallel as cultures matured and synapses formed. In contrast, ChAc, though present, does not always increase with time. The cultures also contain a sodium dependent, high affinity GABA uptake system which can be inhibited by DABA. Parallel autoradiographic studies show a dense uptake of GABA over the somata and processes of many but not all neurons. Exogenous GABA or GABA made from 14C-glucose can be released from the cultures in a calcium dependent manner. Specific binding of 3H-bicuculline has been found and increases as the cultures mature. Additional receptor studies, including autoradiography, are being done with the GABA agonist muscimol.

The cultures contain glycine in concentrations similar to that found for GABA, but the uptake of glycine is less than that of GABA and we have been unable to demonstrate autoradiographic labelling of neurons with glycine. We do not believe that glycine is functioning as a major transmitter. Tyrosine hydroxylase activity is not detectable in the cultures, in keeping with the presumed absence of monoamine cell bodies from cerebral cortex.

Our biochemical demonstration of GABA synthesis, release and uptake together with the physiological and pharmacological evidence that GABA mimics the inhibitory transmitter (Dichter and Biales, this meeting) indicates that GABA is most likely the inhibitory transmitter utilized by mammalian cortical neurons in vitro.

1876 VESICLE CYCLING AND STORAGE OF ACh IN TORPEDO ELECTROPLAQUE SYNAPSES. J. B. Suszkiw*, (spon. T. L. Schwartz) Physiology Section, Biological Sciences Group, Univ. of Conn., Storrs, CT 06268.

Recycling of synaptic vesicles in the electromotor nerve terminals of Torpedo was studied on the ultrastructural and biochemical level. When isolated electric organs were stimulated electrically (1 Hz, 1200-1500 total pulses) in the absence of choline (Ch^+), the number of vesicles in the terminals, total tissue acetylcholine (ACh), and vesicular ACh all were reduced to 30-40% of control. During subsequent recovery in Ringer solution containing 0.1 mM Ch^+, the number of vesicles in the terminals returned to 80% of control after 3 hours and to 97% of control after 5 hours. Over the same time period, the ultrastructural morphology of the terminals returned to normal. The recovery of the vesicular content of ACh was slower than was the return to normal of vesicle numbers, suggesting that: 1) Reformation of vesicles is not contingent upon the supply of ACh. 2) Reformation of morphologically identifiable synaptic vesicles may precede the recovery of the capability for ACh storage by reformed vesicles.

When an extracellular tracer, dextran, was included in the incubation medium during the reformation of vesicles, no entrapment of the dextran in the reformed vesicles could be demonstrated. This and other morphological evidence suggest that after intensified vesicle usage, new vesicles may originate from cytoplasmic membraneous precursor structures rather than by direct retrieval from plasmalemma. The present results, when compared with results obtained by Zimmerman and Denston (Neuroscience 2, 695, 1977) during low frequency (0.1 Hz) stimulation, when vesicle numbers are conserved, and incorporation of dextran into vesicles can be demonstrated, suggest that vesicle cycling during states of low and high vesicle usage may not follow the same pathway. Part of this work was performed in Max Planck Institut, Goettingen, W. Germany. Supported by Max Planck Fellowship and in part by NIH grant NS10388.

1877 EFFECTS OF SEROTONIN (5-HT) ON CALCIUM CURRENT AND CALCIUM DEPENDENT SYNAPTIC PROCESSES IN PRESENCE OF REMOVAL OF FREE INTRACELLULAR CALCIUM WITH VARIOUS SPEED. Clara Torda. N.Y.Center PA. Training, N.Y.,N.Y.,10028.

Iontophoretically administered 5-HT increases spike generation in lateral (LG) and medial (MG) geniculate neurons having low frequency spontaneous activity and inhibit LG and MG neurons with high frequency spontaneous activity (Torda, Gen.Pharmacol.,1978) and cortical neurons (Szabadi, Bradshaw & Bevan, Brain Res., 1977 126:580). In low concentrations 5-HT increased postsynaptic activity and in high concentrations 5-HT decreased postsynaptic activity. Woolley (The Biochemical Bases of Psychosis, or the Serotonin Hypothesis of Mental Disease, Wiley, N.Y., 1961) observed that 5-HT significantly increased the inward flux of Ca^{++}. Since administration of Mn or Lanthanium may prevent 5-HT potentiation of pre- and postsynaptic excitatory or inhibitory activity, the observed effects of 5-HT may be, at least in part, calcium-dependent. The here presented work addresses the problem whether the speed of removal of intracellular (intraneuronal) free Ca^{++} affects the nature of the effect of 5-HT on synaptic activity. -- The effects of iontophoretically administered 5-HT on presynaptic currents and postsynaptic activity has been studied in squid stellate ganglion following the method described by Llinas, Steinberg & Walton, Proc. Natl. Acad. Sci., U.S.A., 1976, 73: 2918). In low concentrations 5-HT increased inward flux of Ca ion into the presynaptic terminal and its cholinergic storage vesicles resulting in increased release of acetylcholine, and increased postsynaptic activity, including increase of the calcium current. In higher concentrations (preferably after a few second exposure) 5-HT inhibited the previously activated processes (e.g. neurotransmitter release, postsynaptic activity, etc.). After having ascertained for each preparation the critical concentration of 5-HT that started to generate inhibitory effects, several substances with Ca++ binding ability (including polypeptides and proteins with small molecular weight) were introduced into the presynaptic neuron. Some of these agents reversed the inhibitory effects of 5-HT to potentiation of excitatory processes. It seems, therefore, that 5-HT dependent effects result, at least in part, from the combined effects of increased inward flux of Ca^{++} and the availability of intracellular (intraneuronal) processes involved with the removal of the incoming free Ca^{++}. Intracellular concentrations of free Ca^{++} below a critical level seems to promote excitation. Free Ca^{++} concentrations exceeding this critical level promote inhibition of activity.

1878 PHYSIOLOGICAL STUDIES OF POSTNATAL RAT SYMPATHETIC NEURONS AND SKELETAL MUSCLE CELLS IN VITRO. E. Wakshull*, M. Johnson and H. Burton, (SPON: A.L. Perlman). Depts. Phys. & Biophys. and of Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

Dissociated embryonic rat superior cervical ganglion neurons (scgn) form cholinergic synaptic connections on each other (O'Lague et al., PNAS 71: 3602, '74; Ko et al., Br. Res., 117: 461, '76), as well as on co-cultured skeletal muscle (Nurse and O'Lague, PNAS 72: 1955, '75). Hexamethonium (C-6), a ganglionic blocking agent, antagonizes the former synaptic potentials more effectively than the latter, while the reverse is true for d-tubocurare (d-tc) (Nurse and O'Lague, '75). Evidence from our laboratory has indicated that normally adrenergic scgn acquire the ability to synthesize and release acetylcholine in vitro (Johnson et al., Nature, 262: 308, '76). Recent work on explants of scg taken from progressively older postnatal rats has suggested the existence of a critical period for the expression of this transmitter "plasticity" (Ross et al., Nature, 267: 536, '77). We have extended this investigation to the study of postnatal dissociated scgn cultures with and without skeletal muscle cells present. The results show that scgn taken from up to 12 week old rats still form cholinergic connections with each other and with skeletal muscle.

The methods for establishing these cultures are described elsewhere (M. Johnson, this volume). Standard electrophysiological techniques were used to study over 900 neurons. Excitatory post synaptic potentials (epsps) were found between scgn even when taken from 12 week old rats. The epsps were Ca^{++}-dependent and were blocked by C-6; a few potentials could be blocked by the β-adrenergic antagonist propranolol. In some cultures, as many as 80% of the neurons penetrated were synaptically interconnected. SCGN from 5 and 10 week old rats elicited d-tc (1 μM) sensitive junctional potentials with skeletal muscle; these were often suprathreshold. Several scgn formed neuromuscular junctions and an autapse. Autaptic potentials were not effectively blocked by 1 μM d-tc. These data suggest that either the small subpopulation of cholinergic sympathetic neurons selectively survive in these cultures, or that, in contrast to explanted scg, dissociated scgn retain a certain degree of transmitter "plasticity" into adulthood. Studies are in progress to differentiate these two possibilities. (Supported by NIH grants 11888 and NS 09809)

1879 EVIDENCE FOR THE LACK OF INVOLVEMENT OF CYCLIC AMP IN MODULATION OF GANGLIONIC TRANSMISSION IN THE GUINEA PIG SUPERIOR CERVICAL GANGLION. James K. Wamsley*, Asa C. Black, Jr., James R. West, and Terence H. Williams. Department of Anatomy, University of Iowa College of Medicine, Iowa City, Iowa 52242.

The effects of stimulation of guinea pig superior cervical ganglia (SCG) in vitro at 37 C with 50 μM concentrations of dopamine, L-norepinephrine, or L-isoproterenol in Eagle's Medium containing 5 mM theophylline were determined. 50 μM dopamine produced no stimulation of cyclic AMP levels, 50 μM norepinephrine produced a doubling of cyclic AMP levels, while 50 μM isoproterenol produced a 6-fold increase in cyclic AMP levels over control values. The increases were blocked by propranolol, indicating that they were due to stimulation of a β-adrenergic receptor--adenylate cyclase complex. In the rabbit SCG, by contrast, 50 μM dopamine produced an increase in cyclic AMP levels of 60% over control values. The possible involvement of the β-receptor complex in the modulation of ganglionic transmission was tested by stimulating ganglia at 10 Hz for 8 minutes. No elevations of cyclic AMP levels were produced in guinea pig SCG, but a doubling of cyclic AMP levels in rabbit SCG was noted. Current concepts of the function of cyclic AMP in neural transmission in the SCG involve a dopaminergic SIF cell, dopamine receptor--adenylate cyclase complex, and the generation of a slow inhibitory postsynaptic potential (s-IPSP). Previous research has indicated that the guinea pig SCG lacks a s-IPSP, and its SIF cells contain norepinephrine rather than dopamine. Since preganglionic stimulation of the guinea pig SCG has now been shown not to cause increased cyclic AMP levels, and since the guinea pig SCG lacks a dopamine receptor--adenylate cyclase complex, we conclude that cyclic AMP is apparently not involved in the modulation of neural transmission in the guinea pig SCG (unlike the rabbit SCG, where it has been shown to have this function). Supported in part by National Research Service Award PHS 1T-32MH15172-01 to J.K.W., a Pharmaceutical Manufacturer's Association Foundation, Inc., Research Starter Grant to A.C.B., and by NS-11650 to T.H.W. from the National Institutes of Health.

1780 EVIDENCE FOR A ROLE OF CYCLIC GMP IN NEURAL TRANSMISSION IN THE GUINEA PIG SUPERIOR CERVICAL GANGLION (SCG). James R. West, James K. Wamsley*, Asa C. Black, Jr., and Terence H. Williams. Dept. Anatomy, Univ. Iowa Coll. Med., Iowa City, Iowa 52242.

Muscarinic cholinergic receptors regulate cyclic GMP levels in bovine SCG, where muscarinic agonists increase cyclic GMP levels of principal ganglionic neurons (PGNs) (1,2). Current thinking suggests that acetylcholine released from preganglionic terminals interacts with muscarinic receptors, causing increased cyclic GMP levels in PGNS, resulting in generation of a slow excitatory postsynaptic potential (s-EPSP) (2). Cyclic GMP may have a role in the potentiation of the s-EPSP in rabbit SCG (3). Since Libet has shown that the guinea pig SCG has a prominent s-EPSP, we determined the effect of pharmacological and physiological stimulation on cyclic GMP levels in this ganglion. Guinea pig SCG were pre-incubated in Eagle's Medium (EM) for 20 min. at 37 C., then transferred to EM containing 5 mM theophylline, 2.2 mM $CaCl_2$, and agonist. SCG were incubated for 2 min. and frozen in liquid N_2. Preganglionic physiological stimulation was performed at 37 C in EM containing 5 mM theophylline and 2.2 mM $CaCl_2$ for 8 min. at 10 Hz using a 0.5 ms rectangular pulse width. Frozen SCG were homogenized in 6% TCA and centrifuged at 12,000 x g for 30 min. at 4 C, extracted 6X with diethyl ether and assayed for cyclic GMP by the Steiner method. Precipitates were analyzed for protein by the Lowry technique.

2 min.	500 μM Carbachol	2.97 ± 0.52 (6)*
2 min.	500 μM Carbachol + 300 μM Atropine	0.60 ± 0.02 (4)*
2 min.	100 μM L-Norepinephrine	0.36 ± 0.12 (6)*
2 min.	Incubation Control	0.47 ± 0.14 (8)*
8 min.	Preganglionic Stimulation (10 Hz)	1.40 ± 0.17 (10)*
8 min.	Stimulation Control	0.50 ± 0.09 (10)*

*Picomoles Cyclic GMP ± Standard Error (Number Ganglia).

We conclude that both muscarinic agonists and preganglionic stimulation increase cyclic GMP levels in guinea pig SCG. Norepinephrine has no apparent effect on the accumulation. We suggest that these data are consistent with a role for cyclic GMP in synaptic transmission in the guinea pig SCG. (1) Bartfai, Study, and Greengard, Adv. Behavioral. Biol., 24:285-295 (1977). (2) Kebabian, Adv. Cyclic Nucleotide Res., 8:421-508 (1977). (3) Libet, Adv. Biochem. Psychopharmacol., 16:541-546 (1977). Supported in part by National Research Service Award PHS 1T-32MH15172-01 to J.K.W., a Pharmaceutical Manufacturer's Association Foundation, Inc., Research Starter Grant to A.C.B., and by NS-11650 to T.H.W. from the N.I.H.

1881 FUNCTIONAL SIGNIFICANCE OF SLOW EPSPs IN MYENTERIC GANGLION CELLS. J. D. Wood and C. J. Mayer*. Physiol. Dept., Univ. Kansas Med. Ctr., Kansas City, KN 66103 and Physiologisches Institut, der Universität München, 8 München 2, Federal Republic of Germany.

Electrical activity was recorded intracellularly from myenteric ganglion cells of guinea-pig small intestine in vitro. Electrical stimulation of interganglionic connectives evoked a slowly rising excitatory postsynaptic potential (slow EPSP) that was prolonged for several seconds after termination of the stimulus in these cells. The slow EPSP was associated with increased input resistance and augmented excitability of the somal membrane. Microiontophoresis of 5-hydroxytryptamine (5-HT) onto these neurons mimicked the slow EPSP. The 5-HT antagonist, methysergide, blocked both the slow EPSP and the action of 5-HT. Focal mapping with a stimulating electrode indicated that the neurons were multipolar. In the absence of the excitatory transmitter substance, the somal membranes either could not be activated to spike discharge by intrasomatic injection of depolarizing current or the spike threshold was high and the cells did not discharge repetitively to depolarizing current. During the slow EPSP, the characteristic postspike hyperpolarizing potentials of these cells were eliminated and the cells discharged continuously at a frequency that was directly dependent upon the intensity of injected depolarizing current. Electrical stimulation of the cell's processes elicited spikes which electrotonically invaded the soma. Spontaneously occurring spike patterns in the processes were also observed as electrotonic potentials in the soma. It was obvious that the excitability of the soma was much lower than that of its processes under these conditions. The probability that the passive current flow from the axonal or dendritic spikes would trigger a somal spike was greatly increased during the slow EPSP. Spike activity appeared to be restricted to the dendritic tree in the absence of the slow EPSP; whereas during the EPSP, when the membrane time constant, space constant and excitability were increased, the soma functioned to relay dendritic information to the axon at the opposite pole of the soma. These axons project to adjacent ganglia. The functional significance of the slow EPSP appears to be provision of a mechanism by which the soma gates the transmission of information between its processes and thereby regulates the spread of excitation within the neural plexus.

Supported by: The Alexander von Humboldt Foundation, NIH AM 16813 and Research Career Development Award AM 70726 to J.D.W.

1882 LEPTINOTARSIN: NEUROCHEMICAL STUDIES OF THE RELEASE OF ACETYLCHOLINE FROM RAT BRAIN SYNAPTOSOMES. J. Yoshino*, D. Baxter*, T. Hsiao* and W. O. McClure. Section of Cellular Biology, University of Southern California, Los Angeles, CA 90007, and Department of Biology, Utah State University, Logan, UT 84322.

Presynaptic neurotoxins which influence the spontaneous release of neurotransmitter, such as black widow spider toxin and β bungarotoxin, are of importance as possible mechanistic probes in examining the process of neurotransmission. Recently we have described (Satin, et al., these Abstracts) the action at the neuromuscular junction of a new presynaptic neurotoxin, leptinotarsin (LPT). LPT is a protein which was discovered by Hsiao and Fraenkel (Toxicon 7, 119, 1969) in the hemolymph of the Colorado potato beetle, Leptinotarsa sp. We have now examined the ability of the partially purified toxin from L. haldemani to stimulate release of acetylcholine (ACh) from rat brain synaptosomal preparations. Synaptosomes were incubated with [^{3}H] choline to label internal stores of both choline and ACh, and were immobilized and washed on Millipore filters. Solutions of LPT were applied to the washed bed of synaptosomes, and the filtrate collected for analysis. In this system LPT induces the release of radioactive material. Fractionation of the released radioactivity indicates that the release of both choline and ACh is stimulated, and that relatively more ACh is released. Heating the LPT abolishes releasing activity. Removing Ca^{++} from both the washing solution and the solution of LPT reduces activity, but does not eliminate it entirely. The data suggest that LPT is a specific stimulant of release of ACh in synaptosomes, as it is at the neuromuscular junction.

Other properties of the release have been defined. Release of radioactivity follows first order kinetics in time. Increasing the concentration of the toxin yields a saturation in the rate of release.

Before being studied in more detail, however, the toxin must be purified to homogeneity. Using the assay described above, isolation of the toxin is now being carried out. Supported by the National Science Foundation (BNS 76-80657), the National Institute of Health (NIH 5 SO7 RR07012), and the Nelson Research and Development Co.

TISSUE CULTURE

1883 IN VITRO SYSTEMS FOR THE STUDY OF EMBRYONIC VISUAL NEURONS. Ruben Adler, Marston Manthorpe* and Silvio Varon. Dept. Biol., Sch. Med., U. Ca. San Diego, La Jolla, CA 92093.

Development and regeneration of neural components of the visual system have been the objects of intensive investigation in vivo. In vitro, aggregate cultures of visual cells have been studied in several aspects, including the increase in choline acetyltransferase (CAT) in co-aggregates of neural retina (NR) and optic lobe (OL) from the chick embryo. However, there is little or no description of retinal or optic lobe neurons in monolayer cultures, the availability of which could permit detailed investigations on trophic influences required for neuronal survival, the interactions between NR and OL cells, and directional instructions for the elongation of NR neurites.

We report here that defined manipulations of the environment in which OL cells are grown make it possible to achieve cultures containing either: (1) an enriched population of neurons in the virtual absence of nonneuronal ("flat") cells; or (2) a purified population of flat nonneuronal cells, free of neurons; or (3) a combined culture containing both cell types. A combination of substrates of higher adhesiveness (polyornithine, unwashed collagen) with a horse serum-containing culture medium tends to produce sparser cultures in which no development of flat cells takes place. On the contrary, substrates of lower adhesiveness (washed collagen, plastic), together with the supplementation of the medium with fetal calf serum produce "clumped" cultures and promote extensive development of nonneuronal cells underlying the neurons. Mechanical removal of the latter makes it possible to obtain purified nonneuronal populations. Preliminary experiments indicate that similar cultures of NR cells can also be obtained. (Supported by USPHS grant NS-07606.)

1884 ULTRASTRUCTURAL CYTOCHEMISTRY OF PLASMALEMMA OF CULTURED HUMAN MUSCLE. Valerie Askanas and W. K. Engel. NIH, Bethesda, MD 20014.

Tissue culture of human muscle provides a valuable tool to study neuromuscular diseases. Since a plasmalemmal defect might be a basic abnormality in some neuromuscular disorders, we used the membrane probes concanavalin A (Con A), ruthenium red α-bungarotoxin, and tannic acid to describe the ultrastructural cytochemistry of the plasmalemma of normal human muscle cells in vitro during different stages of growth. Single spindle-shaped myoblasts have a globular pattern of peroxidase-post-coupled DAB-reacted Con A staining of the plasmalemma, indicating the presence of α-D-glucoside and/or α-D-mannoside groups. After myoblast fusion, the young myotubes and later mature cultured muscle fibers have uniform Con A staining on the entire surface of the plasmalemma. Variation in the staining intensity appeared due to irregularities of the plasmalemma or to obliquity of the section. Penetration of the staining into the cell was not observed when integrity of the plasmalemma was preserved. Omitting the Con A from the incubating medium or pretreatment with α-methyl-glucoside prevented staining. Addition of 25 μg/ml of Con A to the culture medium at day 0 caused agglutination of the myoblasts, but after myoblast fusion addition of Con A did not seem to have an influence on muscle differentiation. Ruthenium red binds to membrane acid mucopolysaccharides. It gave strong uniform staining of the of the plasmalemma at all stages of muscle-cell development. α-bungarotoxin binding, with the immuno-peroxidase technique, identifying acetylcholine nicotinic receptors in the cultured muscle, stained the plasma diffusely and evenly (without hot-spots) at all stages of muscle-cell development, as previously reported (V. Askanas et al., Neurology, 27, 1019-1022, 1977). Tannic acid did not bind to the plasmalemma of single myoblasts or of young myotubes. However, mature cultured muscle fibers had intensive staining of the plasmalemma and of saccular membranes (? t-tubules or plasmalemmal precursors or postcursors) within the muscle fibers (clearly recognized t-tubules do not form in cultured human muscle). (Cultured embryonic chick and rat muscle showed the same differentiation of plasmalemmal tannic-acid stainability with muscle fiber maturity; in addition, the clearly identifiable t-tubules of those mature fibers were darkly stained, as were the t-tubule-originating "lace" of the cultured chick-embryo fibers; an outer layer of the avian leucosis-sarcoma viral C-particles in the cultured chick-embryo was also stained.) Therefore, tannic-acid appears to be a very good probe for studying muscle cell maturation in culture. The described cytochemical staining of normal human muscle during development now provides the basis for seeking plasmalemmal abnormalities in cultured abnormal human muscle.

1885 LABELING CHOLINERGIC NEURONS IN CELL CULTURE. Kate Barald and Darwin Berg. Dept. of Biology, UCSD, La Jolla, CA 92093.

We have previously shown that dissociated chick spinal cord (SC) cells in culture express high affinity choline uptake that has many of the properties expected for cholinergic neurons in vivo. We now report an autoradiographic procedure for selectively labeling neurons that display high affinity choline uptake. The labeled population includes cholinergic neurons both in SC and in ganglionic cultures, while at least some neuronal populations known to be non-cholinergic remain unlabeled.

The success of the autoradiographic procedure depends on the fact that much of the choline taken up by the high affinity system by cells in culture is converted to compounds such as membrane lipids that are retained by conventional fixation methods. Cultures of SC, ciliary ganglion (CG), or dorsal root ganglion (DRG) cells grown with skeletal myotubes were incubated for 1 hr in 0.1 μM (^{3}H)choline (10.1 Ci/mmol) followed by 1 hr in 10 mM unlabeled choline. The cultures were then fixed in glutaraldehyde, post-fixed in osmium, rinsed, coated with photographic emulsion, and developed after 7-11 days.

CG neurons are cholinergic, and in CG-myotube cultures 99% of the neurons labeled. DRG neurons are thought to be non-cholinergic, and in DRG-myotube cultures none of the neurons labeled. Myotubes also remained unlabeled. SC neurons prepared from 4-day embryos displayed a spectrum of labeling when grown with myotubes for 1 wk. About half (56 ± 4%, SEM, 7 expts) labeled substantially. To show that labeled SC cells included cholinergic neurons, extracellular stimulation and intracellular recording were used to identify neurons that had innervated myotubes. The cells were then examined by (^{3}H)choline autoradiography. Out of 17 cases, 16 were clearly labeled. By comparing the number of labeled neurons in cultures incubated with (^{3}H)choline, (^{3}H)γ-amino butyric acid (GABA), or both, it was concluded that most neurons that exhibited little or no (^{3}H)choline labeling could instead be labeled by (^{3}H)GABA, and vice versa. Thus different populations of neurons appeared to be distinguished by the labeling procedures.

In some SC cultures (^{3}H)choline labeling may not be restricted to cholinergic neurons. For example, cultures prepared with SC cells from 7-day embryos are thought from previous work to have many fewer cholinergic neurons than SC cultures from 4-day embryos, yet they appear to have as many (^{3}H)choline labeled neurons, some of which may also label with (^{3}H)GABA. In all cases (^{3}H)choline labeling was blocked by Na^+ deprivation or 10 μM hemicholinium-3. Thus, in addition to cholinergic neurons, the procedure may also label other select populations of neurons, perhaps in rapid growth or in a multi-functional precursor state.

These labeling methods will be useful for distinguishing different neuronal populations and following their development. (Supp. by USPHS Grant #12601, Muscular Dystrophy Assoc., & Am. Heart.)

1886 EFFECT OF GLIAL CONDITIONED MEDIUM ON SURVIVAL AND FIBRE OUTGROWTH FROM CHICK SENSORY NEURONS. Yves A. Barde*, Ronald M.Lindsay*, Denis Monard* and Hans Thoenen, Dept. of Pharmacology, Biocenter of the University, Basel, Switzerland

Process formation can be induced in clonal neuroblastoma cells by a macromolecule termed "glial factor" which has been identified in the medium conditioned by culture C-6 glioma cells. Fibre ougrowth can also be elicited in cultures of peripheral sensory neurons by the well-characterized mouse submaxillary gland nerve growth factor (NGF). We report here that glial conditioned medium (GCM), the source of the "glial factor", can support both survival and fibre formation of isolated chick sensory neurons and that neither NGF nor "glial factor" are responsible for this effect.

Dorsal root ganglia from 10-12 day old chick embryos were dissociated and plated on collagen coated dishes and surviving neurons counted after 48 hours. In the absence of either NGF or GCM, less than 5% of the cells plated survived. The addition of either GCM or NGF led to a marked increase in the number of surviving neurons. The effect of NGF could be completely abolished by specific antibodies to NGF. In contrast, the increased survival of neurons promoted by GCM was not blocked by the NGF antibodies, even when the concentration of antibody used was 100-fold higher than that required to block the effect of NGF. Partially purified "glial factor" did not support the growth of the isolated sensory neurons despite a 100 to 150-fold increase in specific activity determined by the neuroblastoma bioassay. Therefore, it may be concluded that the support of survival and induction of fibre outgrowth in sensory neurons were brought about by a factor other than that which is responsible for neuroblastoma cell process formation, i.e. "glial factor". Digestion of GCM with pronase or chymotrypsin markedly reduced the ability of GCM to support the survival of the sensory neurons, suggesting that this factor might be a polypeptide. Further characterization is now in progress. We are also examining the possibility that this factor might be influencing the survival of sympathetic cells in culture.

1887 MUSCARINIC RECEPTOR BINDING IN SPINAL CORD CELL CULTURES: EFFECT OF MUSCLE-CONDITIONED MEDIUM. Neville Brookes, David R. Burt and Alan M. Goldberg. Dept. Pharmacol. and Exptl. Therap., U. Maryland Sch. Med., and Dept. Envirn. Hlth. Sci., Johns Hopkins U., Sch. Hygiene, Baltimore, MD.

The specific activity of choline acetyltransferase (CAT) in surface cultures of dissociated murine spinal cord (SC cultures) is greatly increased when the cultures are maintained in medium which has been conditioned by murine skeletal muscle cells (Giller et al., J. Cell Biol. 74:16, 1977). Protein content, neuronal cell count and activities of cholinesterase (ChE), glutamic acid decarboxylase and other enzymes are not similarly increased by conditioned medium (CM). It is not known whether this induction of CAT activity is associated with changes in other parameters of cholinergic neurotransmission. The present experiments examine the effect of skeletal muscle CM on the binding of [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) to muscarinic receptors in SC cultures.

Cultures were prepared by mechanically dissociating spinal cords from 13-day-old mouse embryos and then seeding aliquots of the cell suspension onto collagen-coated dishes at a cell density of approx. $2x10^5/cm^2$. [^{3}H]QNB binding was detectable in control SC cultures after 5 days of incubation and it had increased by an average factor of 6.6 times at 21 days, when the specific binding was 340±80 pmole/g protein (mean ± SEM, N=8). Specific [^{3}H]QNB binding was still increasing at 28 days of SC culture. CM-treated SC cultures were fed from day 4 onward with medium which had previously been conditioned by muscle cell cultures for 2-3 days. CAT specific activity in CM-treated SC cultures was increased by a factor of 2.7±0.5 (N=3) times control values at 14-15 days, and 4.2±0.8 (N=3) times at 18-21 days. The corresponding factor for ChE specific activity at 18-21 days was 1.3±0.1 (N=3). In parallel experiments, [^{3}H]QNB specific binding increased only by a factor of 1.6±0.2 (N=8) times control values at 20-21 days. Thus, the effect of muscle CM on muscarinic receptor binding was comparable to the modest effect on ChE activity and did not approach the scale of the marked influence on CAT activity.

(Supported, in part, by grants from the P.M.A. Foundation and from NIEHS.)

1888 DOES THE SAME COMPONENT OF CONDITIONED MEDIUM INDUCE NEURITE OUTGROWTH FROM BOTH SYMPATHETIC AND PARASYMPATHETIC NEURONS? Frank Collins* (SPON: L. Mathers) Dept. Bio. Sci., Stanford Univ., Stanford, CA 94305

Individual ciliary ganglion (parasympathetic) and lumbar chain (sympathetic) neurons from chick embryos extend neurites in heart-cell conditioned medium, but not in unconditioned medium. Nerve growth factor (NGF) will not substitute for conditioned medium under our culture conditions, nor is the activity of conditioned medium affected by antiserum to NGF. Since conditioned medium supports outgrowth both from neurons which are considered to be NGF-dependent (sympathetic) and from those considered to be NGF-independent (parasympathetic), it is important to know whether the same or different components of conditioned medium are involved.

An active component of conditioned medium, which is apparently negatively charged, binds to the positively charged polyornithine-coated culture substratum, so that culture dishes pretreated with conditioned medium will support neurite outgrowth in unconditioned medium. The conditioned medium activity which binds to the culture substratum supports neurite outgrowth from both the sympathetic and parasympathetic neurons. The activity is sensitive to trypsin, but not to collagenase, RNase, or DNase. An ammonium sulfate fraction, which contains a very low percentage of the total protein in conditioned medium, contains the material which binds to the substratum and which supports neurite outgrowth from both classes of neuron.

These results suggest that identical or very closely related components of conditioned medium induce neurite outgrowth from both sympathetic and parasympathetic neurons.

1889 ELECTROPHYSIOLOGY AND PHARMACOLOGY OF STRIATED MUSCLE FIBERS CULTURED FROM DISSOCIATED NEONATAL RAT PINEAL GLANDS. Joseph E. Freschi*, Andrew G. Parfitt* and William G. Shain (SPON: L. H. Masukawa). Armed Forces Radiobiol. Res. Inst., Bethesda, Md. 20014.

Striated muscle fibers have been observed within the pineal glands of several mammalian species including man. We found striated muscle fibers in each of twenty consecutive pineal glands cultured from individual neonatal (2 day) rats. Subsequent experiments were done with dissociated cultures of pineal glands pooled from several litters. Myotubes were first visible after about one week in culture. During the next several weeks the myotubes increased in size, developed cross-striations, and began to twitch spontaneously. The resting membrane potential (RMP) increased with age in culture. All myotubes studied showed delayed rectification. Action potentials either occurred spontaneously or could be evoked if the membrane were sufficiently polarized. No spontaneous end plate potentials were seen. Acetylcholine (ACh) produced a brief, monophasic depolarizing response. Norepinephrine (NE), serotonin (5HT), dopamine (DA), melatonin (MEL), and γ-aminobutyric acid had no effect on the RMP when applied iontophoretically. The ACh response was reversibly blocked by 10^{-6}M d-tubocurarine and irreversibly blocked by 10^{-6}M α-bungarotoxin. Atropine at 10^{-4}M reduced the amplitude and shortened the time course of the ACh response, and 10^{-3}M produced complete but reversible inhibition.

It is concluded that myogenic cells of unknown origin occur within the neonatal rat pineal gland. These pineal muscle fibers are electrophysiologically and pharmacologically identical with peripheral skeletal muscle cells in vitro. Although the pineal gland is devoid of ACh, these striated muscle fibers develop ACh receptors but do not develop receptors mediating electrophysiological responses for NE, 5HT, DA, MEL, or GABA which are known to be present in the pineal. A comparison of the developmental properties of these cells in culture with other systems such as striated muscle fibers in dissociated thymus (Wekerle, et. al. Nature 256: 493-494, 1975) suggests the possibility that these pineal striated muscle fibers may arise from pluripotential stem cells.

1890 **PRECISE NEURITE TRANSECTION AND DE-INSULATION OF PHOTOETCHED MULTI-MICROELECTRODES WITH UV-LASER MICROBEAM TECHNIQUES.** **Guenter W. Gross*, M. Louise Higgins*, and Marilyn Smith* (SPON. J.E. Hines). Dept. Biology Texas Woman's University, Denton, Texas 76204.**

A UV-laser microbeam system is being utilized to a) transect neurites of nerve cells in culture, b) create adhesion patterns on glass or plastic surfaces and c) de-insulate end regions of photoetched gold conductors on the floor of tissue culture chambers. These techniques are being developed to influence the ordered neuronal growth of networks in vitro and to develop a long-term simultaneous single unit recording capability from more than 30 neurons in a network. The three micromanipulations can be easily carried out with a 1 μm resolution by firing UV laser pulses of 8 ns duration and 337 nm wavelength through a microscope objective. This achieves a minimum focus of 0.7 μm and a maximum power density of 10^{12} Watts/cm^2. Successful transections depend on the power density utilized, the exact position of the focus relative to the plane of the neurite, the distance of the transection from the cell body and the size of the neurite. Scattered UV radiation appears to have little immediate effect on neurons and is being investigated on the ultrastructural level. EM studies also reveal blebbing and ultrastructural disruption resulting from shock waves produced by excessive energy densities or vaporization of substrate. Electrode de-insulation is achieved by the laser-induced vaporization of minute amounts of gold from the flat conductor and the concomitant pressure-removal of the overlying insulation. A 10 μm diameter crater in a 3 to 4 μm thick insulation layer has an impedance of approximately 3 megohm at 1 kHz. These recessed gold surfaces have recorded single unit activity from snail brain ganglia resting against the recording matrix in a shallow pool of saline. Giant cells produce 3 mV signals that can be seen by several electrodes simultaneously. The more abundant 40 μm diameter cells produce spike amplitudes of 300 to 500 μV that are seen by only one electrode. With this recessed tip design, simultaneous single unit recording from smaller neurons appears assured if electrode impedances remain below 4 megohm, shunt impedances are above 30 megohm and glia cells are not allowed to re-insulate the electrode craters.

1891 HRP CHARACTERIZATION OF NEURONS IN ORGANIZED CULTURES OF CEREBELLUM. W.J. Hendelman and K.C. Marshall, Depts. of Anat. and Physiol., University of Ottawa, Ottawa, Canada, K1N 9A9.

Organotypic (Maximow) cultures of newborn mouse cerebellum have 3 different regions: the cortical area with its Purkinje neurons (PN), the deep cerebellar nuclear neurons (DN), and a group of cells derived from the brain stem in the peduncular region (BS). Clearly identified neurons were visualized in select mature cultures and injected intracellularly with horseradish peroxidase (HRP, Sigma type VI), using depolarizing pulses equivalent to about 100-120 x 10^{-9}Amp.-minutes of steady current. Perfusion of the cultures in BSS (supplemented with glucose) at 35°C was continued for 4-6 hours, and followed by aldehyde fixation. The reaction in Hanker-Yates solution was monitored microscopically for up to 2½ hours. It was generally observed that the soma and dendrites of smaller neurons (25μ or less) were not stained, though the axons were well filled. The PN axon had a constant diameter of 1-2μ and followed a relatively direct course to the DN area. Most axons emitted a single recurrent collateral. In the DN region the axon arborized in a narrow field (appr. 60 x 150μ) and terminated in knobs (2-5μ);some axon branches had "terminals-en-passant". The DN axons were seen to bifurcate several times, and the diameter of the branches varied in size but was usually 1μ or less. After injection of a single neuron many axon branches were seen entering the cortical region. Several axons followed a sweeping trajectory along the margin, though others coursed directly through the explant. Some had a long, sometimes looping pathway through the cortex, often exhibiting abrupt changes in direction. Evidence of terminals consisted of smooth or beaded excrescences along the axons or along slender side branches. BS neurons: These large cells (~30μ) have 2-4 dendrites which are broad at their origins and emit spines. They taper gradually and often seem to continue as axon-like neurites which may extend for some distance. Some neurons appear to give rise to multiple axons emanating from soma and dendrites. The axons are thin, and characterized by small varicosities along their course. There is usually an extensive plexus of local collaterals, but the longer branches meander erratically into the cortical and the non-neuronal outgrowth regions. This technique has proven useful to demonstrate the axonal branches of a single neuron. These projections corroborate our electrophysiological data on the interconnections in these cultures. Further studies are intended to define the synaptic connections electron microscopically. (Supported by the Medical Research Council of Canada).

1892 SYNAPSE FORMATION BY NEUROBLASTOMA AND HYBRID CELL LINES. Haruhiro Higashida*, Steven P. Wilson, Michael Adler and Marshall Nirenberg. Laboratory of Biochemical Genetics, NHLBI, NIH, Bethesda, MD 20014.

Nineteen neuroblastoma or hybrid cell lines that synthesize acetylcholine (ACh) were cultured with rat striated muscle cells. Myotubes were tested for synapses by intracellular recording using the presence of spontaneous miniature endplate potentials and evoked responses as criteria for functional innervation. Rates of ACh synthesis were 35-460 pmol ACh formed/min/mg homogenate protein. Cells incubated with 100 μM [^{3}H]-choline released 80-6,370 fmol [^{3}H]-ACh/min/mg protein into the medium and contained 38-540 pmol [^{3}H]-ACh/mg protein (Wilson, et al., Fed. Proc. 37, 1784, [1978]). Six cell lines formed synapses with high frequency (Syn^+ lines) (14-63% of myotubes tested were innervated); whereas 13 cell lines formed few or no synapses (Syn^- lines). Some, but not all, Syn^- lines exhibited defects in the coupling of K^+-dependent depolarization with ACh release. Four additional cell lines lack choline acetyltransferase activity and also were unable to form functional synapses with myotubes. Syn^+ cell lines that were examined formed synapses with myotubes rapidly, 20-30 min after cells were added to myotubes. These cells also formed synapses with clonal G8-1 mouse striated muscle cells.

Some Syn^+ cell lines undergo depolarization when activated by ACh, serotonin, or dopamine. Addition of neurotensin, angiotensin-II, bradykinin, or somatastatin by iontophoresis or by diffusion from blunt pipettes resulted in slow hyperpolarization (usually 10 to 20 mV, 10-20 sec) followed by prolonged depolarization (2 to 10 mV, 30-80 sec). The effects of activating these and other species of receptors on synapse formation and function thus can be studied.

Treatment of Syn^+ hybrid cells with dibutyrl-cAMP (Bt_2cAMP) for > 5 days increased markedly the number of synapses formed, the frequency of spontaneous muscle synaptic responses, and K^+-dependent release of [^{3}H]-ACh from cells. These results show that stimulus-dependent ACh release is regulated by Bt_2cAMP, thereby regulating synapse formation and efficiency, and that clonal cell lines can be generated with defects in depolarization-ACh-release coupling and other presynaptic functions.

1893 BULK ISOLATION FROM RAT CEREBRAL CORTEX OF VIABLE NEURONS WHICH RETAIN SYNAPTIC COMPLEXES. W. B. Huttner*, R. Meyermann*, V. Neuhoff* and H.-H. Althaus* (SPON: P. Greengard).
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A new approach to the bulk isolation of rat cerebral neurons has been introduced (1). In the present study, the principles of this isolation procedure were investigated, and EM examinations of the isolated nerve cells were carried out (2). The basis of this procedure is the predisaggregation of the neurons in situ brought about by perfusion of the brain under specialized conditions: 1) a hyperosmolar concentration of hexoses in the perfusate; 2) the presence of collagenase and hyaluronidase; and 3) an elevated capillary pressure of the perfusate. The first condition is a prerequisite for the isolation of neurons whereas the other two conditions improve the morphological integrity of the neurons. Histological and TEM studies of the perfused brain reveal that after perfusion most of the glial cells surrounding the neurons are destroyed. This predisaggregation of the nerve cells in situ, together with the damaging effect of the perfusion on the capillary network, greatly facilitates the subsequent mechanical dissociation of the brain tissue. The cell suspension obtained contains virtually no neuronal nuclei, and 70% of the neurons retain the proximal parts of their processes. Using a Ficoll density gradient with a liquid fluorocarbon as a cushion, a neuronal fraction of 90% purity is obtained yielding 20 x 10^6 nerve cells/g cortex. Upon SEM and TEM examination of the isolated neurons, various cell types can be distinguished, and the plasma membranes and intracellular structures appear well preserved. A novel feature of the isolated, viable neurons is that they still retain some of their synaptic complexes. Having attached presynaptic buttons including mitochondria and vesicles, as well as pre- and postsynaptic membranes and densities, the cells represent neurons retaining synaptosomes on their plasma membranes. Preliminary experiments have determined that the nerve cells can be maintained in culture (Althaus, Neuhoff, Huttner, Monzain and Shahar).

(1) Althaus, Huttner and Neuhoff, Hoppe-Seyler's Z. Physiol. Chem. (1977) 358, 1155-1159.

(2) Huttner, Meyermann, Neuhoff and Althaus, submitted for publication.

1894 ION TRANSPORT AND MEMBRANE POTENTIAL PROPERTIES OF CULTURED RAT BRAIN ASTROCYTES. H.K. Kimelberg*, C. Bowman* and R.S. Bourke. Div. Neurosurg., Albany Med. Coll. and Neurobiology Research Center, State Univ. of N.Y. at Albany, Albany, N.Y.

Primary astrocyte cultures were grown from 1-3 day old rat brains (Booher and Sensenbrenner, Neurobiol. 2, 97, 1972), and were studied after 2-3 weeks in culture. The initial rate of K^+ influx (as $^{86}Rb^+$) at an external $[K^+]_o$ of 4.5 mM under equilibrium conditions at 37°C, was 0.02 to 0.03 μmoles K^+/min/mg protein. Efflux rates calculated from a first order rate constant of 0.03 min^{-1} and a K^+ content of 0.9 μmoles/mg protein were similar. Active ouabain-inhibited uptake of K^+ was 0.006 to 0.01 μmoles K^+/min/mg protein, balancing a Na^+ efflux (based on Na^+ influx) of 0.007 μmoles/min/mg protein. Since $[Na^+]_o$=130 mM this data suggests that the permeability of the membrane to Na^+ is considerably less than for K^+. (Na^++K^+) ATPase activity was 0.016-0.05 μmoles ATP hydrolyzed/min/mg total cell protein. Cl^- influx and efflux (as $^{36}Cl^-$) were both in the range of 0.04 to 0.05 μmoles/min/mg protein and were partially inhibited by the anion inhibitor,4 acetamido-4'-isothiocyanostilbene-2,2'-disulfonate (SITS), suggesting that Cl^- transport in these cells was both passive and mediated. Also the cells lose Cl^- in low Cl^- medium.

The membrane potentials of these cells were measured at 25°C using microelectrodes. Untreated cells were too flat to be impaled but after treatment with DBcAMP the cell bodies rounded up and were easily impaled. Addition of DBcAMP had little effect on transport rates. A number of the cells had membrane potentials (E) of around -70 mV, similar to astroglia in vivo. Groups of cells were pre-equilibrated with media of increasing $[K^+]_o$ at constant $[Cl^-]_o$, and the slope of a plot of the data was 53 mV/10 fold change in $[K^+]_o$ at $[K^+]_o$ values > 35 mM. Extrapolation to E=0 gave a value for $[K^+]_i$ of 150 mM. Below 35 mM $[K^+]_o$ the E values diverged from a straight line, and a permeability ratio (α) for Na:K of 0.04 was calculated. However cells with less negative potentials, due either to heterogeneity or impalement injury, were also found, giving a mean value for E of -51±18 mV (+S.D. n=43) at $[K^+]_o$=4.5 mM. The results from perfusion of impaled cells with media of varying $[K^+]_o$ but constant KxCl product, also showed a slope of 55 mV/10 fold change in $[K^+]_o$. From this a $[K^+]_i$ of 170 mM and α=0.03 was obtained.

Thus these astrocyte cultures, like mammalian astroglia in vivo, show high negative potentials of -70 mV largely determined by K^+, and a (Na+K) pump sufficient to maintain a K:Na ratio of ≥ 10, which makes them suitable as in vitro models. In addition, these cells seem to show both passive Cl^- transport and a mediated, SITS-inhibitable Cl^- exchange component. (Supported by Grant NS 13042 from NINCDS).

1895 PARALLEL CHANGES IN ELECTRICAL AND CONTRACTILE ACTIVITY IN CULTURED MYOCARDIAL CELLS. M. Kitzes* and M. W. Berns* (SPON: J. Sassin). Dept. Dev. & Cell Biol., UC Irvine, Irvine, CA 92717.

Intracellular study of spontaneous activity of over 800 neonatal rat (1-2 day old) ventricular cells in culture provided consistent data to establish normal patterns of spontaneous electrical activity under controlled conditions of the surrounding standard medium. Our results indicate that a certain percentage of the cells showed resting membrane potentials (total range -40mV to -98mV), overshoot and total spike amplitude values comparable to those normally found in neonatal and adult rat heart. A relatively low ratio of pacemaker (40%) to non-pacemaker cells (60%) and low incidence of hyperpolarizing afterpotentials (35%) were found. These findings indicate that reversion of the cultured cells to a younger state may only be partial in our cultures.

Our results show that La^{+++} (.1mM to 3mM) administration always results in diminished contraction frequency and strength with complete block of spontaneous contractility at higher concentrations. These changes, however, were paralleled by marked alteration of electrical activity. Intracellular recordings during this trend towards complete block of contractility show a concomitant progressive cell depolarization, diminished discharge frequency and marked increase in action potential duration with alteration of normal configuration. Action potentials from cells showing slow rhythmic spontaneous contractions in an otherwise immobile monolayer achieved durations of up to 2.5 sec. All cells which showed complete block of contractility after exposure showed resting membrane potentials which ranged from -25mV to -55mV and no action potentials. In all cases, complete recovery of contractility, resting membrane potential and normal action potentials followed replacement of experimental medium by normal medium. Recovery to increased strength and contraction frequency paralleled by increased action potential discharge frequency and levels of membrane polarization was observed frequently.

Our results indicate that in the neonatal rat monolayer culture, La^{+++} is not a specific E-C uncoupler as has been reported previously but has multiple effects upon the normal electrical characteristics of the cultured cells.

This research was supported by NIH grants HL15740, GM23445 and GM22754, and by U.S. Air Force grant AFOSR-77-3136.

1896 GROWTH CHARACTERISTICS OF ISOLATED ADRENAL MEDULLARY CELLS IN CULTURE. Bruce G. Livett, Deanne M. Dean* and Garth M. Bray. Division of Neurology, Montreal General Hospital and McGill University, Montréal, Québec, Canada, H3G 1A4

Peripheral adrenergic neurons and adrenal medullary cells both originate from the neural crest. Adrenal medullary cells, obtained in high yield (10^7 cells/gland) from bovine adrenal medullae by retrograde perfusion with collagenase[1], provide a convenient system for studying adrenergic function and development in-vitro. Cells rich in adrenaline or noradrenaline have been obtained by fractionation on Percoll gradients and plated in DMEM with serum supplements. By two days in culture, most cells had flattened out and developed short processes, some of which had terminal expansions resembling growth cones. By 6 days these processes had extended up to 250μ in length and displayed a varicose appearance. Examination of the cultures by the Faglu fluorescence method[2] revealed high concentrations of catecholamines in the varicosities and growth cones of the cell processes. Transmission electron microscopy confirmed that the processes contained either adrenaline or noradrenaline vesicles. Chemical analysis of the cells after 6 days in culture showed that they contained principally noradrenaline (NA) (NA/A 21.94 ± 0.07, n=5) in contrast to the cells which, when first plated, contained mostly adrenaline (A) (NA/A 0.23 ± 0.07, n=9). Long-lasting contacts were made by the adrenergic processes with the soma and processes of other chromaffin cells.

We conclude that under appropriate conditions, adrenal chromaffin cells can undergo process formation resulting in varicose fiber networks similar to those of adrenergic neurons.

1. Fenwick,E.M., Fajdiga,P.B., Howe,N.B.S. and Livett,B.G. (1978). J. Cell Biol. 76: 12-30
2. Furness,J.B., Costa,M. and Wilson,A.J. (1977). Histochem. 52: 159-170

(Supported by M.R.C.)

1897 MORPHOLOGY AND GFA-IMMUNOFLUORESCENCE OF ASTROGLIA-CONTAINING MONOLAYER CULTURES FROM RAT CEREBRUM. Marston Manthorpe*, Ruben Adler and Silvio Varon (SPON: S.A. Hillyard). Dept. Biol., Sch. Med., U. Ca. San Diego, La Jolla, CA 92093.

Reactive gliosis in response to damage in the CNS leads to scar tissue which is thought to present a barrier to the regenerating neurite. Regulation of proliferation, hypertrophy or fibrotization of astroglial cells, the three main components of gliosis, could be most conveniently analyzed under the control conditions of a monolayer cell culture, particularly if the latter could be made available as a purified, homotypic population. Cell suspensions were obtained from neonatal rat cerebrum by trituration after trypsin treatment. The cells were cultured on glass coverslips, in CO_2/air with fetal calf serum-supplemented medium. Cultures were inspected periodically under phase contrast microscopy for morphology and numerical analyses, and examined at selected time intervals by an indirect immunofluorescence test for the presence of Glial Fibrillary Acidic protein (GFA). The early cultures displayed two readily distinguishable cell categories, each susceptible of further, though less sharp, subdivisions.

(1) Flat cells (thinly spread, phase-light elements, with grossly polygonal contours and no processes) were responsible for practically all of the culture growth, and became the nearly exclusive population in 2 week-old cultures started at appropriate seeding densities. A combined treatment of serum withdrawal and dibutyryl cyclic AMP (DBcA, 1 mM) causes these cells to assume typical astrocytic morphologies, as already described by other investigators. In our hands, this morphological conversion was i) massive, ii) rapid, iii) reversible, and iv) imposable on early and/or sparse cultures, as well as 2-week old confluent ones. Each treatment component, applied separately, appears to elicit distinctive morphological changes. GFA was found to be present in most flat cells before, as well as after, the treatments.

(2) Process-bearing (PB) cells were present as early as the cultures could be analyzed, when they equaled or outnumbered the flat cells, but showed no propensity to proliferate. PB cells were characterized by: i) small, well-contained bodies with either a phase-dark or a phase-bright appearance, ii) discrete, thin processes varying in number and branching patterns, iii) no gross morphological responses to the above treatments, and iv) no detectable GFA antigen, thus far.

Attempts are underway to alter behavior and/or relative numbers of the two cell classes by use of different culture conditions, with the ultimate aim to obtain them as separate subpopulations of cerebral cells. (Supported by USPHS grant NS-07606).

1898 Morphology and electrophysiology of cerebellar neurons in cell culture. E.A. Neale, G. Moonen*, R.L. Macdonald, W. Gibbs*, and P.G. Nelson. LDN, NICHD, NIH, Bethesda, MD 20014

The cerebellar cortex has been used as a model for the study of neuronal development and synaptic specificity. To further examine the morphology and synaptic physiology of specific neurons, it is desirable to grow cerebellar tissue in a monolayer cell culture system where anatomic complexity is reduced and neurons are more readily accessible for intracellular recording and structural tracing. We have obtained, from 17-19 da fetal rat cerebella, long-term (>8 wk) cell cultures which contain several types of neurons. Intact cerebella were passed through Nytex 215 nylon mesh to obtain small clusters of cells which, when plated, spread to form networks of neurons. Neuronal survival in these cultures was greater than in cultures prepared from single cell suspensions, with or without subsequent reaggregation before plating.

Intracellular recordings were obtained from >100 neurons with soma diameters >15 μm. Although membrane potentials were low (20-30 mV), spontaneous action potentials were usually seen. Synaptic activity was recorded in most cells (>90%) and both excitatory and inhibitory postsynaptic potentials were observed.

More than 20 neurons were injected with horseradish peroxidase (HRP) so that entire selected cells might be structurally analyzed. The most common large neuron had a 20 μm rounded soma, one or more dendritic shafts with blunt-ended, spine laden branches, and a single axon that bifurcated several times, but showed few swellings in contact with other neurons. With the electron microscope, these swellings were seen to contain pleomorphic vesicles. Morphologic features of such neurons suggest that they are Purkinje cells whose dendritic arborizations are considerably less complex than in vivo. A second type of large neuron showed a 20 μm rounded soma, long slender dendrites, and an axon with many delicate branches forming numerous contacts on processes of nearby cells. Similar cells, not injected with HRP, displayed nuclear rodlets and subjunctional dense bodies beneath impinging synaptic terminals, features associated with Golgi cells. A third neuronal type was characterized by a 6 μm soma occupied mostly by its nucleus, rich in condensed chromatin, similar to cerebellar granule cells.

Several distinct varieties of synaptic boutons were found in these cultures. It appeared that the shafts of the presumed Purkinje cell dendrites received primarily large boutons marked by pleomorphic vesicles, while smaller boutons filled with rounded vesicles were commonly associated with dendritic spines.

Cerebellar neurons, possessing active membrane properties, can thus be maintained in long-term cultures. The neurons display morphologic features characteristic of in vivo cerebellar neurons, and develop extensive synaptic connectivity.

1899 QUANTITATIVE LONG-TERM SURVIVAL AND DEVELOPMENT OF CHICK CILIARY GANGLION NEURONS GROWN ALONE IN DISSOCIATED CELL CULTURE. Rae Nishi and Darwin Berg. Dept. of Biology, UCSD, La Jolla, CA 92093.

Cell death between embryonic days 8 and 13 in the normally developing chick ciliary ganglion reduces the number of neurons from 6500 to 3200. We have previously shown that quantitative long-term survival of the neurons, including those destined to die in ovo, can be obtained in cell culture when 8-day embryonic ganglia are dissociated and grown with skeletal myotubes. We now report that equivalent survival can be obtained when CG neurons are grown alone in appropriately conditioned medium, and that they develop high levels of choline acetyltransferase (CAT) activity under these conditions.

Ciliary ganglia from 7-8 day chick embryos were removed, gently dissociated, and plated with or without skeletal myotubes or heart cells at a density of ca. 6 x 10^3 neurons (1 ganglion equivalent) per 16 mm collagen-coated Falcon tissue culture well. Culture medium consisted of Eagle's MEM supplemented with 5% chick embryo extract and 10% horse serum. Neurons grown alone received medium diluted 1:1 with conditioned medium obtained from myotube or heart cell cultures. Culture extracts were prepared and assayed for CAT activity by following the conversion of radioactivity from (^{3}H)acetyl Co-A to (^{3}H)acetylcholine, as determined by extraction with tetraphenylboron in heptanone. Surviving neurons were counted with phase contrast optics before extract preparation in each case.

When grown alone in conditioned medium, the number of surviving neurons per culture remained constant for at least 3 weeks. The mean number, per ganglion equivalent used to prepare the cultures, was 6370 ± 170 (SEM, 37 cultures counted) over the entire period. Comparable survival was obtained in conditioned medium lacking embryo extract; no survival was observed for unconditioned medium supplemented only with horse serum. For neuron-myotube cultures, in which we have previously shown that most of the neurons innervate myotubes, levels of CAT activity increased from 25 ± 1.2 fmoles ACh/neuron/hr at 3 days in vitro to 404 ± 36 at 15 days (mean ± SEM, 3 experiments). Neurons grown with heart cells displayed a similar development of CAT activity. CAT activity for neurons grown alone in conditioned medium with embryo extract increased from 29 ± 3.6 fmoles ACh/neuron/hr to 347 ± 12 over the same time period (3 experiments).

Thus factors present in the medium can promote the survival of CG neurons in dissociated cell culture, including those neurons destined to die in ovo. Direct contact with myotubes or heart cells is not necessary for the survival, and the neurons continue to develop under these conditions as indicated by the increases in levels of CAT activity. (Supported by USPHS Grant #12601, The Muscular Dystrophy Association, the The American Heart Association.)

1900 CHOLINERGIC AND NUCLEOTIDE RESPONSES IN CULTURED FROG SYMPATHETIC NEURONS. Ante L. Padjen, Donna L. Gruol, George R. Siggins, and David S. Forman. Dept. Pharmacol. Therap., McGill Univ., Montreal, Canada, LNP, NINCDS, NIH, Bethesda, Md., The Salk Institute, La Jolla, Calif., and NMRI, NNMC, Bethesda, Md.

Several purine and pyrimidine mononucleotides including ATP and UTP produce potential changes in cultured bullfrog sympathetic neurons which are similar to cholinergic muscarinic responses (Siggins et al., Nature 270:263,1977). In the present study we have analyzed the responses of these neurons to muscarine, a cholinergic agonist thought to act primarily at the muscarinic receptor, and compared them to nucleotide-evoked responses. Longterm (4-8 week) cultures of adult bullfrog sympathetic ganglia were studied using conventional intracellular recording techniques and superfusion of drugs (Padjen et al., Neurosci. Abs. 1:813, 1975). Neurons ($\bar{x}$=40x54 μm) displayed stable resting potentials (-35 to -58 mV; $\bar{x}$=45 mV) and generated single or repetitive overshooting action potentials when stimulated with intracellular current pulses. Input resistances calculated from hyperpolarizing I-V curves ranged from 14 to 41 MΩ($\bar{x}$=27 MΩ). Mean R_m, τ_m, and C_m were 1612 Ωcm^2, 5.2 msec, and 3.4 μF/cm^2 respectively. The response to a 15-60 second pulse of 10^{-4}M muscarine was most often (29 out of 40 cells) a slow depolarization ranging from 2-19 mV ($\bar{x}$=7 mV) which lasted several minutes. Occasionally this depolarization was followed by a prolonged secondary hyperpolarization (1-5 mV). In 11 cells there was little or no response (<2mV depolarization). The depolarizing and hyperpolarizing muscarinic responses were associated with either an increase or no change in input resistance. The nucleotide responses were similar to those seen with muscarine: prolonged depolarization sometimes followed by secondary hyperpolarization, both generally accompanied by an increase in input resistance. In 24 cells in which both muscarine and nucleotides were tested, 21 responded to both agents with UTP being the most potent, while 3 responded only to the nucleotides. However, atropine ($3x10^{-6}$-$1.3x10^{-5}$M) reversibly blocked the depolarizing responses to muscarine but had no effect on responses to nucleotides. Thus, the electrophysiological properties and muscarinic receptors of these cultured neurons are similar to those of acutely isolated sympathetic neurons. However, a unique response is seen in the cultured neurons. Research is in progress to test for nucleotide responses in acutely isolated ganglia.

(Supported by NIMH, IRP, Lab. Neuropharmacol. and the MRC of Canada.)

1901 MORPHOLOGY AND ELECTROPHYSIOLOGY OF DISSOCIATED MOUSE HIPPOCAMPAL CULTURES. J. Peacock, D. Rush, and L. Mathers, Stanford University Medical School, Stanford, CA 94305

Dissociated hippocampal cultures from fetal mice (13-18 days gestational age) were grown for up to 2 months in culture. Neurons in mature cultures were round and small (maximal size, 15-20 μM). Their processes were extensively branched but difficult to see with phase contrast optics due to their growth among underlying nonneuronal cells. Processes could be identified with silver staining and by intracellular injection of the fluorescent dye, Lucifer Yellow. Some processes appeared to have spines and others to be beaded. Processes tended to emanate from one side of the soma in 22/28 fluorescent-stained cells either originating at the cell body (11 cells) or from a single trunk (11 cells). Commonly, there were 2-4 orders of branching, but up to 6 orders could occur (as counted centrifugally from the soma).

Spontaneously occurring action potentials (APs), postsynaptic potentials (PSPs), and burst potentials were recorded intracellularly in > 200 cells with K-acetate or Lucifer Yellow filled micropipettes. Repetitively firing APs were also elicited by intracellular stimulation and frequently were preceded by stereotyped prepotentials which suggested their sites of origin were on processes remote from the cell body. In some cells, the AP could trigger long duration depolarizing afterpotentials (0.3-2 sec) with superimposed action potentials.

APs in these highly electrogenic cells were short (0.6-1.2 ms) with peak rates of rise from 320-1333 v/s (mean 696 ± 300 v/s, 24 cells), and corresponding rates of fall from 107-667 v/s (mean 351 ± 167 v/s, 24 cells). Following the AP, the afterhyperpolarization was usually short (< 10 ms), but postburst afterhyperpolarization could last < 2.5 sec).

Synapse formation was demonstrated in the following ways: by spontaneous occurrence of PSPs which were inhibitory (50/72 consecutive cells) or excitatory leading to APs; by reversal of inhibitory PSPs (Vrev ∿-40 mV); by the detection of synaptically coupled cell pairs; and by ultrastructural identification of synaptic junctions. Electronmicroscopy further revealed multiple synaptic contacts on single processes with a predominance of asymmetric over symmetric junctions.

Neural networks which formed in these cultures demonstrated reciprocal innervation (3/19 synaptic pairs) and multiple innervation (2/19 pairs). Widespread synchronous bursting could occur with many cells participating in the bursting (up to 25 cells per microscopic field have been found).

These experiments show that a well developed branching morphology and a broad range of electrophysiologic phenomenology are found in hippocampal neurons which have been grown in culture for 1-2 months. (Supported by NIH Grant NS12151).

1902 CILIARY GANGLION NEURONS MAY FORM INEFFECTIVE SYNAPSES IN DISSOCIATED CELL CULTURE. Guillermo Pilar and Jeremy Tuttle.* Physiology Section, Biological Sciences Group, University of Connecticut, Storrs, Connecticut 06268.

Neurons in dissociated cell culture vary in their ability to form functional synapses. While synapses between sympathetic ganglion cells and among spinal cord cells seem common, functional synapses between dorsal root ganglion cells are either very rare or do not occur. This study examines synaptogenesis between dissociated ciliary ganglion neurons in cell culture.

Electron micrographs of ciliary ganglion neurons in culture include profiles with typical synaptic ultrastructure (bouton-like "endings" with aggregates of clear vesicles along the "presynaptic" membrane; evidence of "post-synaptic" electron density) and these profiles are often found on the cell soma as well as along neurites. However, intracellular recordings from over 100 neurons in these cultures have failed to detect synaptic potentials. Recordings were taken in growth medium, normal saline and high-Ca^{++} saline; and they include simultaneous recordings from cell pairs with direct intracellular stimulation of each in turn, and single-cell recordings using extracellular stimulation of other neurons and processes. The neurons in these cultures have adequate levels of choline-acetyltransferase activity, and will take up ^{3}H-choline from the medium and synthesize ^{3}H-acetylcholine (Ach). Thus the observed lack of synaptic interaction is probably not due to a defect in transmitter metabolism. Rather, the results suggest a defect in synaptic transmission such that the neuron-neuron contacts formed are ineffective.

This possibility was examined by measuring the sensitivity of dissociated ciliary ganglion neurons to iontophoretically applied Ach. When neurons from St. 36-37 embryoes were tested at 24 hrs. after dissociation, most of the cells depolarized and discharged in response to short (3 ms) pulses of Ach. However, only about half of the cells tested at 3 da. in culture were sensitive, and none of a small sample (n=8) tested at 5-11 da. in culture showed a depolarization to Ach. Ach was also iontophoresed onto myotubes co-cultured with some of these neurons, using the same iontophoretic micropipettes. All of the myotubes depolarized in response to much lower doses of Ach, whether or not they were contacted by nerve fibers. While we cannot at this time exclude a defect in the neuronal release of Ach, our findings suggest the failure to record synaptic potentials from these cultured neurons is due to a very low neuronal sensitivity to Ach. This lack of sensitivity to Ach may be a cellular response secondary to the trauma inherent in tissue isolation. Its basis, and the ability of these cells to release Ach is under study. Supported by NIH-NS10338, NS5382, and the Univ. of Conn. Research Foundation.

1903 FORMATION OF CHOLINERGIC SYNAPSES BETWEEN EMBRYONIC CHICK SYMPATHETIC NEURONS AND SKELETAL MYOTUBES DEVELOPING IN DISSOCIATED CELL CULTURE. Barbara Pope* and Paul H. O'Lague (SPON: William L. Byerly.) Dept. Biology, UCLA, Los Angeles, CA 90024.

Monolayer cultures of isolated sympathetic neurons and skeletal myotubes were made by dissociating the superior cervical ganglia of 13-14 day-old chick embryos and adding the resulting cell suspension containing primarily neurons and a small number of non-neuronal cells to the myotubes. These myotubes had formed from myoblasts isolated from 11 day-old chick embryonic pectoral muscle and plated onto collagen-coated coverslips (10^5 cells/cm^2) 4-7 days prior to the addition of the neurons. 10-31 days after the addition of the neurons, neuron-myotube pairs were tested with intracellular microelectrodes for synaptic interaction. In 27 of 150 pairs tested an action potential evoked in a neuron by an intracellular depolarizing current pulse gave rise after a latency (range 2 to 20 msecs) to an excitatory junction potential (e.j.p.) in a myotube. The amplitudes of the e.j.p.'s ranged from 3 to 25 mV. In the few cases tested, the e.j.p.'s were blocked by d-tubocurarine (1 μg/ml) and α-bungarotoxin (5 x 10^{-7} g/ml). The effect of these agents on the neuron-myotube interaction suggests that transmission was nicotinic-cholinergic. In a few experiments neuron-neuron pairs in the cultures were tested for interaction; excitatory synaptic potentials were found. The characteristics of this interaction are presently under investigation. Supported by USPHS Grants NS12901, 5-S07RR07009-12, and a Muscular Dystrophy Association postdoctoral fellowship to B.P.

1904 α-BUNGAROTOXIN BINDING SITES AND ACETYLCHOLINE RECEPTORS IN CHICK CILIARY GANGLION NEURONS IN CULTURE. Peter Ravdin*, Ralph Nitkin, and Darwin Berg. Dept. of Biology, UCSD, La Jolla, CA. 92093.

We have found that neurons from a parasympathetic source, the chick ciliary ganglion, bind α-bungarotoxin (α-Bgt) when grown in dissociated cell culture. Toxin concentrations adequate to saturate sites revealed by fluorescent toxin do not block the sensitivity of the neurons to iontophoretically applied acetylcholine (ACh).

Neurons obtained by trypsin dissociation of 8-day embryonic chick ciliary ganglia were grown for 1-2 weeks in cell culture with chick skeletal myotubes. Tetramethyl rhodamine-conjugated α-Bgt was used to visualize the distribution and relative site densities of toxin binding sites on the neurons. After 1 hour in 0.1 μM toxin, fluorescence microscopy revealed that both the neuron cell bodies and the processes had toxin binding sites. The labelling of individual cell bodies was generally uniform with occasional small clusters appearing on a few of the neurons. A wide range in the intensity of toxin labelling was found among different neurons in many of the culture dishes; some apparently morphologically normal neurons had no detectable labelling, while others in the immediate vicinity were labelled quite brightly. Fluorescent toxin binding could be blocked by 140 μM d-tubocurarine or excess unlabelled toxin. Prelabelling with 0.1 μM unlabelled toxin blocked most of the fluorescent toxin bindign, suggesting that the sites had been largely saturated. [^{125}I]α-Bgt binding studies indicated a mean site density of ca. 10^6 per neuron.

The neurons showed a wide variability in their sensitivities to iontophoretically applied ACh even after normalization for differences in resting potential (mean: 51 mV) and input impedance (mean: 45 MΩ). Preincubation with 0.1 μM α-Bgt for one hour followed by sensitivity measurements in the presence of toxin resulted in a complete blockade of ACh sensitivity on the myotubes but caused no significant change in the sensitivity of the neurons. The sensitivities for neurons in control cultures was 104 mV/nC (SEM=23, n=18) and in toxin treated cultures was 82 mV/nC (SEM=19, n=12).

Thus chick parasympathetic neurons have a high affinity binding site for α-Bgt that is distinct from the active site of the ACh receptor, as has been shown for sympathetic neurons (Carbonetto et al., PNAS 75: 1016 (1978)) and a pheochromocytoma cell line (Patrick and Stallcup, PNAS 74: 4689 (1977)). While the relationship between the toxin binding site and the ACh receptor in the membrane of ciliary ganglion neurons remains to be determined, it is clearly different from that observed for skeletal myotubes. (Supported by USPHS Grant #12601, The Muscular Dystrophy Association, and The American Heart Association.)

1905 IN VITRO MAMMALIAN PACEMAKER NEURONS. Michael S. Raybourn and Cornelius A. Tobias. Radiation Biophysics Group, Donner Lab/LBL, Univ. of California, Berkeley, Berkeley, Ca. 94720

We are currently investigating the cellular bioenergetics of neuronal pacemaker cells in mammalian tissue cultures. In our roller-tube cultured explants from rat cerebellum, we can record the spontaneous electrical activity of Purkinje cells during liquid and/or gas phase presentations of various agents. Application of synaptic blocking agents results in a complete cessation of spontaneous activity in about one-half of the Purkinje cells (non-PM). These cells presumedly derive their driving force from their synaptically-mediated inputs. The rest of the Purkinje cells revert from their normal stochastic firing patterns to a clock-like pacemaker (PM) rhythmicity. These latter cells must possess an endogenous pacemaker for such activity.

Using various agents to interrupt the intracellular electron transport chain activity (hypoxia, carbon monoxide), we are attempting to correlate the threshold sensitivity of a given Purkinje cell subtype (i.e. PM,non-PM) to these insults. Presumed differences in metabolic requirements due to the presence or absence of an endogenous pacemaker mechanism may well be resulting in significant differences in susceptibility to such insults. We have found dose-dependent effects on Purkinje cell spontaneous activity due to gas-phase hypoxic and carbon monoxide insults. These effects are generally photodisassociable and we are currently attempting to determine the action spectra for this in order to localize the site of direct cellular toxicity. In addition, we are using the protein synthesis inhibitor, Anisomycin, in order to ascertain what role, if any, that protein synthesis might play in determining the endogenous activity of pacemaker Purkinje cells.

1906 COORDINATE BIOCHEMICAL AND MORPHOLOGICAL CHANGES DURING PHENOTYPIC CONVERSION OF HUMAN NEUROBLASTOMA CELLS. R.A. Ross & D.J. Reis (Lab. Neurobiol.,Cornell Univ. Med. Coll., New York, NY) and B.A. Spengler* & J.L. Biedler* (Lab. of Cellular & Biochemical Genetics, Memorial Sloan-Kettering Cancer Center,New York, NY)

The continuously cultured human neuroblastoma cell line SK-N-SH was analyzed to study the relationship between its biochemical and morphological properties. Biochemically, the cell line is adrenergic, expressing activities for tyrosine hydroxylase (17.3 ± 2.2 pmol/hr/mg) and dopamine- -hydroxylase (DBH)(14.66 ± 0.42 nmol/hr/mg), but not choline acetyltransferase or glutamate decarboxylase. Morphologically, the line consists of two different cell types: a neuroblast-like cell with neuritic processes and a small rounded cell body and an epithelial-like cell without processes and with a flattened cell body. Biochemical analysis of clones of these two cell types shows a clear correlation between the cell's morphology and its DBH activity: a neuroblast-like clone (SH-SY5Y) having high DBH activity (11.13 ± 0.4 nmol/hr/mg) and an epithelial-like clone (SH-EP) having no DBH activity. A clone with intermediate morphology (SH-IN) has intermediate DBH activity (7.63 ± 0.4 nmol/hr/mg). During long-term culture of the neuroblast-like clone, a small proportion(5%) of the cells began to express an epithelial-like morphology, suggesting that these two cell types may interconvert in cell culture. To test this hypothesis, subclones were isolated from the SH-EP cells which had begun to show morphological divergence from their usual epithelial appearance. The presence of a unique chromosomal marker in SH-EP cells was used to confirm that the subclones were derived from the SH-EP cells. Nine subclones were isolated and all contained the unique chromosomal marker. All were assayed for DBH activity. The two epithelial-like subclones were without DBH activity while the 3 with neuroblast-like morphology expressed DBH activity (5.77 ± 0.14 nmol/ hr/mg). Four subclones with intermediate morphology expressed intermediate levels of DBH activity (3.67 ± 0.09 nmol/ hr/mg). Thus changes in cell morphology were directly correlated with changes in DBH activity.

Using morphological and biochemical markers for neuronal differentiation, we have shown that the two widely differing cell types of the human neuroblastoma cell line SK-N-SH show coordinate morphological and biochemical bidirectional interconversion in culture. We speculate that these two cell types may represent extremes in a spectrum of phenotypic expression , one representing a differentiated neuroblast with a small, rounded cell body, neurite-like processes, and neurotransmitter-synthesizing enzymes and the other an undifferentiated neuroepithelial cell with a flattened cell body and lacking neuritic processes and neurotransmitter-synthesizing enzyme activity. Supported by grants HL 18974, NS 03346, NS 06911, CA 08748, & CA 18856.

1907 PINEAL CELLS ENHANCE CHOLINE ACETYL TRANSFERASE ACTIVITY IN MONOLAYER COCULTURE WITH CELLS DERIVED FROM SUPERIOR CERVICAL GANGLIA. Vernon Rowe* and James Parr* (SPON: James Couch). Neurology Research Lab, VA Hospital, Kansas City, MO 64128 and Dept. of Neurology, Univ. of Kans. Med. Ctr., Kansas City, KS 66103.

Monolayer cultures of cells derived from neonated rat pineal glands and superior cervical ganglia (SCG's), by trypsinization and trituration, were plated in replicate fashion. The replicates contained cells derived from either pineal, or SCG, or both. All cultures were plated onto collagen, and were maintained in identical culture media containing 2.5S nerve growth factor under identical conditions. Tyrosine hydroxylase (TH), choline acetyltransferase (CAT), and serotonin N-acetyltransferase (NAT) activities were measured at 18 days of culture age. At this time, TH activity was very low in all replicates (0.2-0.3 nmoles/mg protein/hr). NAT activity did not vary markedly among the cultures (800 to 1300 pmoles/dish/hour). CAT activity in the cocultures, however, was up to 10 times that observed in the SCG's cultured alone (180 vs. 18 pmoles/mg protein/min). Pineals cultured alone did not contain detectable CAT activity.

These data are consistent with the interpretation that adrenergic target cell influence is insufficient, in itself, to develop and maintain adrenergic specification in the developing peripheral autonomic nervous system. Indeed, adrenergic target tissue, in the absence of presynaptic influences, can produce cholinergic specification in immature sympathetic neurons.

1908 ELECTROPHYSIOLOGIC PROPERTIES OF HUMAN DRG NEURONS IN CELL CULTURE:EFFECT OF POTASSIUM AND DEVELOPMENTAL STAGE.Brian S. Scott, Ted L. Petit and Lawrence E. Becker.* Surrey Place Centre, Toronto, Ontario, Canada M5S 2C2.

The electrical properties of human neurons have been characterized quantitatively through an extensive intracellular electrophysiologic investigation of cell cultures of dorsal root ganglia (DRG). In three cases, cultures were prepared from fetal DRG (13, 23 and 27 weeks of gestation) and in one case from more mature DRG (3 months post-natal). No neuropathology was detected in these cases.

The DRG were softened in collagenase, dissociated and plated on collagen coated coverslips. The culture medium consisted of 10% fetal calf serum in CMRL-1415 with both normal (4mM) and elevated (20mM) potassium (K). The later has been found to enhance neuron survival.

After periods in culture ranging from 6 to 62 days, cultures (both 4 and 20mM medium) were transferred to 4mM K and the following features examined:resting membrane potential (Vm); cell input resistance (Ri), specific membrane resistance (Rm), rheobase (Irh), voltage change at rheobase (Vrh=Irh x Ri), duration of the action potential (APt), time constant (TC, determined from strength duration data), and absolute refractory period (ARP). The table below illustrates the results obtained from all four specimens grouped according to the K concentration of the culture medium.

K	Vm**	Ri**	Rm	Irh**
mM	(mV)	(Mohm)	(ohm·cm^2)	(nAmp)
4	48.7±.79(222)	22.5±.79(178)	1074±.39(176)	.65±.03(167)
20	52.7±.60(307)	19.5±.81(205)	1008±.46(202)	.91±.04(206)

K	Vrh*	APt**	ARP**	TC**
mM	(mV)	(msec)	(msec)	(msec)
4	12.3±.55(156)	3.34±.13(135)	3.51±.12(126)	3.68±.14(164)
20	14.5±.74(171)	2.38±.11(152)	2.77±.11(143)	2.81±.12(192)

* P<.05, ** P<.01, $\bar{X}\pm$ S.E.M., N in brackets

The changes in Ri and Irh were due to an increase in cell size in elevated K. However analysis of variance with cell size as a covariate showed that K exerted a small but significant effect, independent of cell size, on the other significantly different variables.

The data, limited to four cases with respect to developmental stage, showed that cell size also increased with age. Rm, APt, ARP and the TC were also found to increase with age independent of cell size, but more cases are required to confirm this phenomenon.

Supported by a grant from the Physicians' Services Incorporated Foundation.

1909 PROLIFERATION OF GLIAL CELLS IN CONTINUOUS CELL CULTURES FOLLOWING KAINIC ACID LESIONS OF RAT STRIATUM. V.K. Singh, E.M. Bohn*, and D. Van Alstyne*. Immunology Unit, Children's Hospital, Vancouver, B.C. V5X 1X2, Canada.

Glial cells grow to confluency in vitro following their dissociation from kainic acid lesioned striatum of normal adult rat brain. The procedure involved the induction of a specific neuronal degeneration, achieved by stereotaxic injections of 5 nmoles of kainic acid into caudate-putamen nucleus (Singh et al., Brain Res. 147: 1978), dissection and mincing of the tissue in the presence of growth medium (MEM containing 0.2% glucose, 10% fetal calf serum 5 units/ml penicillin and 5 µg/ml streptomycin) and distribution of the dissociated tissue in glass tissue culture dishes.

The cells from kainic acid lesioned striatum proliferate to form complete monolayers within 4-5 days and exhibit contact inhibition. The cell growth, which is sensitive to actinomycin D and colchicin, is correlated with an active synthesis of DNA, RNA and protein. Morphologically several distinct cell populations, including astrocytes and oligodendrocytes, are observed. The cells are characterized by the presence of glial fibrillary acidic protein (GFAP) localized through immunofluorescent and immunoperoxidase techniques. This cell line appears to be more susceptible to lysis by rubella virus than are the more commonly used rabbit or baby hamster kidney cell lines.

(Supported by a grant from the Multiple Sclerosis Society of Canada).

1910 ACETYLCHOLINE RECEPTOR AND ESTERASE: EVIDENCE FOR DISTINCT PATHWAYS OF INTRACELLULAR TRANSPORT. Henry Smilowitz* (SPON: Yvonne Grimm-Jorgensen), Dept. Pharmacol., U. Conn. Health Ctr., Farmington, CT 06032.

Most of the acetylcholinesterase (ACHE) that is synthesized by chick embryo pectoral muscle cells in culture is released into the culture medium [Wilson B.W. et al., Dev. Biol., 33: 285-299, 1973]. We have found that ACHE release (measured as ACHE activity appearing in diisopropyl fluorophosphate (DFP) treated culture medium) can be stimulated 60% by 5×10^{-8}M of the calcium ionophore A23187 and can be inhibited by the carboxylic ionophores which transport monovalent cations. For example, release of ACHE is inhibited 50% by 4×10^{-8}M Monensin, or 1.6×10^{-8}M Nigericin or 7.5×10^{-8}M X537A [Smilowitz, H., Fed. Proc. 37: 788, 1978]. The inhibition of ACHE release by the ionophores is not due to an overall inhibition of protein synthesis. And both the inhibition of ACHE release and the accompanying accumulation of ACHE activity by the cells can be fully reversed within 2-4 hours after removal of the drug.

The rate of appearance of cell surface acetylcholine receptors (AChR) (measured as the rate of appearance of I^{125} α-bungarotoxin binding sites) is unchanged by concentrations of ionophore which maximally inhibit ACHE release. Experiments employing ACHE histochemistry and electron microscopy reveal intracellular membrane vesicles in ionophore treated cells which stain heavily for ACHE. Further, the ACHE of ionophore treated cells is less accessible to degradation by trypsin and collagenase. Preliminary cell fractionation experiments indicate that the ACHE which accumulates in ionophore treated muscle cells can be found on membranes that are distinct from plasma membrane. These experiments suggest that the ACHE accumulates on intracellular membranes in ionophore treated cells that are inaccessible to externally applied agents, while the acetylcholine receptor proceeds normally to the plasma membrane and remains accessible to externally applied α-bungarotoxin. We propose that the acetylcholinesterase and the acetylcholine receptor are transported intracellularly by distinct membranous pathways; one is profoundly inhibited by the monovalent ionophores, the other is unaffected. Supported by NIH NS13860.

1911 MODULATION OF cAMP-INDUCED PROCESS FORMATION AND ConA-INDUCED CAP FORMATION IN CULTURED GLIOMA CELL LINES. Barry H. Smith, Theodore M. Liszczak*, Rogers Pleasants*, and Paul L. Kornblith*. Neurosurgical Service, Massachusetts General Hospital, Boston, MA 02110

The molecular mechanisms of cyclic AMP-induced process formation as well as conconavalinA-induced cap formation have been studied in a cultured human glioma cell line (Mi) and four clones derived therefrom.

The parent cell line, as well as all derived clones, form processes in the presence of 10^{-3}M dibutyrl-cAMP, although the percentage of cells exhibiting such processes varies from clone to clone (i.e., from 50 to 80% of the population at 48 hrs.). Ultrastructurally such processes are characterized by prominent bundles of microfilaments as well as by microtubules all arrayed in the long axis of the process.

Local anesthetic agents (dibucaine 10^{-4}M, lidocaine 10^{-4}M, and procaine 10^{-4}) which are membrane-active agents as well as microfilament-active agents, block this process formation with dibucaine being the most effective (∿ 100%). The microtubule-active agent vincristine (.0005 mg/ml) also blocks such processes but cytochalasin B, (12.5 µg/ml), which interferes with microfilament function, results in very short, highly-branched processes, suggesting that microfilaments are important for elongation. Protein synthesis, as evidenced by the addition of 10^{-6}M puromycin, is not required for process formation. Metabolic inhibition (Na azide, 10^{-2}M), although it reduces the total number of cells as well as the proportion of cells with processes, allows process formation to occur. 10^{-3}M Ca^{++} in the medium results in the formation of extremely long processes. Phosphodiesterase inhibitors (theophylline, 10^{-3}M and papaverine, 10^{-3}M) enhance process formation when added with dibutyrl-cAMP. Papaverine alone produces bipolar cells with extremely long, wide processes, whereas theophylline alone is without effect. Relative movements of surface-bound molecules were monitored by binding with ConA (5-100µg/ml) which reliably produces patching and capping in this glioma line. Formation as well as internalization of the caps has been followed by light, scanning, and transmission (electron microscopy). Microfilaments (40-60 Å) appear necessary for cap formation and internalization, but microtubules, perhaps by virtue of a role in stabilizing the cytoskeleton, may be inhibitory. These postulates are supported by the fact that dibucaine (10^{-4}M) blocks cap formation as does dibutyrl cAMP, which has been associated with microtubule assembly. In the case of dibucaine treatment, aborted caps appear to form on short, microfilament-free processes.

We conclude that microfilaments, microtubules and associated membrane states are critical elements in cAMP-induced process formation, as well as conA-induced cap formation, in our glioma cell lines, although the two processes differ in their mechanisms.

1912 ELECTROPHYSIOLOGICAL PROPERTIES OF ISOLATED SYMPATHETIC NEURONS DEVELOPING IN MICROCULTURES. Trisha Suppes and Paul H. O'Lague. Dept. Anatomy and Dept. Biology, UCLA, Los Angeles, CA 90024.

Intracellular microelectrodes were used to study the electrophysiological properties of sympathetic neurons developing in microcultures. These cultures consisted of small (<300 microns in diameter) islands of cardiac myocytes and one or a few (<5) neurons isolated from mechanically dissociated superior cervical ganglia of newborn rats as described previously (Furshpan et al., PNAS 73: 4225, 1976). 18-90 days after plating the neurons, their electrophysiological properties were examined during continuous perfusion of the cultures (see above reference for methods and perfusion media); all neurons tested (>100) had resting potentials in the range -55 to -70 mV, action potential amplitudes of 65 to 90 mV, and maximum rates of rise of 200 to 480 V/sec. These values are comparable to those reported for the neurons in mass cultures (O'Lague, P.H., Potter, D.D., and E. J. Furshpan, Devel. Biol., in press) and for sympathetic neurons in superior cervical ganglia of adult rats (Perri et al., Pflugers Arch. 14:40, 1970). The ionic basis of the action potential in the neurons was studied with the pharmacological agents tetrodotoxin (TTX), a blocker of voltage-sensitive Na^+ channels, tetraethylammonium (TEA), a blocker of voltage-sensitive K^+ channels, and Co^{++} and Mn^{++}, both blockers of voltage-sensitive Ca^{++} channels. TTX (0.5-3 µM; 40 cases tested) abolished the action potential evoked by a current pulse; the remaining depolarization was graded with the strength of the current pulse and could be transformed into an all-or-none response by addition of TEA (5-25 mM) to the TTX perfusion media. This all-or-none response was dependent on extracellular Ca^{++} (16-20 mV per 10 fold change), was independent of changes in extracellular Na^+ and abolished by Co^{++} (2-5mM, 4 cases) or Mn^{++} (1mM, 2 cases). This evidence suggests that Na^{++} and Ca^{++} carry inward current during the action potential. In many neurons, usually in older cultures (>3 weeks), the action potential was followed by a long lasting (150-450 msec) afterhyperpolarization (LAH) of 5-15 mV peak amplitude. This LAH was due to an increase in K^+ permeability triggered by Ca^{++} ions entering during the action potential. Evidence for this is: 1) the LAH was accompanied by a substantial decrease in membrane resistance (in some cases up to 30%); 2) it reversed at the K^+ equilibrium potential; and 3) it was greatly reduced in amplitude and duration by lowering the extracellular Ca^{++} or by addition of Co^{++} and Mn^{++} at the same concentrations which block the Ca^{++} action potentials in these cells. Whether in a single neuron the expression of the above electrophysiological properties changes as it develops in culture and whether electrical activity affects this expression are presently under investigation.

Supported by USPHS Grant NS12901.

1913 NEURONAL SURVIVAL AND CAT ACTIVITY IN DISSOCIATED CELL CULTURES OF CILIARY GANGLION. Jeremy Tuttle*, March Ard*, and Janusz Suszkiw*, (spon: J. Alanis). Physiology Section, Biological Sciences Group, Univ. of Connecticut, Storrs, Connecticut 06268.

This study intends to better define the culture conditions necessary for the survival and development of ciliary ganglion neurons, and to describe the developmental pattern of choline-acetyltransferase activity in culture.

All cultures were prepared from ganglia dissected at embryonic stage 29-32. The effect upon subsequent cell survival was determined for the following other variables: 1) Plating density. No effect of plating density was noted between 9000 and 30,000 neurons cm^2. 2) Basic medium. Cell growth was most rapid when supplemented MEM was used. Supplemented L-15, L-15, DMEM, and highly supplemented MEM were not as suitable. 3) Serum additions. Bovine serum (newborn and fetal), and human serum (from a single source and pooled), (10% V/V), were all unsatisfactory, for survival at 2-4 days in culture was <20% and zero at 1-2 weeks. Heat-inactivated horse serum (HS, 10% V/V) allowed neuronal survival at reduced levels for no more than ten days. 4) Chick embryo extract (CEE) was necessary for long-term survival. 10% (V/V) CEE in supplemented MEM without serum could support 30% survival for three weeks in culture. At 1% CEE, without serum, all cells died in 5 days. When 10% CEE was combined with 10% HS, up to 90% of the neurons plated could survive three or more weeks in culture. 5) 50% of plated neurons could survive at least one month in the absence of other cells in fresh 10HS10CEEMEM, but only 20% remained at 3 weeks in conditioned medium. Neurons survived best when grown on collagen along with myoblasts, for >80% survived at least one month.

The cholineacetyltransferase (CAT) activity in these cultures was also determined over three weeks in culture. CAT activity per cell increased 100-fold during the first two weeks in culture both with and without myoblasts. Cultures in conditioned medium showed a marked but lesser rise in CAT activity. This development of CAT activity in dissociated cell culture differs from that in vivo as follows: 1) CAT activity increases at a later time in cell culture. This may reflect a period of recovery after dissociation. 2) In vivo, the rise of CAT activity continues past embryonic stage 40 to much higher levels while the initial sharp rise in activity in vitro is followed by a slower increase or drop, depending upon the culture substrate used. These results suggest: 1) Some component of CEE is critical for the survival of ciliary neurons in culture. 2) Interactions with other cells or other unknown factors are necessary for the full expression of normal CAT development in vitro. Supported by NIH-NS10338, NS5382 and The Univ. of Conn. Research Foundation.

1914 TROPHIC AGENTS DIRECTED TO CILIARY GANGLIONIC CELLS IN MONOLAYER CULTURES. Silvio Varon, Ruben Adler and Marston Manthorpe* (SPON: A.L. Miller). Dept. Biol., Sch. Med., U. Ca. San Diego, La Jolla, CA 92093.

Neurons dissociated from 8-day chick embryo ciliary ganglia (CG) will not survive in monolayer cultures with medium supplemented only with serum. Other investigators have reported their survival on polyornithine substrata in the presence of heart-conditioned medium (Helfand et al, Dev. Biol. 50, 541, 1976), on pre-established cultures of myotubes (Nishi & Berg, PNAS 74, 5171, 1977), or on collagen in medium supplemented with embryo extract and horse serum (Tuttle, Soc. Neurosci. Abst., III, 529, 1977). We report here the detection of different trophic agents (directed to CG neurons) in chick embryo extract (CEE), heart-conditioned medium (HCM), and fetal calf or horse sera. CG's were dissociated after mild trypsin treatment, and seeded in dishes coated with either a highly adhesive collagen or poly-ornithine (P-ORN). One-day cultures were counted differentially for total and neurite-associated large phase-bright (LB) cells, and analyzed for choline acetyltransferase (CAT). CEE was produced from 12 day whole embryos. HCM was obtained by incubating serum-containing medium for 48 hr over 2-day old cultures of 8-day chick embryo heart cells.

CEE supplementation (25 µg protein/ml) provided: i) in the absence of serum, no survival of LB cells on either collagen or P-ORN, ii) in combination with 10% fetal calf, high numbers of LB cells on both substrata but some neuritic outgrowth only on collagen, or iii) in combination with 10% horse serum, high LB cell numbers on both substrata and high neurite production on collagen but not on P-ORN. HCM supplementation (at 50% dilution) yielded: i) with no serum present during conditioning, no LB support on either substratum even after serum addition, ii) with fetal calf serum (both before and after conditioning), high numbers of LB cells and extensive, elaborate neurites on P-ORN but lower numbers and no neurites on collagen, or iii) with horse serum throughout, slightly lower LB cell numbers than with fetal calf serum and the same neurite development on P-ORN but no neurites on collagen. The occurrence of different active agents in the two trophic sources (CEE and HCM) is indicated by their dependence on different substrata for neuritic promotion (even though both substrata are competent with the correct trophic source), as well as by their different serum requirements. In addition, serum constituents are necessary, though not sufficient, contributors to the trophic effectiveness of either source. Lastly, comparison of CAT activities in these several culture situations suggests that regulation of this enzyme may be partly independent from cell numbers and/or neurite production. (Supported by USPHS grant NS-07606).

1915 LARGE-SCALE CULTURE OF NON-NEURONAL CELLS AS AN ADJUNCT TO SPINAL CORD RECONSTRUCTION. J. Wrathall*, C.C. Kao* and D.Rigamonti (SPON: M. DeSantis). Department of Anatomy, Georgetown University Schools of Medicine & Dentistry, Washington, D.C. 20007.

In a previous report (Kao et al. J. Neurosurg. 46:757, 1977) on a delayed nerve grafting technique for spinal cord reconstruction, proliferation of Schwann cells and fibroblasts from the grafted nerve was observed one week after placement of the graft. These cells grew into the cut ends of the spinal cord and appeared to facilitate axonal outgrowth from the spinal cord and subsequent bridging of the transection. To attempt to enhance this process, we have prepared large-scale cultures of mitotically active non-neural cells from peripheral nervous tissue to be used as an adjunct to the reconstruction procedure.

Dorsal root ganglia, or segments of sciatic nerve, from the adult cat were minced, incubated with trypsin or trypsin-EDTA solutions, and dissociated by mechanical agitation with a vortex mixer. The cell suspensions obtained were plated on collagen-coated dishes and cultured in a medium consisting of Eagle's MEM, non-essential amino acids, 6 mg/ml glucose, 10% fetal calf serum and 50 µg/ml Gentamicin. The cultures were maintained at 36.5° in an atmosphere of 5% CO_2 - 95% air, and subcultured weekly with the aid of trypsin-EDTA solution. One cell line derived from sciatic nerve appears to consist of larger fibroblast-like cells and smaller, spindle-shaped, refractile cells with elongated oval nuclei. This mixture of cell types has been maintained over 10 subcultures to date. Two cell lines derived from dorsal root ganglia in separate experiments, were morphologically similar to one another. These cultures consist almost exclusively of small, spindle-shaped, refractile cells with elongated oval nuclei and resemble cultured Schwann cells described by others. Electron-microscopic examination of pellets of trypsinized cells showed that these cells have irregular nuclei, dilated rough endoplasmic reticulum containing material of a density that is similar to the cell matrix and well-developed Golgi. These cell lines, and that derived from sciatic nerve, have been successfully frozen in liquid nitrogen using 10% dimethyl sulfoxide. Cells derived from both tissue sources have been used individually, or in combination, in spinal cord reconstruction experiments. For this purpose, cultures were harvested using trypsin-EDTA solution, the cells counted in a hemocytometer and viability estimated by trypan-blue exclusion. Cells were washed with a balanced salt solution by centrifugation and pellets containing about 5×10^7 viable cells were prepared. Portions of the pellets have been applied to the cut ends of the spinal cord just prior to the placement of the nerve graft, in a cat transected cord model system.

(Supported by: NIH NS14413-01)

1916 OUTGROWTH OF NEURONAL PROCESSES IN VITRO FROM THE RETINAL TISSUE INTO THE TECTAL TISSUE, EXPLANTED FROM THE ADULT GOLDFISH. Myong G. Yoon, Dept. of Psychology, Dalhousie Univ., Halifax, N. S., Canada, B3H 4J1.

The patterns of neuritic outgrowths from the retinal tissue into the tectal tissue are investigated in vitro under various experimental conditions by culturing the neural tissues, explanted from the visual pathways of the adult goldfish. A rectangular piece of the retinal tissue is dissected free and implanted on a culture dish in a predesignated orientation. Its topographically matching area of the optic tectum is also dissected from the same fish, and co-cultured with the retinal explant in various geometric configurations within the same culture dish. Under favorable culture conditions, the retinal tissue shows vigorous outgrowths of neuronal processes as early as 12 hrs after explantation. These retinal neurites have bush-like growth cones which contain several philopodia at the advancing tips. Autoradiographic examination reveals that if (H^3) L-proline is injected into the eyeball about 24 hrs before explantation of the retinal tissue, the sprouting neuronal processes from the retinal explant later show intense labelling throughout their entire extent, including the growth cones at their advancing tips. The labelling in the retinal neurites persists as long as the culture is maintained for up to 6 weeks. The direction of neuritic outgrowths from the retinal explant in vitro seems to follow the regularity in the embryonic development or regeneration of the axons of retinal ganglion cells in vivo: the retinal neurites tend to grow in the radial direction towards the optic disc at the center of the retina, especially at an early stage in culture. Some of the retinal neurites eventually grow into the co-cultured tectal tissue. It is not known as yet whether these retinal neurites would establish functional connections with appropriate visual neurons of the tectal tissue in vitro. In contrast to the retinal tissue, the tectal explant does not show any neuritic outgrowth in the same culture dish for a period up to 6 weeks.

(Supported by grants from NRC and MRC of Canada.)

TROPHIC FUNCTIONS

1917 ADVERSE EFFECTS OF TETRODOTOXIN ON EARLY DEVELOPMENT AND SURVIVAL OF POSTSYNAPTIC CELLS IN SPINAL CORD CULTURES. Gregory K. Bergey*, Robert L. Macdonald and Phillip G. Nelson. Laboratory of Development Neurobiology, NICHD, NIH, Bethesda, Md. 20014.

Dissociated fetal mouse spinal cord cultures were used to assess the effects of chronic action potential blockade on neuronal survival. Dorsal root ganglion (DRG) cells and spinal cord (SC) cells in culture are easily distinguished morphologically and electrophysiologically. Intracellular recordings show that SC cells commonly exhibit ongoing action potentials and postsynaptic potentials, whereas DRG cells, while electrically excitable and capable of establishing synapses with SC cells, are electrically quiescent and receive no synaptic input when grown in culture. Previous electron microscopic examination has shown cultured SC somata to be heavily invested with synaptic terminals; DRG somata appear devoid of innervation.

Exposing combined SC-DRG cell cultures to 10^{-6}M or 10^{-7}M tetrodotoxin (TTX), a specific blocker of sodium conductance channels in neurons, completely blocked all spontaneous electrical activity. When cultures were grown in medium containing these concentrations of TTX from day 1 or day 8, examination of the cultures at 5 weeks revealed a marked reduction in the number of SC neurons while DRG cell counts were unaffected. SC cells larger than 30μ were reduced in number to less than 9% of controls ($p<.001$) while DRG cell counts were not significantly different from controls. If treatment with TTX was delayed until 10 weeks in culture no significant diminution of either SC or DRG cell counts was evident. Tetrodotoxin treatment did not affect the electrical characteristics of the DRG cells; resting membrane potentials and input resistances did not differ from the control values. The remaining SC cells were fragile and had lower resting membrane potentials than normal, but some surviving SC cells revealed evidence of synapse formation in TTX with both IPSPs and EPSPs being recorded. TTX is reported not to affect axoplasmic transport and no non-specific toxic effects have been reported at the concentrations used in these experiments. The specificity seen in the present experiments with regard to cell type and age of culture argues against such non-specific effects.

These results indicate that blockade of electrical activity in co-cultured mammalian DRG and SC neurons markedly affects the development and survival of neurons that normally receive synaptic inputs in culture. This suggests that action potentials and synaptic activity play crucial roles in central neuronal development during early stages of differentiation and synapse formation; at later states neuronal survival is less affected by blockade of electrical activity.

1918 RAPID LOSS OF JUNCTIONAL ACETYLCHOLINE RECEPTORS AFTER DENERVATION OF RAT SKELETAL MUSCLE. R. S. Brett* and S. G. Younkin. Dept. Pharmacology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106.

Previous studies of the mouse diaphragm (Porter & Barnard; Exp. Neurol. 48:542, 1975) and the rat soleus (Frank et al.; Cold Spr. Harb. Symp. 4:275, 1976) have found no decrease in the number of junctional acetylcholine receptors (AChR) in the early period after denervation. In this study we examined junctional AChR after denervation of rat diaphragm and extensor digitorum longus (EDL) muscles by measuring the binding of (^{125}I)α-bungarotoxin. Junctional AChR were evaluated by one method in diaphragm and another in EDL. Innervated and denervated (1,2,3,5 and 7-9 days) left hemidiaphragm muscles were labelled with toxin in vitro and dissected into a piece containing end-plates and several 1-mm wide pieces adjacent to the piece containing end-plates. The number of extrajunctional AChR in the end-plate-containing piece was estimated on the basis of the extrajunctional AChR found in the immediately adjacent end-plate-free piece and junctional AChR were calculated by subtracting this number from the total number of AChR in the end-plate containing piece. Junctional AChR were significantly ($P = 0.01$) decreased by day 5 and at 7-9 days after denervation were reduced to 58% of the innervated level. EDL muscles were denervated unilaterally and 7-8 days later AChR in denervated and contralateral innervated muscles were labelled in situ using the tibialis muscle to construct a pool which held a small volume of rat Ringer's solution containing 2.0 μg/ml (^{125}I) α-bungarotoxin. Rats were allowed to recover and 5 days later the radioactivity in each muscle was measured. At this time virtually all extrajunctional receptors have been degraded so residual radioactive toxin is bound almost exclusively to junctional AChR. In these experiments denervated muscles contained only 65% of the radioactivity measured in contralateral innervated muscles ($P = 0.001$ by the paired t-test). Using the methods described, we find decreases of 35% and 42%, respectively, in the number of junctional AChR in rat EDL and diaphragm muscles denervated for 7-9 days. The apparent discrepancy between this and earlier reports may be related to differing experimental conditions or may reflect a difference between slow (mouse diaphragm, rat soleus) and mixed or fast (rat diaphragm and EDL) muscles. Our results support the concept that innervation plays a significant role in maintaining the normal high concentration of junctional AChR as well as in suppressing extrajunctional AChR. (Supported by USPHS NIH grant NS 13027.)

1919 REGULATION OF ACETYLCHOLINE RECEPTOR METABOLISM BY DIRECT ELECTRICAL STIMULATION. Diana J. Card* and Douglas M. Fambrough. Carnegie Institution of Washington, Baltimore, MD 21210

As a result of denervation skeletal muscle synthesizes large numbers of extrajunctional acetylcholine receptors. Many studies have shown that extrajunctional ACh receptor numbers (or ACh sensitivity) are regulated by the contractile activity of the muscle. The aim of this study is to identify the mechanism by which electrical stimulation regulates ACh receptor metabolism. We have measured the effect of electrical stimulation on the synthesis (appearance in the extrajunctional muscle membrane) of ACh receptors and their subsequent degradation (or removal) from membranes of denervated rat skeletal muscles maintained in organ culture. ACh receptors were measured by specific and irreversible binding with radioactive α-bungarotoxin.

The rate of de novo biosynthesis was measured by determining the rate of appearance of ^{2}H, ^{13}C, ^{15}N-containing ACh receptors when muscles were cultured in medium containing ^{2}H, ^{13}C, ^{15}N-amino acids. Cultured 5-day denervated extensor digitorum longus (EDL) and soleus muscles were found to synthesize new receptors for several days in organ culture. Stimulation at 100 Hz for 1 sec every 80 sec, producing visible contraction, but not maximal tetanic tension, barely altered the rate of incorporation of new ACh receptors into extrajunctional plasma membrane of EDL and soleus muscles, even when applied for 5 days. Supra-maximal stimulation with the same stimulation pattern produced a rapid decline of 10-20% in rate of new receptor production and a corresponding decline in overall protein synthesis. Stimulation beyond 18-24 hr (up to 68 hr) resulted in a further decrease in new receptor production to about 30% of control rate, but no more. Stimulation for longer than 16 hr produced less than 5-10% decrease in overall protein synthesis compared with control muscles.

The degradation rate of extrajunctional ACh receptors was estimated by irreversibly labeling ACh receptors with radioactive iodinated α-bungarotoxin and measuring the rate of release into the culture medium of mono- and diiodo-tyrosine, breakdown products of the radioactive α-bungarotoxin. The rate of this proteolytic process which reflects the average ACh receptor lifetime was 22 hr. Electrical stimulation at 100 Hz for 1 sec was applied every 80 sec for up to 30 hr to produce maximal tetanic tension and had no effect on the apparent degradation rate of receptors in denervated EDL muslces.

Strong electrical stimulation, producing frequent tetanic contractions, may therefore, decrease receptor biosynthesis and thereby contribute to previously reported reduced ACh sensitivity.

1920 NGF-INDEPENDENT DEVELOPMENT OF MOUSE EMBRYO SYMPATHETIC NEURONS IN CELL CULTURE. Michael D. Coughlin and Ira B. Black, Dept. of Neurology, Cornell University Med. Coll., N.Y.,N.Y.10021.

The superior cervical ganglion (SCG) of the 14 gestational day (E14) mouse embryo extends neurites and differentiates biochemically when cultured in the absence of added nerve growth factor (NGF). In contrast, ganglia from newborn mice (NB) require added NGF for survival in culture. Co-culture of the E14 ganglion with target submandibular gland radically alters nerve growth fiber outgrowth and development of tyrosine hydroxylase (T-OH) activity in the ganglion. By 5 days in culture, ganglia grown with target tissue, even in the presence of anti-serum to NGF (Anti-NGF), exhibit a 2-fold increase in T-OH activity over ganglia grown alone or with non-target tissues. Ganglia grown with target salivary glands exhibit increased elaboration and directionality of nerve fiber outgrowth, even in the presence of Anti-NGF.

In the above experiments, ganglion support cells and/or target tissue may have transferred NGF to neurons through an antibody-resistant mechanism. To determine whether E14 and NB ganglion neurons are intrinsically different, ganglia were trypsin-dissociated and neurons were grown in single cell culture. E14 neurons, in medium without added NGF, attached to poly-ornithine coated culture dishes and extended neurites within 4 hours. Approximately 20% of the neurons plated survived for 24 hours. In direct contrast, NB neurons exhibited neurite extension only in the presence of added NGF. Heart cell conditioned medium (CM) enhanced survival and neurite extension in both E14 and NB neurons; the mechanisms involved, however, differed. In CM, 50% of the E14 neurons exhibited neurite extension at 24 hours and survival was not significantly affected by Anti-NGF. A similar percentage of NB neurons survived in CM, but this effect was abolished by Anti-NGF. Thus, E14 neurons, unlike NB neurons, are capable of surviving in cell culture without added NGF and without support cells. Moreover, E14 neurons, unlike NB neurons, respond to CM factor(s) which are insensitive to Anti-NGF.

(This work was supported by the NIH, the NSF, the Dysautonomia Foundation Inc. and the Hirschl Trust Fund.)

1921 A FACTOR RELEASED FROM NERVE BY STIMULATION PREFERENTIALLY INCREASES END-PLATE ACETYLCHOLINESTERASE. B. Davey*, S. G. Younkin, and L. H. Younkin. Dept. Pharmacology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106.

Supramaximal neural stimulation of a single sciatic nerve-extensor digitorum longus (EDL) muscle preparation at 7 Hz for 30-60 min releases a factor which causes a significant increase in the cholinesterase (ChE) activity of EDL muscles denervated in vivo for 3 days and then maintained in organ culture for 1 day. The release of this factor is not diminished when d-tubocurarine is used to block muscle contraction in the neurally stimulated preparation, but its release is diminished after presynaptic block with magnesium (Younkin et al., Science, in press). In this study we show (1) that the increase in ChE caused by this factor is virtually completely due to an increase in true acetylcholinesterase (AChE), (2) that the factor has a preferential effect on end-plate AChE, and (3) that protein synthesis is not required for the factor to be effective. To show that the increase in ChE caused by this factor is due to an increase in AChE the ChE of organ-cultured EDL muscles was measured without inhibitors present as described by Davey and Younkin (Exp. Neurol. 59, 168-175, 1978) and with either iso-OMPA (10^{-4} M), which selectively inhibits butyrylcholinesterase, or BW284C51 (10^{-5} M), which selectively inhibits AChE, present. The increase in total ChE (no inhibitors present) caused by the factor released by neural stimulation was 0.70 ± 0.11 umoles acetylthiocholine hydrolyzed per min per g muscle. Virtually all of this increase was due to an increase in AChE because the increase in iso-OMPA resistant ChE was 0.75 ± 0.05 and the increase in BW284C51 sensitive ChE was 0.64 ± 0.17. To assess the effect of the factor on end-plate and background AChE we examined segments of hemidiaphragm which were denervated in vivo for 3 days and then cultured for 1 day in control medium or in medium conditioned with neurally stimulated EDL muscle. In these experiments the factor released by nerve stimulation caused a dramatic (102%) and highly significant ($P<0.001$) increase in end-plate AChE but had essentially no effect on background AChE. Cycloheximide (10^{-5} M) was added to control medium and to medium conditioned with neurally stimulated EDL muscles to determine the extent to which the effect of the factor released by neural stimulation is dependent on protein synthesis. Cycloheximide decreased the AChE of 3-day denervated muscles cultured for 1 day in control or supplemented medium to about 60% of normal, but muscles cultured in the presence of factor continued to have an AChE which was significantly ($P<0.05$) higher (20-30%) than that of muscles cultured in the corresponding control medium. (Supported by USPHS NIH grant NS 13027.)

1922 ANATOMY AND PHYSIOLOGY OF AXOTOMIZED CAT ABDUCENS MOTONEURONS. J. M. Delgado-Garcia*, R. Baker, K. Alley and R. McCrea. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., N.Y. 10016; Case West. Reserve Univ. Med. Ctr., Cleveland 44106.

During experiments designed to study internuclear neurons in the abducens nucleus (Abd.n.), it was observed in Nissl material that either intracranial or peripheral section of the VIth nerve did not produce retrograde chromatolysis and degeneration of Abd. motoneurons (Mn). Subsequently, a study was implemented to follow activity of Abd Mns from onset of axotomy to reinnervation. The results complement, but more often contrast with, the classical picture provided in other axotomized Mns. Experimentally, stimulating electrodes were placed bilaterally and intracranially on the VIth nerve. The magnetic search coil technique was used to record eye movement. In two experiments tungsten microelectrodes were implanted in the Abd.n. to chronically record antidromically-elicited field potentials following axotomy. Four to seven days following axotomy, the antidromic field potential was reduced by more than 90%. This reduction remained until reinnervation of the muscle (16-25 days) at which time the field potential returned to normal amplitude. During either eccentric lateral gaze or natural vestibular stimulation, the field potential amplitude could be facilitated up to 50%, but it never reached control level. In acute electrophysiological experiments carried out 10 days after axotomy, the antidromic field potential was also depressed. Intracellular recording from Abd Mns exhibited the presence of an M spike; however, unless the cell was depolarized, an IS-SD invasion did not occur. Preliminary studies indicate that failure of antidromic invasion is likely due to a decrease in membrane resistance including the axon hillock area most prominently. In similarity with synaptic deafferentation in spinal Mns, the somatically located vestibular IPSP was either completely, or nearly completely, removed in all axotomized Mns; however, the contralateral vestibular EPSP always remained with only moderate modification in time course and amplitude. Large changes in dendritic excitability were not observed. To corroborate the above findings, we injected HRP intracellularly in axotomized and normal Mns for light and electron microscopy. To date, the dendritic spread of reconstructed axotomized Mns does not differ from that of normal Mns nor is there evidence for any other unusual dendritic morphology. The soma and proximal dendritic tree of the axotomized Mns are covered with fibrillar astrocytic processes and the number of synapses is reduced 50-60% from normal. We conclude that the classical chromatolysis picture as ascertained in other mammalian Mns is not without exception and that Abd Mns may be a useful model for study of trophic relationships in the mammalian CNS. (Supported by USPHS grants NS-13742, EY-02007 and NS-00147)

1923 ALTERATION OF SKELETAL MUSCLE MEMBRANE BY SUBPERINEURAL INJECTIONS OF BATRACHOTOXIN - FURTHER EVIDENCE FOR NEUROTROPHIC CONTROL OF MUSCLE. S.S. Deshpande*, R.J. Boegman, and E.X. Albuquerque. Dept. Pharm. & Exp. Ther., Sch. Med., Univ. MD., Baltimore, MD. 21201.

Substances that block axonal transport such as batrachotoxin (Ochs, Science 187:1087, 1975) are of interest to further understand axonal transport of trophic factors and their effect on the muscles. Although BTX increases Na^+ permeability and instantaneously blocks axoplasmic transport, the mechanism is poorly understood. BTX (2.0-10 mM) was injected subperineurally into peroneal nerves about 10 mm (close site) or 35 mm (distant site) away from the entrance of nerve into extensor digitorum longus (extensor) muscle of the female Wistar rats. The radial front of bulk concentration of toxin at either site was ± 5 mm. The vehicle, 10% dextrose in 0.9% saline, was injected at similar sites on the contralateral side. The muscle membrane was significantly depolarized (5-10 mV) at the endplate at 50 min after BTX injection at close site; distant site required about 160 min. Neither MEPP frequency nor amplitude were affected at this time or even up to 6 hrs when muscles were still paralyzed. The observed membrane depolarization was not a result of direct depolarization by BTX on the muscle membrane nor was it reversible with bath applied tetrodotoxin (TTX 3 µM). Similar injections of TTX (2 µg) into nerve which failed to block axonal transport also failed to cause membrane depolarization up to 24 hrs although muscles were paralyzed and the normal transmitter release was still present. Therefore early depolarization seen at the endplate region with BTX injections was not due to muscle inactivity. There was total cessation of spontaneous and evoked transmitter release in muscles studied at 18 hrs when almost complete recovery of membrane potential and partial recovery of axonal transport had occurred. At 48 hrs and 5 days after BTX injection no transmitter activity was evident but endplates were significantly depolarized. Thus, possible interpretations for these findings are: 1) block of the trophic factor(s) responsible for maintenance of membrane potential and transmitter activity occurs simultaneously but the effect on the latter is only evident when available stores of transmitter are depleted. 2) Although recovery of transport resulting in membrane repolarization occurs, depletion of transmitter could possibly trigger proliferation of Schwann cells and nerve terminal degeneration. 3) Early depolarization seen with BTX injections at two sites and the onset of its occurence that is related to the site of injection positively support neurotrophic hypothesis. (Supported by NIH grants NS-12063, and funds from Muscular Dystrophy Association and the Paralyzed Veterans of America.)

1924 ENHANCEMENT OF AXONAL OUTGROWTH FROM SPINAL CORD EXPLANTS BY CONDITIONED MEDIUM. Lori B. Dribin, and John N. Barrett. Dept. Physiol. and Biophys., Univ. of Miami Med. Sch., Miami, Fla. 33152.

Results indicate that a large molecule(s) (molecular weight greater than 20,000 daltons) produced by rat muscle, lung, or fibroblast cells in tissue culture is capable of enhancing neurite outgrowth from rat spinal cord slices.

Slices of 15 to 16 day fetal rat spinal cord, freed from all spinal ganglia and meninges were plated on collagen plus poly-L-lysine coated glass coverslips. 5-Fluorodeoxyuridine was sometimes included in the culture medium (modification of Hams F12) to reduce non-neuronal cell outgrowth. Twenty four hours after explantation, medium previously conditioned 3 to 4 days by dissociated fetal or newborn rat muscle, lung, or fibroblast cultures was added to the spinal cord slices. Medium conditioned in the presence or absence of 10% fetal calf serum was effective in increasing the area covered by fiber outgrowth by more than 100% over control values. The maximal effect of the conditioned medium was observed one week after plating.

In order to determine the approximate molecular weight of the factor, the conditioned medium was filtered with Pellicon molecular filters PTHK or PTGC (nominal molecular weight cutoffs 100,000 and 10,000 respectively). The neurite growth enhancing activity was found exclusively in the higher molecular weight fractions. Controls consisted of fresh, non-conditioned medium filtered in the same manner as the conditioned medium. Since the neurite growth promoting activity was retained in the higher molecular weight fractions even after 2 washes with serum free fresh medium using the PTHK filter, the molecular weight of the active fraction must be at least 20,000 daltons.

The molecule(s) involved is probably not the classical Nerve Growth Factor (NGF), since spinal cord slices cultured in 5, 10, 15 or 30 biological units of NGF per milliliter of medium were similar to controls. Supported by NIH grants NS12207 and NS07044.

1925 NEUROTROPHIC CONTROL OF CHLORIDE CONDUCTANCE IN CULTURED MUSCLE. J. K. Engelhardt, K. Ishikawa*, and Y. Shimabukuro*. Grant Neuroscience Laboratories, Dept. of Neurology, USC School of Medicine, Los Angeles, Ca. 90033.

A neurotrophic factor is known to reduce the membrane resistance (R_m) of cultured chick skeletal muscle while leaving the membrane capacitance ($C_m \simeq 10\ \mu F/cm^2$) unchanged (Engelhardt et al., Brain Research 126: 172-175, 1977). The product $R_m \times C_m$ is equal to the electrical time constant (τ_m) of the muscle fiber; therefore, τ_m measurements can be used to estimate membrane conductance ($G_m = C_m/\tau_m$). When τ_m measurements are made in Normal Cl^- and Low Cl^- bathing solutions, component ionic conductances of the resting muscle membrane can be estimated using the following relations:

$$G_m(\text{in Normal } Cl^-) = C_m/\tau_m(\text{Normal}) \simeq G_K + G_{Cl}$$
$$G_m(\text{in Low } Cl^-) = C_m/\tau_m(\text{Low } Cl^-) \simeq G_K$$
$$G_{Cl} \simeq G_m(\text{Normal}) - G_m(\text{Low } Cl^-)$$

When τ_m measurements from pure muscle cultures are compared with those from muscles that have been co-cultured with spinal cord explants, the above assumptions result in the conductance values (in μsiemens/cm^2) tabulated below:

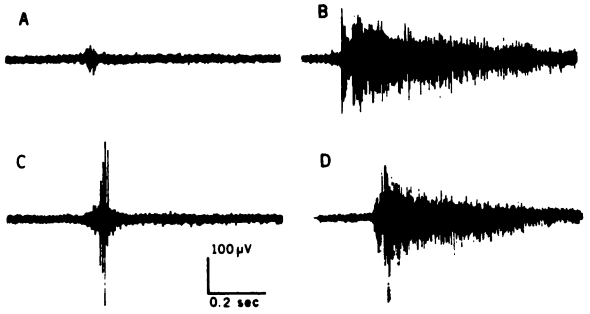
	G_m	G_K	G_{Cl}
Pure Muscle	575	510	65
Muscle + Nerve	1020	518	502

These results indicate that the neurotrophic factor is reducing R_m (increasing G_m) through an increase in G_{Cl}.

(Supported by grants from The Amyotrophic Lateral Sclerosis Society of America (ALSSOA), The Myasthenia Gravis Foundation, and The Wright Foundation)

1926 MYOTONIA IN NORMAL COMPLEXUS MUSCLE AND MUSCULAR DYSTROPHY OF THE CHICKEN. Richard K. Entrikin, William R. Randall** and Barry W. Wilson. Depts. Pharmacol. and Avian Sciences, Univ. California, Davis, CA 95616

Abnormal EMG (electromyographic) activity, similar to that in various forms of myotonia, is a characteristic of pectoralis major muscles of genetically dystrophic chickens. We have observed similar activity in complexus ("hatching") muscles of normal chicks at 5 days ex ovo. "Insertional" activity was elicited from pectoralis major and complexus muscles by slight movement of a 31 gauge concentric needle electrode inserted into muscles of anesthetized (pentobarbital, 30 mg/Kg, i.p.) chicks. The activity in A below was recorded from the pectoralis major of a 5-day-old normal chick. This short duration, low amplitude, "immature" pattern contrasts markedly with the prolonged "myotonic" pattern recorded from the complexus muscle of a 5 day normal chick (B). The short duration, high amplitude pattern from the pectoralis major of a 28-day-old normal chicken is shown in C, and the prolonged "myotonic" pattern from the pectoralis of a 28-day-old dystrophic chicken is shown in D. The results show that insertional EMG activity in the normal complexus at 5 days ex ovo (B) is very similar to the "mature" dystrophic pattern (D), and is markedly different from both "immature" (A) and "mature" (C) normal activity. Another important feature of normal complexus muscles is the hypertrophy of fibers on days 16-20 in ovo, followed by atrophy until day 7 ex ovo (Ashmore et al., J. Histochem Cytochem. 21:266-278, 1973). The complexus muscle of the normal chick appears to offer unique possibilities for studying the relationship between myotonic EMG activity and skeletal muscle development. (Supported by the MDA).



1927 NEUROTROPHIC CONTROL OF 16S ACETYLCHOLINESTERASE AT THE NEUROMUSCULAR JUNCTION. Hugo L. Fernandez and Barry W. Festoff. Neurobiology Research Lab, VA Hospital, Kansas City, MO 64128 and Dept. Neurology, Univ. of Kansas Med. Ctr.

The mechanism(s) underlying neural control of skeletal muscle acetylcholinesterase (AChE) is not fully understood, but nerve-evoked muscle activity, ACh and regulatory substances released by nerves have all been proposed. The precise role and relative importance of these factors are unclear, partly because some might be involved in the control of junctional AChE and others of non-junctional AChE. Junctional versus non-junctional AChE may now be clearly distinguished, since of three AChE molecular forms in skeletal muscle only one (16S, sedimentation coefficient) is associated with endplates. Neurotrophic influences which are exerted independent of nerve-evoked muscle activity may be demonstrated by showing that denervation changes develop sooner if the nerve is cut close to, rather than far from, the muscle. The present work was undertaken in an effort to determine which factor(s) is involved in "trophic" regulation of junctional 16S AChE.

Obturator nerves of male Sprague-Dawley rats (150-200g) were transected on one side either at their point of entrance to anterior gracilis muscle (short stump) or 3.5-4cm central to that point (long stump), leaving the contralateral nerve intact (control). At varying postoperative times gracilis endplates, which are localized in two well-defined regions (3-4mm wide), were separated from non-innervated muscle regions and processed for AChE molecular forms (sensitive to BW284C51) on sucrose gradients. Endplate enriched zones of control gracilis contained 16,10 and 4S AChE, while muscle regions devoid of endplates had only 10 and 4S AChE. The amount of 16S AChE (% of control) gradually decreased in both long and short stump preparations, reaching the same level (10-20% of control) 6 days after denervation. The time of onset of these effects, however, was different; enzymatic decay started considerably earlier in short stump (12-24h) as compared to long stump (3-4d) preparations.

These and other results indicate that the time of onset of 16S AChE decrease, induced by denervation, is dependent on the length of nerve stump that remains attached to the muscle. In as much as muscle becomes inactive at the time of nerve section, regardless of nerve stump length, our findings cannot be attributed to muscle disuse. Rather, they imply that "trophic" regulation of 16S AChE primarily involves a neurogenic factor(s) which is independent of nerve-evoked muscle activity. Whether such a factor(s) is 16S AChE itself, also found in peripheral nerve, other axoplasmic molecule(s), or even ACh, is currently under investigation.

(Supported by the Muscular Dystrophy Assn. and the Medical Research Service of the Veterans Administration).

1928 EXCHANGE OF PROTEIN BETWEEN GLIA AND NERVE CELL AXONS IN CRAYFISH R.M. Grossfeld and G.D. Bittner. Zool. Dept., University of Texas, Austin, TX 78712.

In crayfish peripheral nerves, the distal stumps of cut motor axons undergo relatively few ultrastructural and physiological changes for long periods after separation from their cell bodies. The glial cells surrounding these axons exhibit a progressive hypertrophy and hyperplasia, as well as a prolonged increase in incorporation of radioactive leucine. Parallel histological results have been obtained for the medial giant axons and their adjacent glial sheaths in the crayfish central nervous system. We hypothesize that the long-term survival of severed motor axons and certain CNS axons depends, in part, upon their exchange of essential macromolecules with adjacent glial cells. We have, therefore, investigated the transfer of newly synthesized proteins from glia to adjacent axons.

Central nerve cords or limb nerves were isolated. These tissues were incubated in van Harreveld's solution containing H^3-leucine and then in a comparable ("chase") solution in which non-radioactive leucine replaced radioactive leucine ("normal incubation conditions"). Tissues were fixed with formaldehyde and autoradiographs were prepared from plastic-embedded sections. The autoradiographic grains overlying glial sheaths and the axoplasm of adjacent axons were quantified. Axonal areas were measured by planimetry.

Protein synthesis was linear for at least 2 hours. The washout of unincorporated radioisotope from the tissues attained 75% of the maximal value by 10 minutes and reached the maximal value by 40 minutes in the "chase" solution. Addition of puromycin, cycloheximide, and chloramphenicol to the labeling and "chase" solutions gave a 99% inhibition of protein synthesis without significantly altering the uptake of radiochemical. Under these conditions of protein synthesis inhibition, the grain counts for areas overlying the tissues were reduced to background. We have performed experiments, under normal incubation conditions, in which we varied only the time in the "chase" solution or the calcium concentration of this solution. Preliminary results indicate that there is a transfer of protein from glia to adjacent axons and that this transfer is sensitive to the extracellular calcium concentration.

(Supported by BRSG funds from U.T.-Austin to RMG and NIH RCDA 00070 and NINCHDS research grants # NS-11861 and NS 14412 to GDB).

1929 ALTERATIONS IN THE IN VITRO PHOSPHORYLATION OF NUCLEAR PROTEINS AFTER DENERVATION OF SKELETAL MUSCLES. Irene R. Held and Hock C. Yeoh*. Neuroscience Research Laboratory, VA Hospital, Hines, IL 60141 and Loyola Univ. Med. Ctr., Maywood, IL 60153.

Previously, we reported that the endogenous activities of nuclear protein kinases are stimulated in skeletal muscles which have been denervated for 48 hours. In this report, the changes occurring after denervation in the differential phosphorylation of nuclear proteins from two different types of skeletal muscle are demonstrated.

Nuclei are isolated as previously described by us from soleus and EDL (extensor digitorum longus) muscles which have been denervated for 48 hours by cutting the sciatic nerve in the mid-thigh of the rat. The sham-operated, contralateral muscles are used as the source of "control" nuclei. Intact nuclei (100-400 µg nuclear protein) are incubated for 10 minutes at 37°C. in a phosphorylation media (0.25 ml) consisting of 50mM Tris:HCl, pH 7.5, 0.1M NaCl, 2mM $MgCl_2$, 20 µCi γ - ^{32}P-ATP and 1uM cyclic AMP, when added. After termination of the reaction with excess, cold ATP and $Na_2P_2O_7$, aliquots are taken for determination of the levels of endogenous protein kinase activities by measuring the specific radioactivities (dpm/µg nuclear protein) of TCA-precipitable protein and also for the evaluation of the differential phosphorylation of hot, SDS-solubilized, nuclear proteins resolved by SDS-acrylamide electrophoresis and identified by assays of the radioactivity of gel slices.

When the gel electrophoregrams of the SDS-solubilized nuclear proteins from soleus and EDL muscles of the rat are compared, marked quantitative and qualitative differences are seen in the 10-12 discrete, protein-staining bands that are resolved. In addition, the phosphorylation profile also differs. At least four of the major protein bands of soleus nuclear proteins have high levels of ^{32}P radioactivity. The endogenous nuclear protein kinase activity of EDL muscle, however, appears to be more than fivefold lower than that of soleus muscle. After a denervation period of 48 hours, the nuclear protein kinase activity (dpm/µg protein) is significantly stimulated in both muscles and an increased radiolabeling of several discrete protein bands is found. No change is observed in the protein pattern after denervation. Alterations in phosphorylation induced by pre-incubation of skeletal muscle nuclei with cyclic AMP are being studied.

The factors responsible for the mediation of the observed alterations in the phosphorylation of nuclear proteins after denervation of skeletal muscles remain unidentified. Supported by NINCDS Grant NS-11755 and by the Medical Research Service of the Veterans Administration.

1930 I-125-αBUNGAROTOXIN BINDING TO DENERVATED MUSCLE: A SURVEY STUDY USING LIGHT AND EM AUTORADIOGRAPHY. R. H. Loring and M. M. Salpeter. Cornell Univ., Ithaca, N.Y., 14853.

The appearance of extrajunctional acetylcholine receptor following denervation of a muscle has commonly been studied by measuring the increase in I-125--αBungarotoxin (αBGT) binding to the muscle. It is often assumed that most of the increased binding of labeled toxin to denervated muscle represents binding to acetylcholine receptors in the plasma membrane. This study was undertaken to test this assumption by localizing αBGT binding at both the light and electron microscope levels by autoradiography.

Labeling was performed in vivo, by bathing the exposed sternomastoid muscle of a mouse in αBGT (Fertuck et al, J. Cell Biol. 1975: 66, 209). αBGT was used at $3x10^{-6}$ M for 2 to 3 hrs, which was found to fully inactivate receptors. Three mouse sternomastoid muscles denervated for eight days were compared with two normal sternomastoid muscles. Non-saturable binding was determined on two eight-day-denervate muscles that were bathed for 3 hrs in $3x10^{-6}$ M unlabeled toxin before bathing for 3 hrs in labeled toxin. All the muscles were prepared for both light and EM autoradiography.

We find that: 1) αBGT binding to the postjunctional membrane (PJM) of the denervated endplate is not significantly different from that at the innervated PJM, and is decreased more than 90% by pretreatment with unlabeled toxin; 2) Denervated muscle shows a large fiber to fiber variability in the label on the extrajunctional plasma membrane (EJM). The site density on the EJM of adjacent fibers may differ by as much as 10-fold; 3) The average αBGT density at the denervated EJM is 200 sites/μ^2 (compared with the normal EJM site density of less than 40 sites/μ^2) and these αBGT binding sites are evenly distributed with no "hot spots" in areas of the muscle within 5 mm of the endplate; 4) A fraction of αBGT is bound in the "cytoplasm" of denervated muscle. Preliminary EM autoradiographic analyses, corrected for radiation spread, suggest that more than 50% of the internal sites are associated with the muscle triads, presumably representing sites on the T-system. If this assumption is correct then the actual site density of T-system membrane is probably only about 1/3 the site density at the EJM; 5) Prelabeling with cold toxin eliminates 90% of both the cytoplasmic and EJM label; 6) In denervated muscle, αBGT is also bound to collagen and invading non-muscle cells. This binding is not saturated by pretreatment with unlabeled toxin. These results indicate that care should be taken to show that αBGT is actually bound to muscle acetylcholine receptors when interpreting experiments designed to study receptor spread following denervation.
Supported by NIH grant NSO9315.

1931 NERVE GROWTH FACTOR IN THE SERUM AND SUBMANDIBULAR GLANDS OF MICE WITH MUSCULAR DYSTROPHY. Richard A. Murphy, Nicholas J. Pantazis* Dept. Anat., Harvard Med. Sch., Boston, MA 02115.

Nerve growth factor (NGF) is present in high concentrations in the submandibular glands of adult mice but the reason for this is not understood. Removal of the salivary gland, for example, has no effect on serum levels of NGF so the gland does not appear to be secreting this protein into the circulation. On the other hand, high levels of NGF are present in saliva and this result suggests that the gland is an exocrine organ with respect to NGF. In spite of this information, however, we still have no clue as to what role NGF plays in the gland or in saliva.

Recently, Furukawa et al. (BBRC 76:1202, 1977) have shown that extracts of salivary glands from mice with muscular dystrophy are less active in the bioassay for NGF (biological units/mg protein) than are extracts from control animals. This result could arise for several reasons: 1) there may be less NGF than in the salivary glands of dystrophic mice than in glands from normal animals; 2) the structure of the NGF molecule in the dystrophic gland may be altered and therefore less active biologically; 3) there may be substances in the submandibular gland which inhibit NGF's actions in the bioassay. In this study, we have begun to examine these alternatives.

Submandibular glands from sexually mature 129 B6/f_1/Jdy mice were frozen and subsequently homogenized and the supernatant fractions examined using a radioimmunoassay made against 2.5S NGF. The data indicate that glands from male dystrophics contain less NGF (2.21±.29µg/mg protein) than glands from unafflicted littermates (4.58±.38µg/mg) ($p<0.001$). Female dystrophic mice show even greater differences in gland NGF levels (0.021±.01µg/mg) when compared to control animals (0.25±.06µg/mg). Serum levels of NGF, however, are not altered in the afflicted animals of either sex. Furthermore, the deficit in the glands is not limited to NGF. Concentrations of epidermal growth factor (EGF) measured by RIA are reduced by amounts comparable to that for NGF. On the other hand, β amylase, a digestive enzyme in the submandibular gland, is normal in dystrophic animals.

The molecular properties of NGF from the salivary glands was analyzed by gel filtration. The NGF from normal and dystrophic animals (male and female) eluted identically as a single peak (m.w. approx. 140,000) on two different resins. Furthermore, NGF from dystrophic glands is biologically active at concentrations which are similar to those of the NGF from normal animals. Hence, we are unable to detect any differences in the chemical and biological properties of the NGF from the glands of normal and dystrophic animals. We conclude, therefore, that submandibular glands from dystrophic mice indeed contain less NGF (and EGF) than do glands from normal animals, for reasons unknown.

1932 THE EFFECT OF PROPYLTHIOURACIL ON THE DEVELOPMENTAL MORPHOLOGY OF THE MESENCEPHALIC NUCLEUS OF DUCK EMBRYOS. Y. Narayanan* and C.H. Narayanan (SPON: R.B. Malloy). Dept. Anat., LSU Sch. Med., New Orleans, LA 70119.

This report deals with the effects of propylthiouracil (PTU) on the development of the mesencephalic nucleus of the trigeminal nerve of duck embryos from eight to twenty-six days of incubation age. Embryos were made hypothyroid by the in vivo injection through membrane blood vessels of single or multiple doses of 0.2 to 0.5 cc of 0.2% PTU beginning from the eighth day of incubation. PTU treated embryos and a corresponding number of saline injected control embryos were examined at one day intervals until hatching. Recordings of the frequency of beak movements showed a significant reduction in the treated embryos at all stages of development. Thyroid follicles in the treated embryos had very little or no colloid in them, and the follicular epithelial cells were enlarged as determined by mean acinar heights. In a previous study on the normal development of the mesencephalic nucleus, based on the morphological changes observed, the cells have been classified into three stages from early appearance until hatching. We have followed the same sequence of changes in the present study in our evaluation of the effects of PTU on the neurons of the mesencephalic nucleus during (a) the midincubation period, 13 to 18 days; (b) the last phase, from day 19 to hatching. The neurons of the mesencephalic nucleus in the treated embryos showed a general retardation of structural development. This was particularly reflected in a reduction of the Nissl and neurofilaments. A reduction in the size of neuronal somata and a reduction in the number of cells were observed. Our results suggest that thyroid hormone is essential for the normal development of structure and functional relations of the mesencephalic nucleus of the trigeminal nerve.

Supported by USPHS Grant DE04258-03.

1933 IMMUNOLOGICAL DETECTION OF A NERVE GROWTH FACTOR-LIKE MOLECULE IN MOUSE MILK. Nicholas J. Pantazis*, Richard A. Murphy and Michael Young*. (Spon. Barry G.W. Arnason) Dept. Anatomy, Harvard Med. Sch., Boston, MA 02115, and Lab. of Physical Biochemistry, Mass. Gen. Hospital, Boston, MA 02114.

The submandibular glands and saliva of adult mice contain large amounts of nerve growth factor (NGF). High concentrations of the protein are secreted via saliva into the digestive tract of the adult animal, and it is reasonable to suspect that the protein's presence there is of biological importance. If this is true, however, then intriguing questions arise concerning the newborn animal. It is known that NGF does not appear in abundance in the salivary gland or saliva until puberty, so the newborn may require NGF from other sources. This reasoning led us to question whether or not the diet of the newborn mouse could provide this protein. Consequently we have examined mouse milk to determine if it contains NGF.

Using a radioimmunoassay for 2.5S-NGF, we have detected in milk a molecule which is immunologically indistinguishable from salivary gland NGF. The material is present in concentrations ranging from 100-600 ng/ml, and it is detectable in milk collected from mothers nursing from 3 to 16 days postpartum. Over this time period, no consistent differences in the concentration of this material in milk were noted.

The chemical properties of the immunoreactive molecule were studied on columns of Sepharose 6B. The material elutes from the column as a large molecular weight species (500,000-600,000 daltons) and it does not dissociate at low concentrations (ng/ml). In solvents containing guanidine hydrochloride, the material displays a molecular weight of 40,000-50,000. This value differs significantly from the molecular weight of the immunoreactive component of salivary gland NGF (13,000) determined under identical experimental conditions.

Thus far we have been unable to obtain a positive response in the ganglion bioassay with the NGF-like molecule detected in milk. Several explanations could account for this result. It is possible that the molecule measured by radioimmunoassay is not NGF but rather an immunochemically cross reacting species. Alternatively, the NGF we detect in milk may be present in a biologically inactive form. Finally, milk may contain other factors which interfer with neurite outgrowth in the ganglion assay. These alternatives are presently under study.

Supported by a grant from the National Foundation, March of Dimes to RAM and NIH grants to RAM and MY.

1934 SODIUM-STIMULATED, NERVE GROWTH FACTOR DEPENDENT HEXOSE UPTAKE BY CHICK EMBRYO GANGLIONIC NEURONS. Stephen D. Skaper* and Silvio Varon. Dept. Biol., Sch. Med., U. Ca. San Diego, La Jolla, CA 92093.

Nerve Growth Factor (NGF) is essential for development of sympathetic and sensory neurons. Dorsal root ganglionic (DRG) cells, when incubated in vitro in the absence of NGF undergo a general metabolic degeneration which is preceded by certain changes in permeation properties. To determine whether NGF deprivation could affect the capacity of these cells to take up an important energy source, namely glucose, cells were incubated with or without NGF for different times, and then presented with the factor and tested for uptake of [^{3}H]labeled 2-deoxy-D-glucose (2DG). The pulsed cells were collected on moist glass fiber filters under vacuum suction. The filters were washed with ice-cold incubation medium, dried and counted via liquid scintillation techniques. The standard incubation medium consisted of 40mM Hepes or Tris-Hepes pH 7.4, 140mM NaCl, 5mM KCl, 1mM $MgCl_2$, 0.1mM $CaCl_2$, and 1% bovine serum albumin.

2-Deoxy-D-glucose uptake by DRG cells was reduced by NGF deprivation and restored by delayed NGF administration (up to 6-hr). Calculation of apparent uptake constant (K_t) and maximal velocity (V_{max}) in NGF-maintained and -deprived cells showed about 2-fold differences between NGF-controlled and NGF-independent hexose uptakes. This NGF-dependent portion (about one-third) of the total specific hexose uptake was also dependent on the presence of Na^+ ions, with the apparent K_t for 2DG varying inversely with the Na^+ concentration (V_{max} was unaffected). Preincubation of DRG cells with 1mM ouabain for 30 min, followed by a pulse with [^{3}H] labeled 2DG, also resulted in a 53-63% reduction of the NGF sensitive uptake. The NGF-controlled hexose uptake was also energy dependent, being inhibited 47-67% by a 30 min preincubation with 1-2mM 2,4-dinitrophenol. Other transport substrate activities (α-amino-isobutyric acid, uridine) for DRG cells which exhibited NGF regulation were likewise Na^+ sensitive. These results indicate that NGF may provide for the maintenance and development of its embryonic target neurons by modulating permeation properties which regulate the availability of major energy substrates (and other nutrients), and may conceivably do so by acting through a sodium gradient across the cell membrane.

(Supported by U.S. Public Health Service grant NS-07606.)

1935 A NEUROTROPHIC INFLUENCE ON ADULT MUSCLE WHICH IS INDEPENDENT OF ELECTRICAL ACTIVITY. L. Ziskind* and A.J. Harris*(SPON: J.I. Korenbrot). Dept. Physiology, Otago University Medical School, Dunedin, New Zealand.

Strips of denervated adult mouse diaphragm and segments of embryonic spinal cord were pinned to collagen-coated dishes and immersed in 0.2 ml of culture medium enriched with foetal calf serum, human plancental serum and chick embryo extract.

1-2 weeks after explanting spinal cord segments spontaneous depolarising potentials were recorded in some muscle fibres. These were reversibly blocked by d-tubocurarine (dTC, 5 x 10^{-6} gm/ml) but were not affected by tetrodotoxin (TTX, 2 x 10^{-7} gm/ml). In denervated muscle fibres action potentials were insensitive to TTX, whereas sensitivity to the toxin was restored upon reinnervation of the cultured muscles. Such TTX-sensitive action potentials were detected only in fibres which showed miniature end-plate potentials (m.e.p.p.'s), indicating that a spinal cord explant in the bath was not sufficient to initiate the restoration of toxin sensitivity.

Strips of denervated diaphragm which were chronically stimulated for 4 days did not regain TTX sensitivity. In contrast, the action potential mechanism became sensitive to TTX in muscle fibres which had developed synapses in the continual presence of dTC (5 x 10^{-6} gm/ml). The observations indicate that restoration of TTX sensitivity in reinnervated adult muscle results from some trophic influence of the nerve which is independent of both muscle activity and the action of transmitter on postsynaptic receptors.

VESTIBULAR SYSTEM

1936 NECK MOTOR RESPONSES TO VERTICAL ROTATIONS IN THE ALERT CAT. John H. Anderson and Costas Pappas*. Lab.Neurophysiol.,Dept.Physiol., U.Minn., Mpls., Minn. 55455

Vestibular reflexes involving the neck musculature play an important role in controlling posture and mediating eye-head coordination. Due to the mechanical linkage of the head and neck, one important aspect is for the control of head position in the vertical planes. Therefore, as part of our efforts at determining the dynamic characteristics of the vestibulo-nuchal reflexes, we have examined those initiated by roll (lateral) and pitch (forward-backward) rotations, in which cases both vertical semicircular canal and otolith inputs are present. The motor outputs measured were the EMG activities of the sternomastoid (a head rotator and ventral flexor) and biventer cervicus (dorsal flexor) muscles. These were chosen since they can function either synergistically (during roll) or antagonistically (during pitch) and therefore might demonstrate the reciprocal organization of vestibular reflexes involving flexor and extensor motoneurons.

Alert cats, whose heads and spinal vertebrae (C_{7-8}) were rigidly fixed, were subjected to sinusoidal rotations in the frequency range 0.04-4.0 Hz. In this situation there was a modulation of the tonic activity in the biventer cervicus which, if the head were free to move, would have subserved compensatory movements: during roll the responses lagged the contralateral angular acceleration and during pitch they lagged downward acceleration. The phases showed lags of 140 to 150 degrees at the lowest frequencies tested, whereas at the higher frequencies this became less: about 120 deg. at 1.0 Hz and only 50-85 deg. at 4.0 Hz. Since the sternomastoid muscle was usually silent in the alert situation, for some recording sessions the cats were given small doses of ketamine hydrochloride (5-8 mg/kg). There was then a background level of activity in the sternomastoid. The phase of the modulated activity was largely in agreement with that of the biventer.

From these results we conclude that the otolith inputs make important contributions to the neck motor outputs at the lower frequencies, whereas the canal inputs are more significant at the higher frequencies. Furthermore, the rising phase curves and rather small phase lags for frequencies above 1.0 Hz suggest that in addition to "indirect" pathways involving integration of the vestibular inputs, there is likely to be also a significant contribution to the motor outputs by the direct pathways at the higher frequencies. This is most probably necessary to prevent large phase lags in head position which would otherwise be introduced by the viscoelastic properties of the head-neck system in the freely moving animal.

1937 FREEZE-FRACTURE STUDIES ON THE SYNAPSE OF THE TYPE I HAIR CELL IN THE VESTIBULAR SYSTEM OF THE GUINEA PIG. D. Bagger-Sjöbäck* and R. L. Gulley. Illinois Eye and Ear Infirmary, University of Illinois, Chicago, Illinois and Department of Anatomy, Case Western Reserve University, Cleveland, Ohio.

Synapses of type I hair cells with calyceal terminals of dendrites of vestibular ganglion cells were studied in the maculae and cristae using thin section and freeze-fracture techniques. In thin sections, very few synaptic junctions are found along the apposition of these cells. Infrequently, synaptic bodies are adjacent to the hair cell plasmalemma, and the opposed calyceal plasmalemma is lined by a prominent postsynaptic density. In freeze-fracture replicas, the plasmalemmae at these junctions are identical to those of the ribbon synapse in the inner hair cell of the organ of Corti (Gulley and Reese, '77). Our failure to find such junctions in replicas of most type I hair cells, many of which had very large areas of their membrane exposed, suggests that very few of these junctions are present in these hair cells, and that some type I hair cells may even lack this junctional structure. The apposition of the hair cell and dendrite, however, is specialized. The hair cell is invaginated by cytoplasmic protrusions of the dendritic calyx. The number, shape and depth of these invaginations varies between different cells. These variations are not related to the location of the hair cell in the sense organ. In non-invaginated regions of the apposition, the plasmalemmae are separated by a 25-30 nm extracellular space. The extracellular space narrows at the invaginations, where the apposed plasmalemmae are separated by 6 to 7 nm. A thin paramembranous density lines the plasmalemmae along the region of widened extracellular space adjacent to the invagination. In freeze-fracture replicas, neither the hair cell nor the calyceal plasmalemma within the invaginations have a special distribution of intramembranous particles on either membrane leaflet. However, at the base of the invaginations of the hair cell, a large aggregate of widely-spaced, large, angular particles surrounds the invagination on the external membrane leaflet. The corresponding region of the calyx has no special distribution of particles on either membrane leaflet. Typically, chemical and electrical junctions are characterized by a special distribution of intramembranous particles at the junctional region. The regions of the type I hair cell where the plasmalemma is most closely opposed to the calyx, have no particle specialization, but instead are surrounded by large intramembranous particles. The scarcity, or lack, of chemical synaptic junctions along this calyceal apposition and the unique arrangement of particles around a region of plasmalemma closely apposed to the postsynaptic cell may indicate an unusual mode of conduction between the hair cell and ganglion cell dendrite.

(Supported by NIH grant NS-13889-01 to RLG.)

1938 VESTIBULAR AND OPTOKINETIC PROPERTIES OF NEURONS IN THE VESTIBULAR NUCLEI OF RABBITS. N. H. Barmack and V. E. Pettorossi*. Neurological Sciences Inst., Good Samaritan Hosp. & Med. Ctnr., Portland, OR. 97209

The vestibular nuclei receive a direct projection from primary otolithic and semicircular canal afferents. In addition these nuclei receive indirect visual projections from the cerebellar flocculus, as well as extracerebellar visual projections of unknown origin. We have examined how these classes of afferent information converge on single cells in the vestibular nuclei in paralyzed and unanesthetized rabbits. Rabbits were mounted with heads placed near the center of a biaxial rate table, which could be sinusoidally oscillated about the vertical axis activating the horizontal semicircular canals, and about the longitudinal axis, activating the anterior semicircular canals and utricles. Monocular optokinetic stimulation in the horizontal and vertical planes was delivered, by projecting a random dot pattern off two mirrors mounted on galvonometers and onto a rear projection tangent screen subtending 72 x 72 deg. All vestibular units tested evinced both a vestibular and visual responsiveness. Most cells which were primarily sensitive to rotation in the vertical plane differed from cells which were sensitive to rotation in the horizontal plane, in that the discharge rate of vertically sensitive cells could be modulated at low frequencies of stimulation (.001-.02 Hz), as well as at higher frequencies of stimulation (.02-.8 Hz); indicating that vertically responsive cells receive convergent otolith and semicircular canal inputs. Cells which had a type I vestibular response also had a visual directional selectivity for ipsilateral optokinetic stimulation which was synergistic. For example, type I cells in the medial vestibular nucleus were excited by ipsilateral horizontal acceleration and by posterior-anterior optokinetic stimulation of the ipsilateral eye. The effect of optokinetic stimulation of the contralateral eye was always weaker and sometimes not synergistic. The vestibular and optokinetic interactions of type II cells were less predictable, and some type II cells were more strongly driven by optokinetic stimulation of the contralateral eye. The optokinetic velocity sensitivity for vestibular cells had a broad range, extending from .25 deg/sec to 50 deg/sec. This broad range far excedes the range of neurons in one of the possible sources of indirect visual input to the vestibular nuclei; the dorsal cap of the inferior olive. Electrolytic lesions of the dorsal cap made during recording from optokinetically modulated type I vestibular nuclei neurons, reduce the overall level of activity of these cells but do not abolish their directional selectivity. We conclude that the immediate visual responsiveness of vestibular nuclei neurons originates from extraolivary sources. (Supported by PHS Grant EY-00848 and The Oregon Lions Sight Foundation).

1939 VESTIBULO-SPINAL SYSTEM ADAPTATION TO SUDDEN UNILATERAL VESTIBULAR LESIONS. F. Owen Black and Conrad Wall III. Department of Otolaryngology, Eye and Ear Hospital, University of Pittsburgh, Pittsburgh, Pennsylvania 15213, USA.

Otherwise normal patients with sudden unilateral vestibular disturbances develop a characteristic nystagmus and ataxia which gradually improves. In contrast to the vestibulo-ocular system, quantitative characteristics of the compensating vestibulo-spinal system have received very little attention. We are presently investigating vestibulo-spinal system adaptation using a computerized force platform to record and analyze patients' postural stability. (Black, et al. 1977).

Serial force platform recordings from two groups of patients will be presented: 1) patients with temporary vestibular disturbances as a consequence of otosclerosis surgery (stapedectomy) and 2) patients with sudden, complete loss of peripheral vestibular function (labyrinthectomy). Data analysis was accomplished by 1) x-y displacement amplitude plots, 2) polar vector amplitude and angle fluctuations, 3) phase plane plots and 4) power spectral analysis. Preliminary results indicate that the two groups of patients recover at significantly different rates, (and probably by different mechanisms) when account is taken of patient age at the time of the lesion. General inferences regarding possible adaptive mechanisms will be presented.

References

Black, F.O., O'Leary, D.P., Wall III, C., and Furman, J. The Vestibulo-Spinal Stability Test: Normal Limits. Trans. Amer. Acad. Ophth. and Otol. 84: 549-560, 1977.

1940 VESTIBULAR, AUDITORY, AND SOMATOSENSORY RESPONSES OF NEURONS IN VENTRAL BASAL AND POSTERIOR THALAMUS OF THE CAT. Paul Blum and Sid Gilman. Dept. Neurol., Columbia Univ., New York, NY 10032 and Dept. Physiol., Thomas Jefferson Univ., Phila., PA 19107.

Investigations of the rostral projections from the vestibular system in the cat using evoked potential techniques have shown that activity originating in the vestibular nerve reaches the ventral basal (VB) and posterior (PO) nuclei of the thalamus. In this study, investigations were undertaken of the responses of single units in VB and PO to: 1) electrical stimulation of the vestibular nerve; 2) electrical and natural stimulation of the auditory nerve; 3) electrical stimulation of the ipsilateral forepaw and contralateral forepaw (CFP); and 4) natural stimulation of cutaneous, muscle, and joint receptors throughout the body surface. Forty-two percent of 333 units recorded were responsive to these sensory stimuli. Units that were activated at short latency (<30 msec) following CFP stimulation or with receptive fields confined to the contralateral body surface were termed "somatic non-convergent units." These units (38% of responsive units) were found preferentially in VB. Units responding exclusively to auditory stimuli were termed "auditory non-convergent units" (4% of responsive units) and were found in the medial geniculate nucleus. A third class of units were termed "convergent" (43% of responsive units). These units either: 1) responded to auditory and somatic stimuli; 2) responded to CFP stimulation at excessive latency (>30 msec); or 3) had a bilateral receptive field. Convergent units were located throughout PO. Fourteen percent of the responsive units were activated by vestibular nerve stimulation. Seven of these units were activated by stimulation of the vestibular nerve at a stimulus intensity that was low enough so that the stimulus did not spread and activate fibers in the cochlear nerve. Another group (13 units) was activated at higher intensity vestibular nerve stimulation. There was no differences that could be identified between the two groups of vestibular-activated units on the basis of location or characteristics of the response to sensory stimuli. Vestibular-activated units were found throughout PO and along the border between PO and VB. The range of latencies following vestibular nerve stimulation was from 4 to 25 msec, and 71% of these units also were activated by auditory or somatosensory stimuli. This data suggests that vestibular activity is processed by the thalamus in PO. This data also shows that there is considerable convergence of vestibular information with somatosensory and auditory information at the level of the thalamus.

(Supported by USPHS Grant NS 11307)

1941 A METRIC SPACE DERIVED FROM NEURONAL FIRING PATTERNS. W. Bouris and A. A. Perachio. Yerkes Reg. Primate Res. Ctr., Emory Univ., Atlanta, GA 30322.

Recordings from second-order cells of the vestibular system that were responsive to linear acceleration (translational motion) were examined for dynamics in their responses that would reflect adaptation to repetitive stimulation and for quantitative comparisons among such cells. Since these cells can exhibit nonlinear responses, such as rectification, the requirements for linear analysis of the data cannot be met satisfactorily. Also, the experimental conditions that would allow for the linearization of such nonlinear data are extremely difficult to achieve for this type of vestibular stimulus (French, A.S., et.al., Kybernet. 11(1972), 15-23; Gardner, E.P. and Fuchs, A.F., J. Neurophysiol. 38(1975), 627-650; Spekreijse, H. and Oosting, H., Kybernet. 7(1970), 22-31).

An alternative method is developed which is based on first deriving a geometric space in which a point is an unambiguous representation of a cell's firing pattern, and conversely any observed firing pattern is a unique point in this space. First, consider the interval of time taken to observe a cell's response to a stimulus. This interval is a subspace of the X-axis. The list of firing times that occur in this interval is a finite subset of it. Any such finite subset has a maximum number of firing times, determined by the length of the interval and the maximum firing rate possible. The collection of all such finite subsets forms the space we are interested in.

Next, consider the metric of the X-axis. The distance between two times of firing is simply the length of interval between them. (This is the usual notion of distance for one-dimensional space.) A new metric is derived from this one whose units are the same (msecs) with which, given two firing trains, A and B, the distance between A and B can be measured. The calculation of this distance requires the operations subtraction, minimum of a list and maximum of a list. It satisfies the definition of a metric: $d(A,B) = d(B,A)$, $d(A,B) + d(B,C) \geq d(A,C)$, if $d(A,B) = 0$, then A is B; and if A is B, then $d(A,B) = 0$. Suppose A is 50 msecs from B. One way to view this is that a clock whose resolution is finer than 50 msecs would be required in order to distinguish A from B.

Programs have been written for the LINC-8 Computer to calculate these distances. Algorithms are also utilized to compute a new pattern that is in some sense minimally distant from a given population of observed firings, i.e., a kind of "typical" pattern.

(Supported by NIH Grant #RR 00165 and NASA Grant #NGR 11-001-045.)

1942 THE VESTIBULAR CORTEX IN THE ALERT MONKEY: NEURONAL ACTIVITY IN AREA 2v DURING ROTATION IN THE DARK AND OPTOKINETIC STIMULATION.
U.Büttner*, U.W.Buettner* and V.Henn* (SPON: T.T. Kennedy). Dept. of Neurology, University of Zürich, CH 8091 Zürich (Switzerland).

Single unit activity was recorded from the lower end of the intraparietal sulcus (area 2v) of the rhesus monkey seated on a turntable with his head fixed. For vestibular stimulation the monkey was rotated sinusoidally around a vertical axis in complete darkness at frequencies between 0.005 and 1 Hz. During optokinetic stimulation a cylinder covered with vertical black and white stripes, which completely surrounded the monkey, was rotated sinusoidally at frequencies between 0.01-1 Hz around the stationary animal. Horizontal and vertical eye position was recorded with chronically implanted DC silver-silver chloride electrodes. For quantitative analysis phase and gain of neuronal activity relative to turntable velocity was determined using a Fourier analysis program.

About half of the neurons responding to vestibular stimulation were classified as type I neurons, since they were activated during rotation to the ipsilateral side and inhibited during rotation in the opposite direction. The remaining half were type II neurons with a mirror-like behavior. At frequencies between 0.1 and 1 Hz cortical neurons showed a phase advance of 0-20° relative to turntable velocity. At lower frequencies the phase advance increased and reached about 90° at 0.005 Hz. The phase characteristics were compared with the phase advance of the simultaneously recorded nystagmus.

Nearly all vestibular cortex neurons responded also to optokinetic stimulation. In order to obtain similar responses the cylinder, or the turntable, had to move in opposite directions, which elicits nystagmus into the same direction. The response to optokinetic stimulation was instantaneous and often as strong as for a comparable vestibular stimulus. It was noticed that neurons still responded at high frequencies (0.5-1 Hz) with small cylinder displacements.

Supported by Swiss Nat. Foundation (3.672.77) and Deutsche Forschungsgemeinschaft (Bu 379/2).

1943 VARYING COMBINATIONS OF VISUAL AND VESTIBULAR INPUTS ALTER RESPONSES OF OTOLITH UNITS IN CAT VESTIBULAR NUCLEI. N.G. Daunton, D.D. Thomsen*, and C.A. Christensen*. NASA-Ames Research Center, Moffett Field, CA 94035 and Vassar College, Poughkeepsie, NY 12601.

Many neurons in the cat vestibular nuclei which are sensitive to linear acceleration are also influenced by moving visual stimuli (Daunton and Thomsen, Neurosci. Abstr. II, 2, 1526, 1976). The majority of units which respond to linear acceleration in a given direction also respond to visual stimulation in the opposite direction. These two inputs provide congruent information about the cat's linear self-movement.

The present study examined the response of otolith units in the vestibular nuclei to stimulus configurations which differ from the naturally occurring condition of congruent visual and vestibular stimulation. Chronically prepared cats were relaxed with Flaxedil and artificially ventilated. Single units responsive to linear acceleration in the dark were tested for visual sensitivity. Units responsive to sinusoidal visual and vestibular stimulation in the same axis were tested in three conditions of combined visual and vestibular stimulation: 1) congruent stimulation obtained by moving the animal in the lighted, but stationary visual enclosure; 2) visual and vestibular stimulation applied in opposite directions resulting in twice the image displacement of congruent stimulation; 3) coupled visual-vestibular stimulation resulting in no seen visual movement relative to the animal.

The results show that, compared with vestibular stimulation in the dark, almost all cells displayed increased gain to linear acceleration when they were stimulated in the light, even in the condition in which there was no seen movement. This finding conflicts with the results of Waespe, et al. (Pflug. Arch., S373, R87, 1978) who noted that in monkey sensitivity to angular acceleration was decreased by the coupling of visual and vestibular inputs. In the present study the majority of units showed a greater increase in gain when the visual stimulation was congruent with the linear acceleration than when the visual stimulation provided no image displacement. There was also a tendency for the gain of units to be greater for congruent stimulation than for the double displacement condition. These results are similar to those reported by Berthoz, et al. (Exp. Br. Res., 23, 471-489, 1975) for human observers tested in conditions resembling those studied here. Within-unit analyses of phase relationships showed minimal changes in phase under the coupled stimulus condition relative to the vestibular, dark condition. However, with congruent stimulation, detectable phase shifts were observed. These data suggest that vision can provide both a specific and non-specific influence on units responding to linear acceleration in the vestibular nuclei, and that visual and vestibular inputs about self-motion do not combine additively.

1944 VESTIBULAR AND NECK SENSORY INPUT TO UNITS IN THE VESTIBULAR NUCLEUS OF ALERT CATS. James H. Fuller. Biol. Sci. Group, Univ. Connecticut, Storrs, CT 06268.

Single units in the vestibular nucleus having a semicircular canal and neck sensory input were recorded in cats executing an eye-head movement task. The cats were trained to align their heads and/or eyes with a target and to maintain this alignment within $\pm 2.5^{o}$. In the experiments to be presented, the target was stepped from one side to the other, with an excursion of 20 to 60 degrees in the horizontal plane. Proper alignment was determined by a rotary potentiometer connected to the shaft restraining the head or by the oculogram. Canal input was identified by passive rotation of the whole animal about the vertical axis, and by latency measurements of firing rate changes following a $2,000^{o}/sec^{2}$ deceleration of the head during active movements; latencies were 5-10 msec. Neck sensory input was characterized by fixing the animal's head in space and passively rotating the body about the head. Firing rate modulation was both dynamic and static in most units; latency to rapid pulses of the body was approximately 20 msec.

Spindle, joint, or tendon sensory discharge may be influenced by internally generated muscle contraction as well as by passive movement of the neck; furthermore, direct, internally generated inputs can influence unit firing rate independent of neck muscle activity. Two basic procedures were employed to segregate these sources of firing rate modulation. During active head movements, static friction loads were randomly applied to the rotary shaft. In the other procedure, the animal's head was fixed, and the horizontal head torque (measuring attempted head movement) signal was substituted for the rotary potentiometer signal. The gain of the torque signal was randomly adjusted to require greater effort on some trials while the animal performed the task in one of three paradigms: 1) head fixed to the stationary platform; 2) head fixed to the platform, which was moved by the servo system, with the torque signal serving as the input; and 3) head fixed in space, with the body moved about the head by the servo system as in item 2. Individual trials in which a greater effort was required were segregated and analyzed for qualitative and temporal differences in onset or offset of firing rate changes. Preliminary data indicate that the input to these neurons, located on the medial-descending border of the vestibular nuclei 1-2 mm caudal to the abducens nucleus, is mainly of vestibular and neck sensory origin.

1945 TRANSFER FUNCTION ANALYSIS OF RHESUS MONKEY HORIZONTAL VESTIBULOOCULAR REFLEX USING PSEUDORANDOM BINARY SEQUENCE AND SINUSOIDAL ROTATIONAL STIMULATION. Joseph M. Furman, Dennis P. O'Leary. Dept. Otolaryngology, Div. of Vestibular Disorders, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15213 and James W. Wolfe. USAF School of Aerospace Medicine, Brooks AFB, San Antonio, Texas 78235.

A transfer function analysis of the alert rhesus monkey horizontal vestibuloocular reflex (VOR) was determined using pseudorandom binary sequence (PRBS) rotational acceleration about the vertical axis in the dark. The PRBS period length and state duration were chosen to deliver vestibular stimuli at frequencies from 0.008 Hz to 0.66 Hz simultaneously. Eye movements were recorded with chronically implanted electrooculographic electrodes which were connected to a DC coupled amplifier. The electrooculogram was sampled at 100 Hz by a PDP 11/40 computer using a 12 bit analog to digital converter. The nystagmoid eye position data was then analyzed by a software algorithm which replaced saccadic movements with estimated slow phase eye position. The transfer function of the VOR was then estimated using a cross-spectral analysis technique. Single frequency sinusoidal vestibular stimulation was also used to calculate gain and phase.

The PRBS technique has the advantage of allowing a characterization of the monkey's VOR at many frequencies simultaneously during a short time interval (about 5 minutes). This minimizes the effects of physiological nonstationarities on the estimate of the VOR transfer function.

Preliminary results using the PRBS technique reveal that response decline is minimal even after an hour of stimulation. Also, response variability cycle to cycle using sine wave stimulation exceeds response variability during PRBS stimulation.

1946 RHYTHM GENERATION IN TONICALLY ACTIVE LABYRINTHINE PRIMARY AFFERENTS OF THE TOADFISH, OPSANUS TAU. S. Highstein & A. Politoff. Dept. of Neurosci. A. Einstein Coll. Med., Bronx, N.Y. & Dept. of Physiol., Boston U. Sch. Med., Boston, Mass.

Action potentials (AP) were recorded with a hook electrode from physiologically identified tonically active posterior semicircular canal primary afferents, in vitro. Recording sites were less than 1mm from the labyrinthine sensory epithelium. >200 units were studied. Following each AP a baseline hyperpolarization-depolarization sequence (H-DS) leading to the subsequent spike is seen. H-DSs are stereotyped for a given unit firing at a stable frequency. However, ramp-like depolarization does not invariably lead to spiking as an occasional AP is dropped. Notable in these cases of dropped APs is the absence of an H-DS at the predicted interval of AP occurrence. Tetrodotoxin (TTX, 1µm bath applied) blocks the APs; H-DSs at the frequency of spiking before TTX application are never observed. Thus, H-DSs are invariably associated with APs and are absent if APs are absent.

Frequency of spontaneous APs and H-DSs are increased by de- and decreased by hyperpolarizing currents applied directly to the recorded nerve filament. These currents almost certainly modulate frequency directly by flowing in an antidromic direction thru the afferent fiber into the extracellular space. Changes in frequency are also produced by currents passed across the ampullary wall. As transmission between Type II hair cells and primary afferents is chemical, lumen positive or negative transepithelial pulses presumably act directly on presynaptic secretory membrane to increase or decrease transmitter release respectively, thus modulating discharge frequency orthodromically. In regular units changes in frequency of APs and H-DSs increase and decrease in parallel whether they are caused by direct or transsynaptically applied polarization.

In speculation upon the mechanism of rhythm generation in tonic primary afferents, Calvin's relaxation oscillator model is compatible with the experimental results. Tonically active primary afferents have fine highly branched dendrites which should be subject to ongoing transmitter release from numerous hair cells simultaneously. This electrotonically remote depolarization will be passively conducted towards the spike initiation region producing a steady depolarization at rest. When threshold is exceeded, a spike will be initiated and will spread passively back into the dendritic tree causing hyperpolarization at the spike initiation region of the primary afferent. As the hyperpolarization dies away, the membrane potential will rise again because of the steady synaptic depolarization, and another spike will occur. Polarizing currents passed directly into the nerve are presumably acting on this same spike initiation region to affect spontaneous frequency.

1947 EFFECTS OF IPSI AND CONTRA-LATERAL CALORIC STIMULATION ON THE 8th NERVE RESPONSE IN ALERT MONKEYS. A.W. Louie* and J. Kimm. Depts. of Physiology and Biophysics and Otolaryngology, Uni. of WA., Sch. Med., Seattle, WA 98195.

Activity of single nerve fibers of the vestibular nerve in alert monkeys were recorded during ipsi-lateral and contra-lateral caloric stimulation. Eye movements were recorded simultaneously with these single fiber recordings. Caloric stimulation of the ipsi-lateral canal increased the firing rate of the units when the stimulus was above core body temperature and decreased the firing rate when the stimulus was below core body temperature. Concurrent with these changes in the firing rate of the unit, nystagmic eye movement responses were recorded. As expected, with an increased firing rate of the nerve, the fast phase of nystagmus was toward the stimulated side, the converse was seen when the nerve firing rate was decreased below the resting rate. Contrary to what was expected, nystagmic eye movements were not closely coupled with the firing rate of the 8th nerve. In many cases, the nystagmus would stop and then reverse its direction before the nerve fiber returned to its pre-stimulated resting rate. To a contra-lateral caloric stimulation, the 8th nerve firing rate could also be altered from its resting rate. Irrigations of 20^{o} C of the contra-lateral ear caused a change in firing rate in 22 of the 24 fibers observed. In response to irrigation of 52^{o} C, 12 of 22 units also showed a change in their firing rate. Those fibers which were affected by the contra-lateral caloric stimulation also showed a high correlation to those units which exhibited a diminished phase lag re-acceleration with an increased frequency of oscillation.

Supported in part by NIH Grant RR-00166. Special acknowledgment to LIFE TECH INSTRUMENTS for the use of the Air Caloric Stimulator used in this study.

1948 DIFFERENTIAL RESPONSE OF THE CRISTA TO SINUSOIDAL ROTATION. Jay W. McLaren and Dean E. Hillman, Dept. Physiol. and Biophys., The University of Iowa, Iowa City, IA. (Present addresses: Dept. Physiol. and Biophys., Mayo Clinic, Rochester, MN 55901 and Dept. Physiol. and Biophys., New York University Med. Sch., New York, NY 10016)

The horizontal semicircular canal crista is approximately three times as wide at its dorsal end as at its ventral end, while the cupula's thickness conforms to this asymetric shape. Recent studies have shown that during rotation the thin region of the cupula is displaced through about 50% greater distance than thicker regions (McLaren and Hillman, Neurosci. Abst. 3: 544, 1977). One might expect afferent ampullary nerve fibers to also show response characteristics that are dependent on the position of their associated receptor cells along the length of the crista. In this study, electrophysiological characteristics of single unit afferent fibers from bullfrog horizontal ampullary nerve were examined during rotation and correlated with their site of innervation. Single fibers were dissected from the surface of the main nerve bundle with steel hook electrodes and their action potentials recorded during sinusoidal rotation (rotation amplitude: 0°-40°; frequency: .2-.8 Hz). Fibers were classified as belonging to one of three groups according to their projection into the crista. The first group innervated receptor cells on the narrow end of the crista, adjacent to the thin portion of the cupula, the second group innervated a section of the crista just to the center of the broad end, while the third group innervated the broad end. Fibers that projected to the narrow crista (group 1) showed: 1) high maximal firing rate per unit angular velocity, 2) low angular velocity thresholds, 3) peak firing rates that reached a maximum and leveled off or decreased as peak angular velocity increased from 0 to 150°/sec, and 4) mean phase lead of about 33° relative to angular velocity. In contrast, fibers that innervated the opposite end of the crista showed: 1) relatively low peak firing rates per unit angular velocity, 2) high angular velocity thresholds, 3) firing rates that increased linearly with peak angular velocity (between 0 and 150°/sec), and 4) a mean phase lag of about 17° relative to angular velocity. Characteristics measured from fibers of group 2 were generally between those of groups 1 and 3. The spatial distribution of these response characteristics along the crista is consistent with the distribution of the cupula's mechanical response as measured during similar rotation. It is concluded that separate channels of information carried by the ampullary nerve arise at least in part from the differential mechanical response of the cupula. (Supported by USPHS, grant NS-13742 from NINCDS).

1949 VISUAL "CONTEXT" DETERMINES THE OCULAR RESPONSE TO HIGH FREQUENCY (3 HZ) HEAD OSCILLATION IN SUBJECTS ADAPTED TO PROLONGED VISION REVERSAL. G. Melvill Jones, P.R.T. Davies* and A. Gonshor*. Aviation Medical Research Unit, Dept. of Physiol., McGill University, Montreal, Quebec, Canada.

Previous work has shown that prolonged optical reversal of vision produces large adaptive changes in the vestibulo-ocular reflex (VOR) as tested by low frequency sinusoidal head rotation in the dark. The present results indicate that vision much improves the adapted ocular response to head rotation, even at an oscillatory frequency which exceeds the upper limit of purely visual following. For example, when the one-year adapted cat was oscillated in the dark at 3.0 Hz and 8°/sec amp. (peak to peak), the smooth component of ocular response had a gain (eye vel./head vel.) of 0.44 and phase of +20° (relative to normal compensation). The same head oscillation with lights on and reversed vision produced markedly different values of gain and phase, namely 0.94 and +149° respectively. With head stationary, the same relative oscillatory movement of the visual field produced no measurable ocular response. In all cases, the visual field had approximately the same angular constraint as that provided by the reversing optical system.

A similar, but not identical, phenomenon has been observed in man. When an adapted human subject oscillated his head at 3.0 Hz and 3° amp. in the light, the ocular response was quite different according to whether the reversing prisms were off or on. With prisms off, the eyes moved in approximately the normal direction at about half gain (gain≈0.4; phase≈ +10°). With prisms on they moved in an approximately reversed direction (gain≈0.4; phase≈ +145°). Therefore, in the adapted subject the oculomotor response was substantially affected by vision according to whether it was normal or reversed. Yet, at this frequency (3.0 Hz), but at approximately half the amplitude of movement of the visual field relative to the stationary head, no eye movements were produced in the same adapted subject. Significantly, the unadapted subject always produced non-reversed ocular responses of gain 1.0 and phase zero, independently of whether the prisms were off or on.

It seems that in both cat and man the adaptive process includes modifications which permit the visual "context" to influence the Visual-Vestibular-Interactive-System at frequencies that are beyond the normal visual tracking capabilities, and in ways which are not available to the unadapted subject.

Supported by Canadian MRC Grant No. MT-5630.

1950 ROTATION EFFECTS ON BEHAVIOR DEVELOPMENT AND RECOVERY OF FUNCTION FOLLOWING LESIONS OF LATERAL VESTIBULAR NUCLEUS IN RATS. D. Modianos, T. McCormack*, L. Garrett,* W. McLauchlan*, and R. Catera*. Psych. Dept., Fordham Univ., Bronx, N.Y. 10458.

The lateral vestibular nucleus (LVN) is the origin of the lateral vestibulospinal tract. This tract has been extensively studied neuroanatomically and neurophysiologically. Lesions in LVN produce severe deficits in tests of posture and movement in adult rats (Modianos and Pfaff, Brain Res.106:31, 1976). Since Thoman and Korner (Dev.Psychobiol.,5:92, 1971) have shown that vestibular stimulation affects behavior development in infant rats, the present study investigated (1) effects of rotation upon recovery of performance on several motor tasks following unilateral LVN lesions in weanling rats, and (2) development of, and effects of rotation upon, the acquisition of the same tasks used to evaluate recovery of performance following lesions. One group of rats was rotated for twenty minutes each day beginning at birth, while the second group was identically handled but not rotated. Between day 10 and weaning, each rat was evaluated for ability to balance and traverse a beam, for occurrence of the righting reflex, and for the ability to regain an upright position after being suspended upside-down from a wire grid ("climb over" task). After weaning, all rats received unilateral electrolytic lesions in LVN. One group consisted of rats rotated since birth and after lesions, one group was rotated after lesions only, and a third group was never rotated. Developmentally, rotation significantly facilitated performance on the righting reflex task, had a slight (but statistically significant) effect on the climb over task, and did notaffect ability to balance on or traverse a beam. Following unilateral LVN lesions, rotation significantly facilitated recovery of performance, but only on some tasks and only in rats which had been rotated since birth. Rats rotated since birth recovered righting reflex ability quickly following lesions. Rotation,either since birth or after lesions only, did not affect time spent on the balance beam. However, rats rotated since birth were more able to traverse the balance beam and less resorted to balancing by clinging in an inverted position than either rats rotated only after lesions or rats which were never rotated. There was no effect of rotation upon recovery of performance in the climb over task. We conclude that (1) rotational stimulation has effects on both behavior development and recovery of function following vestibular lesions,(2) knowledge of rotation effects on development does not always predict rotation effects on recovery of function and (3)facilitation by rotational stimulation of recovery of function following vestibular lesions is evident only in animals rotated since birth.

1951 STROBE LIGHT PREVENTS SHORT-TERM VOR ADAPTATION TO VISION-REVERSAL IN CAT. R. Notkin*, O. Panyszak*, G. Melvill Jones, and G. Mandl. (SPON: R. Capek).
Aviation Medical Research Unit, Department of Physiology, McGill University, Montreal, Quebec, Canada.

Exposure of the alert, initially unadapted, but vision-reversed cat to 4 hours forced oscillation in normal light produced marked (over 50%) and highly significant ($P < 0.001$) attenuation of the vestibulo-ocular reflex (VOR) as tested by sinusoidal rotation in the dark at 1/4 Hz and 20°/sec amp (peak to peak).

Identical forcing stimuli performed on the unadapted animal with vision reversal, but in strobe light of 3 μsec flash duration at 4 Hz flash frequency, produced an insignificant ($P > 0.2$) adaptive change in the dark-tested VOR. Thus, prevention of continuous image slip on the retina by means of strobe light was associated with prevention of short-term VOR adaptation to vision reversal. This finding suggests that, unlike in man (Melvill Jones & Mandl, 1977, Neurosci. Abst. Vol. III, No. 1726), retinal image slip is necessary for activation of the short-term adaptive process in cat.

Interestingly, in contrast to humans (Mandl & Melvill Jones, 1977, Neurosci. Abst. Vol. III, No. 1812), the unadapted vision-reversed cat showed no evidence of reverse tracking episodes during head oscillation in strobe light. Thus, an alternative explanation for the absence of adaptation in cats exposed to strobe light may be the absence of reversed, i.e. VOR opposing, eye movements during adaptation.

Supported by Canadian MRC Grants No. MT-5630 and MT-3557.

1952 NONLINEAR AFFERENT RESPONSE PROPERTIES FROM THE ISOLATED GUITARFISH SEMICIRCULAR CANAL. Dennis P. O'Leary and Conrad Wall, III. Dept. Otolaryngology, Div. of Vestibular Disorders, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15213.

Investigations of receptor mechanisms of the semicircular canal have been aided in previous studies by the use of linear system analysis of afferent responses to controlled rotational acceleration. These studies have resulted in models in the form of differential equations or linear transfer functions. However, it is possible that receptor transduction and subsequent spike train modulation are influenced by intrinsic nonlinearities, such as those resulting from viscoelasticity and spike generating mechanisms, that are reflected in the observed afferent response dynamics.

We tested for the presence of certain nonlinear afferent response components by applying a high frequency rotational acceleration signal in combination with a low frequency modulation stimulus. Such dual inputs have been used successfully for identifying certain nonlinearities in engineering control systems (e.g. Atherton, D.P., Nonlinear Control Engineering, Van Nostrand, New York, 1975). Low-amplitude rotational acceleration of frequencies $\geq$ 30 Hz were combined with either (1) single frequency sinusoidal oscillations of frequencies $\leq$ 1 Hz, or (2) pseudorandom binary sequence rotational acceleration spanning bandwidths from 0.04 to 4.0 Hz. Responses to the dual inputs were compared with those resulting from only the lower frequency inputs, in the same horizontal canal afferents. Time domain cross-correlation and frequency domain complex spectral analyses techniques were used to obtain system descriptors.

Results showed that the afferent response dynamics were modifiable reversibly and systematically by application of dual inputs, indicating significant nonlinear effects. Specific effects noted included: (1) reduction of the dominant time constant of afferent responses, (2) enhancement of response "adaptation", (3) systematic changes in low-frequency gain and phase, and (4) changed patterns of background (unmodulated) activity toward lower coefficients of variation. These results form the basis for an extended model of semicircular canal transduction, which, in addition to linear mechanics, includes specific types of nonlinear mechanisms.

Supported by NIH grant NS12494.

1953 RESPONSES OF SECOND-ORDER VESTIBULAR NEURONS DURING LINEAR ACCELERATION ALONG HORIZONTAL AXES IN RAT AND SQUIRREL MONKEY. A. A. Perachio, W. Bouris, S. Anschel, and D. H. Rice. Yerkes Reg. Primate Res. Ctr., Emory Univ., Atlanta, GA 30322.

The response of neurons, in and around the vestibular nuclei of anesthetized rats, to sinusoidal linear acceleration along a vertical (Z) axis is sinusoidally modulated (Perachio, A. A., et. al., Soc. for Neurosci., Abst. 1733, 1977). A nearly linear gain relationship between that response and the input acceleration over a limited (0.2-0.5 Hz) frequency range is indicated by a decrease in gain as acceleration increases. A similar finding has been reported for forelimb muscle (EMG) activity in decerebrate cats accelerated along the fore-and-aft (X), side-to-side (Y) and vertical axes (Anderson, J. H., et. al., Brain Res., 120 (1977), 1-15).

The activity of neurons in the region of the vestibular nuclei was recorded, in both anesthetized rats and alert squirrel monkeys, while the animals were subjected to sinusoidal translational motion along their longitudinal or transverse axes. Head orientation was fixed, pitched nose down 22° from the stereotaxic horizontal plane. Neurons responded either to one direction of movement alone or exhibited some degree of sensitivity to acceleration along either axis. Most neurons, responsive to acceleration along both horizontal axes, were sensitive either to forward and ipsilateral (x^+, y^+) or backward, contralateral (x^-, y^-) motion, indicating a resultant direction of maximum sensitivity.

A relatively small number of neurons was encountered whose activity modulated with oscillating visual stimuli. In general, linear acceleration sensitive neurons did not appear to respond during spontaneous eye movements. In cases where sensitivity was tested, gain appeared to decrease with increasing stimulus frequency (acceleration) resembling previous findings for Z-axis-sensitive neurons.

(Supported by NIH Grant #RR 00165 and NASA Grant #NGR 11-001-045.)

1954 TRANSFER FUNCTIONS OF CAT SEMICIRCULAR CANAL EIGHTH NERVE AFFERENTS. Robert J. Peterka*, Dennis P. O'Leary and David L. Tomko. (SPON: R.F. Dunn). Depts. Otolaryngology & Pharmacology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15213.

Responses to yaw rotation of 94 primary afferent neurons innervating the horizontal semicircular canal were recorded with micropipettes in barbiturate anesthetized cats. Pseudorandom binary sequences (PRBSs) of rotational acceleration (bandwidth 0.0078 to 3.70 Hz) were delivered to a rate-of-turn table under computer control. The transfer function observed at each first order afferent (i.e., gain and phase) was determined using a cross spectral analysis between the input PRBS and the output neural response. Since implementation of the cross spectral analysis required that an estimate of the firing rate of the neuron be specified in equally spaced time intervals, a digital filter, the French-Holden algorithm (French & Holden, Kybernetik, 1971, 8, 165-171) was used. In addition to the transfer function estimates, cross spectral analysis provided a determination of the coherence function, a measure of the linear correlation between the stimulus and the response. Typically, the coherence function at frequencies less than 0.1 Hz had values near unity, indicating a linear response to the PRBS stimulus. At higher frequencies, coherence was generally less than 0.5 indicating either the presence of noise or a nonlinear response to the stimulus.

Results indicated that gain and phase varied widely for different cells at the same test frequencies. Because others have noted a correlation of response dynamics with the degree of regularity of discharge of a cell, we have divided the population into two groups based on the coefficient of variation (CV) of the unit at rest. Cells with resting CVs greater than 10% in general showed an average of two to four times higher gain across the bandwidth tested and 10 to 20 degrees less phase lag (re acceleration) than cells with CVs less than 10%. The differences in gain and phase between the two subpopulations were more pronounced at frequencies above 0.5 Hz. Gains and phases computed from application of sinusoids were in agreement with those obtained from the PRBS stimulus application in the same cells.

Analytic transfer functions of various forms were fit to the averaged gain and phase data for the two populations to provide a quantitative data summary. Neurons with CVs less than 10% were best fit by a two pole, one zero transfer function similar in form to that obtained for this type of unit in the squirrel monkey by Goldberg and Fernandez (J. Neurophysiol., 1971, 34, 681). Irregularly discharging *neurons (CV greater than 10%) on the other hand, were best fit by a transfer function with a fractional exponent term.*

Supported by NIH grants NS12308 and NS12494.

1955 ACTIVATION OF VESTIBULAR AND RETICULAR NEURONS DURING VESTIBULAR REFLEXES INDUCED BY SINUSOIDAL POLARIZATION OF SEMICIRCULAR CANAL AFFERENTS. B.W. Peterson, K. Fukushima, N. Hirai*, R.H. Schor, V.J. Wilson. Rockefeller Univ., New York, N.Y. 10021.

When labyrinthine afferents are activated by sinusoidal horizontal rotation in decerebrate cats, vestibular reflex activation of neck[1] and limb[2] muscles lags 40-80° behind the discharge of second order (2°) vestibular neurons. Thus 2° neuron activity is not sufficient to produce the reflex activation. To investigate the role of vestibulospinal and reticulospinal neurons in vestibular reflexes we have developed a technique for inducing these reflexes by sinusoidal electrical polarization of single semicircular canal nerves. In decerebrate, partially cerebellectomized cats fine wires were implanted at locations near horizontal or anterior canal ampullae where stimuli evoked pure horizontal or vertical eye movements. Modulated, continuous currents were applied monopolarly to polarize canal nerve terminals. The modulating waveform was either a single sinusoid at 0.01-5 Hz or nine superimposed sinusoids covering the range 0.018-6.1 Hz. (To minimize interactions between them, the nine frequencies were all relatively prime, odd multiples of the same fundamental frequency). Action potentials of vestibular or reticular neurons were recorded with micropipettes, discriminated and processed by computer to obtain cycle histograms at each of the applied frequencies. Fourier analysis was used to determine the gain and phase of the neural response at each frequency plus the amount of harmonic distortion and noise.

Responses were typically sinusoidal, except where discharge was cut off at threshold, and response amplitude was related to stimulus amplitude indicating that the responses were linear. Responses of 2° neurons were all similar regardless of whether the neuron projected to the spinal cord. Peak discharge was approximately in phase with peak stimulus negativity at frequencies below 0.2 Hz. Above 0.2 Hz the gain increased and a phase lead developed which reached 40-50° at 6 Hz. This behavior probably reflects accomodation in the nerve terminals which makes them sensitive to rate of change of stimulus current. Higher order vestibulospinal and reticulospinal neurons were also modulated by sinusoidal polarization. Some behaved like 2° neurons but for others the response lagged 30-80° behind the 2° response and the gain fell over the frequency range 0.18-1.5 Hz. Responses of these neurons thus resemble those of neck muscles described by Wilson et al.[3] Supported by N.I.H. NS 02619 and EY 02249.

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3. Wilson, Fukushima, Hirai, Peterson & Uchino, Neurosci. (1978).

1956 THE INFLUENCE OF BILATERAL PLUGGING OF THE ANTERIOR OR LATERAL SEMICIRCULAR CANALS ON VERTICAL AND HORIZONTAL VESTIBULOOCULAR REFLEXES IN THE RABBIT. V. E. Pettorossi* and M. H. Barmack, (SPON: R. S. Dow) Neurological Sciences Inst., Good Samaritan Hosp. & Med. Cntr., Portland, OR.

In contrast to the horizontal vestibuloocular reflex (HVOR), the vertical vestibuloocular reflex (VVOR) of the rabbit has a higher gain (eye velocity/head velocity) and a smaller phase lead (eye position +180° re: head position), at low frequencies of sinusoidal angular accelerations (± 10 deg, .005-.05 Hz). That the low frequency gain of the VVOR can be attributed to a utricular input can be demonstrated by turning the rabbit 90 deg, "nose-up" so that its longitudinal axis is parallel with rather than orthogonal to the linear acceleration of gravity. Under this condition the gravity vector is orthogonal to the plane of polarization of hair cells of the utricular maculae, and the gain and phase of the VVOR are similar to the phase and gain of the HVOR. It is possible to derive a contribution of the utricle to the VVOR by subtracting the VVOR obtained when the rabbit is "nose-up" from the VVOR obtained when the rabbit is prone; assuming a linear combination of the canal and otolith signals. In the present report we examine this assumption by plugging the anterior canals bilaterally and thereby obtaining a direct measurement of the residual contribution of the utricle to the VVOR. The anterior semicircular canals were plugged with bone wax or with wires which compressed a portion of the membranous canal. This bilateral plugging caused a decreased VVOR gain and an increased phase lag at higher stimulus frequencies (.05-.8 Hz). The magnitudes of the reduced gain and increased phase lag, were dependent on the proximity of the canal plugs to the ampullae. When the anterior canal plug was greater than 500 μ from the ampulla, there was residual anterior canal function. The gain and phase of the VVOR following bilateral anterior canal plugging are consistent with the prediction which assumed a linear combination of utricular and anterior canal inputs. Plugging the anterior canals also reduced the gain and increased the phase lead of the HVOR. Conversely plugging the horizontal canals, although eliminating the HVOR, did not cause as big a reduction in the VVOR. We conclude that the blockage of the free flow of endolymph in one of the canals, impedes the flow of endolymph in all canals. The extent of this impedance is different for the lateral and anterior canals. It might be attributed to the different spatial relationships of these canals to the membranous duct which joins their ampullae to the utricle, thereby comoleting their endolymph "circuits". (Supported by PHS Grant EY-00848 and The Oregon Lions Sight Foundation).

1957 ABSENCE OF STRUCTURAL CHANGES IN GRAVISTATIC RECEPTORS DURING VESTIBULAR COMPENSATION. Christopher Platt & Earl Y. Jew*. Dept. Biological Sci., Univ. Southern California, Los Angeles, CA 90007.

Plasticity of vestibular function is shown by vestibular compensation, in which recovery of symmetrical upright posture is restored some time after a unilateral vestibular lesion. Partly because of the relatively short time course of compensation, it has been considered a central phenomenon. However, whether peripheral changes might be necessary along with the central changes has not been tested directly.

Fishes were used for this study because their known capability for both growth and reorganization of adult afferent sensory systems suggests they might have greater structural plasticity in their peripheral vestibular systems than most other vertebrates. Conversely, if such plasticity is not present in fishes, it seems far less likely to be a mechanism used by higher vertebrates.

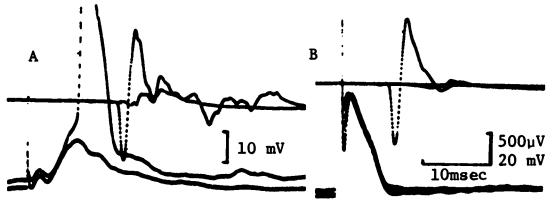
The gravistatic organ, the utricle, was removed from one side of an anesthetized goldfish's head to provide the control specimen. After plugging the wound with saline agar, each fish was allowed to recover. Motor behavior, including body tilt, swimming attitude and eye-roll, was monitored to evaluate the degree of compensation after the initial grossly asymmetrical disorientation. Animals were sacrificed at intervals from 1 to 14 days after the operation, and the remaining utricle prepared for scanning electron microscopy (SEM), as were the controls.

Compensation was largely complete within 5 days. SEM observations on the control and "compensated" utricular sensory maculae showed no changes in a) morphological orientation of hair cell ciliary bundles, which indicate directional sensitivity; b) distribution of the different forms of hair cell ciliary bundles; nor c) relative proportions of macular area covered by outward versus inward-facing hair cells, which are not 1:1 but asymmetrical in the normal utricle.

We conclude that structural changes in the asymmetrically organized gravistatic receptors of one side are not necessary for restoration of symmetrical postural motor output after a contralateral vestibular lesion. Instead, the central processes involved in vestibular compensation must be both sufficient and necessary.

1958 MAUTHNER CELL OF THE BULLFROG TADPOLE MEDIATES RAPID ACTIVATION OF CONTRALATERAL TAIL MUSCULATURE. Michael K. Rock. Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

The Mauthner cell of the bullfrog tadpole (Rana catesbeiana) is a large nerve cell located in the medulla at the level of the VIII nerve as in other species. Stimulation of the anterior branch of the ipsilateral VIII nerve produced short latency EPSP's and firing of the M-cell. An action potential in the M-cell was followed by extracellularly recorded activity in contralateral tail muscle with a latency of about 10 msec (Fig. A). A similar tail response was elicited after direct intracellular stimulation of the M-cell. Variability in the latency and amplitude of the tail response was observed with VIII nerve and intracellular stimulation. Repetitive intracellular stimulation at a frequency of 1 Hz evoked a tail response to the initial M-cell spike with no response to subsequent M-cell activity (Fig. B). The characteristic response in the contralateral tail to VIII nerve stimulation could not be elicited after the M-cell was damaged and inactivated. Stimulation of the contralateral VIII nerve produced depolarizing PSP's in the M-cell soma. However, the tail response elicited by VIII nerve stimulation was eliminated by prior stimulation of the opposite VIII nerve. These results indicate that the tadpole Mauthner cell mediates a rapid activation of contralateral tail musculature in response to ipsilateral VIII nerve stimulation as in teleost fish. (Supported by USPHS Grant NS 09367.)



1959 VESTIBULAR EFFERENT NEURONS OF PIGEONS INNERVATE SEVERAL CRISTAE AMPULLARES WITH AXON COLLATERALS. Dietrich W.F. Schwarz, Irmgard E. Schwarz* and R.David Tomlinson*. Lab. Otoneurol., Depts. Otolaryngology and Physiology, University of Toronto, Toronto, Canada.

Retrograde axonal transport of either ^{3}H-adenosine or horse radish peroxidase (HRP) injected into cristae ampullares of semicircular canals in pigeons, identified perikarya of efferent vestibular neurons. These are located bilaterally within less than 1/2 mm^3 in the nucleus reticularis pontis caudalis just ventro-latero-caudally to the abducens nucleus. Neurons projecting to each of the six canal cristae appaered to be not topographically seperated and neurons projecting to either the utricular or lagenar macula were found at the same location, whereas cochlear efferent neurons are located within and medio-ventral to the superior olivary nucleus. Numbers of cells found labelled after injection of individual cristae were remarkably constant, although cell counts after anterior canal crista injections were lower than for lateral and posterior canals (range 95 to 150 cells per crista). Injections into all three canal cristae of one side yielded similar cell counts, whereas the numbers of cells labelled by injections into all six cristae were increased by ca. 50%. Injections into the lateral canal perilymph yielded no labelled cells which suggests that no effective diffusion of marker from one crista to an other one has occured. Thus our data suggest that efferent cells send collateralizing axons to more than one crista. This conclusion is corroborated by the fact that a few neurons could be shown to be labelled with both, ^{3}H-adenosine and HRP when one crista was injected with the first and an other one with the second marker. It can be concluded that activity of efferent neurons is unlikely to provide a control on afferent vestibular input which is specific for head movement parameters.

Supported by the Medical Research Council of Canada.

1960 LOSS OF BOTH THE QUICK COMPONENT OF HEAD NYSTAGMUS AND THE ABILITY TO RIGHT IN THE AIR FOLLOWING PONTINE LESIONS IN THE RAT. David W. Sirkin, Timothy Schallert*, and Philip Teitelbaum. Program in Neural and Behavioral Biol., and Dept. Psychol., Univ. of Illinois at Urbana-Champaign, Champaign, IL 61820.

Vestibular compensation for passive angular motion is very marked in lower vertebrates. A fish swimming in a rotating bowl circles in the opposite direction to that in which the bowl is turned. A frog sitting on a turntable turns its head to the left if the turntable is rotated clockwise. These responses tend to stabilize the orientation of the animal's head in space. A vestibular response can also be elicited by spinning an animal many times quickly, and then stopping the motion abruptly. In this case, the animal turns in the same direction as the original motion. These vestibular responses to angular motion, shown by the eyes as well as the head and body, are modified in mammals by quick movements of the eyes and head in the direction opposite to that of the vestibular compensatory response. These "anticompensatory" movements are normally triggered before the vestibular response has progressed very far. During a rotation, and following an abrupt stop of a series of rapid rotations, compensatory and anticompensatory movements typically alternate in a rhythm, or nystagmus. The compensatory movement is called the slow component, and the anticompensatory movement, the fast component.

It has long been known that paramedian pontine reticular formation (PPRF) lesions abolish the quick component of ocular nystagmus in monkeys and cats. We report here that lesions of a homologous region in the rat brain abolish the quick component of head nystagmus. (Preliminary observations indicate that the quick component of ocular nystagmus is also lost.) Our observations indicate that when anticompensatory mechanisms are abolished the vestibular compensatory responses of the head and body in the rat are at least as strong as in lower vertebrates. A rat with a right PPRF lesion, if grasped by an investigator and turned clockwise, turns its head to the left until its nose touches its left thigh. When stopped abruptly after rapid counter-clockwise spinning, the rat shows a steady turn of the head to the left, followed by tight counter-clockwise circling. Rats with bilateral lesions show these responses bilaterally. Strong responses are shown long after the lesions are made--at least 9 months in bilaterally lesioned rats--and are not affected by the application of visual occluders.

A second result of bilateral PPRF lesions in the rat is the complete loss of the ability of the animal to right when released in the air with dorsal surface down. Unilateral lesions result in a partial impairment of this reflex. It is interesting that a single lesion releases one vestibular reflex while abolishing another. Supported by NSF SER 76-18255 and NIH RO1 NS 11671.

1962 DYNAMIC EVALUATION OF HUMAN VESTIBULO-OCULAR FUNCTION USING WHITE NOISE ROTATION STIMULUS AND LINEAR SYSTEM PARAMETER ESTIMATION TECHNIQUES. Conrad Wall III, F. Owen Black and Dennis P. O'Leary. Dept. of Otolaryngology, Div. of Vestibular Disorders, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15213.

White noise rotational stimulation has previously been used to describe the response of first order vestibular afferents in animals and the vestibulo-ocular reflex (VOR) in normal humans (Wall, et al, 1978). The results from a cross spectral calculation give frequency domain dynamic response estimates of gain and phase which can serve as one form of inter test comparison.

A more compact form of system descriptor or model is provided by fitting linear system parameters (Eykhoff, 1975) to these experimentally determined gain and phase points. This method yields a small set of coefficients that can be directly related to mathematical models of the semicircular canal or of the VOR (Peterka, et al, 1978). We are applying these two techniques for identification of human vestibular abnormalities.

In the first approach, the data from a small group of patients having known vestibular disorders are compared to normal data. These comparisons provide the basis for initial decision criteria which are used to separate any new data into categories without other information concerning the patient. This prediction is then compared with an independent medical assessment which includes a vestibular test series and post-operative surgical information.

The second approach attempts to classify the fitted parameters of steady state gain and linear system time constants of patients having known disorders into groups which are outside the limits for normal human subjects. The results of these two approaches will be presented and discussed.

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1961 EFFECT OF HEAD TILT ON RECEPTIVE FIELD ORIENTATION OF SIMPLE CELLS IN AREA 17 OF UNANESTHETIZED CATS. David L. Tomko, N. M. Barbaro* & F. N. Ali*. Dept. Pharmacology, Sch. Med., Univ. of Pittsburgh, Pittsburgh, PA 15261

Electrophysiological evidence for convergence of visual and vestibular information in visual cortical cells has existed since 1960 (Grüsser & Grüsser-Cornehls, Pfluger's Arch., 1960, 270, 227-238). Results in the past few years regarding the quantitative nature of such convergence have been to some extent contradictory, with some authors finding that the use of natural vestibular stimuli results in significant alterations in receptive field (RF) orientation of area 17 cells (c.f., Denny & Adorjani Exp. Brain Res., 1972, 14, 312-317), and others finding no effect (Schwartzkroin, Exp. Neurol., 1972, 36, 498-506).

The response properties of 28 single units with simple RF properties and histologically verified to lie in cytoarchitectural area 17 were determined in paralyzed, unanesthetized cats. RF properties were first mapped using a hand held ophthalmoscope to define the RF type, its boundaries, and center. Subsequently, they were determined quantitatively by presenting in random order for 0.6 seconds each, 18 different stationary slit orientations. The random order was repeated five times, and the stimulus was presented monocularly while recording the time of occurrence of action potentials. Then the animal was tilted 45^{0} around the naso-occipital axis and the procedure repeated. When possible (11 cells) the RF properties were redetermined when the animal was returned to the horizontal position. Ten of these 11 replications reproduced the original RF properties within 10^{0}. Data were averaged off-line for the five presentations of the 18 different orientations, the orientation giving the maximal response was determined, and three tuning curves were constructed with responses expressed as per cent of maximum. The tuning curves defined the orientation of the RF first with the animal horizontal, second with the animal tilted 45^{0}, and third after the return to the horizontal position. Comparison of the first two tuning curves enabled comparison of the actual amount of RF tilt with the expected amount of RF tilt due to head tilt (i.e., 45^{0}).

The RF orientation of 10 of the 23 cells (43.5%) after head tilt was within 5^{0} of the expected tilt of the RF due to the head tilt. Of the remaining 13 cells (56.5%), 7 RFs tilted from 15 to 35 degrees more than the expected 45^{0} tilt, and 6 RFs tilted from 15 to 45 degrees less than the expected 45^{0} tilt. These results are interpreted as an indication that the vestibular system exerts a tonic influence over the response properties of at least some of the neurons of the primary visual cortex. (Supported by NIH grant NS12308, and the University of Pittsburgh Medical Alumni Association).

1963 MEMBRANE VOLTAGE NOISE AND CILIARY MOTILITY IN THE APLYSIA STATOCYST. Michael L. Wiederhold. Neurobiology Dept. Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

The Aplysia statocyst is a spherical balance organ. The wall of the cyst consists mainly of 13 receptor cells with motile cilia projecting into the fluid filled cyst lumen. Dense statoconia settle to fill the bottom third of the cyst lumen, but are kept in continual random motion by the beating cilia. A receptor cell is excited when the preparation is tilted sufficiently to bring its cilia into contact with the statoconia. The excitation consists of a depolarization and an increase in membrane voltage noise.

An analysis of the membrane noise caused by excitation is presented, which indicates that the noise is a superposition of many discrete depolarizing events whose average amplitude is between 1.5 and 2.3 mV. These are interpreted as arising from collisions between the moving statoconia and the beating cilia. With a receptor cell positioned so that only occasional collisions between the statoconia and cilia would occur, isolated discrete events of the size predicted by the analysis are seen. The conductance change associated with these events is about 10 times larger than estimates of single ionic channel conductance from other studies.

When a preparation is treated with seawater containing 10 mM $NiCl_2$ (which has been used in other studies to paralyze the cilia of paramecium), the motility of these cilia is reduced or blocked, and both the depolarizing and noise-increase responses to tilting are either greatly reduced or abolished. Another agent which blocks ciliary motility is a factor in the serum of cystic fibrosis patients. When such serum was mixed with seawater, results similar to those with $NiCl_2$ treatment were obtained. The mechanisms by which the ciliary motility might contribute to the sensory function of these cells are discussed.

1964 ANALYSIS OF CENTRAL VESTIBULOCOLLIC PATHWAYS BY MEANS OF MODULATED POLARIZATION OF VESTIBULAR AFFERENTS. V.J. Wilson, K. Fukushima, N. Hirai*, B.W. Peterson and Y. Uchino*. Rockefeller University, New York, N.Y. 10021.

Sinusoidal stimulation of single ampullary nerves with modulated continuous current[1] has been used to evoke activity of contralateral dorsal neck muscles in decerebrate cats. The stimulus typically consisted of nine superimposed sine waves covering the range 0.018-6.1 Hz. Rectified EMG activity usually exhibited a considerable phase lag re input negativity at 0.18-0.37 Hz, with a phase advance at higher frequencies that sometimes resulted in a lead at 3-6 Hz. At frequencies below 0.18 Hz there was great variation and often little or no lag. Gain usually decreased with increasing frequency. Central phase lag was measured by recording simultaneously from second-order neurons and muscle, or by taking the mean difference between the two in many experiments. The magnitude of the lag, which may approach 60-70° at 0.18 Hz, shows that this method of stimulation activates complex neural circuitry, presumably the same circuitry activated by natural stimulation. The phase lag at lower frequencies is clearly not produced by second-order neurons, which in these preparations respond to the same stimulus with a phase lead, but it may be produced by vestibular and reticular neurons that project to the spinal cord but do not receive short-latency canal input[1].

The MVST, which contains the crossed disynaptic excitatory canal pathways, plays no essential role in producing the muscle response at low frequencies, but such a direct pathway may be a more important contributor to the phase advanced response at higher frequencies[2]. We have tested this hypothesis by evoking vestibular reflexes, with superimposed sine wave and square wave stimulation before and after transection of the MLF just rostral to the obex. This lesion, which interrupted the MVST, had minor, inconsistent effects. Therefore, pathways other than the disynaptic one are sufficient to produce all components of the response of the muscle, at least when the reflex is studied in the decerebrate cat with the head immobilized. Supported by N.I.H. and NSF grants NS 02619 and BMS 75-00487.

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1965 EFFECT OF ACETYLCHOLINE DRUGS ON RECEPTIVE FIELD PROPERTIES OF RABBIT RETINAL GANGLION CELLS. Michael Ariel* and Nigel W. Daw, Dept. Physiol., Washington Univ. Sch. Med., St Louis, MO 63110.

We investigated the effects of the cholinergic nicotinic antagonist mecamylamine, and the acetylcholinesterase inhibitor physostigmine, on the activity of ganglion cells in the rabbit retina. The drugs were infused into the internal maxillary artery in the intact animal. Most types of ganglion cell were not much affected by mecamylamine, indicating that acetylcholine synapses represent a small fraction of their input. An exception was on center X cells, in agreement with the results of Masland and Ames (J. Neurophys., 39, 1220 (1976). We showed that both color coded X cells and on center X cells that are not color coded had their activity reduced by mecamylamine: this was true of both spontaneous activity and the response to light in either the center or the surround of the receptive field. Directionally sensitive cells also had their activity reduced by mecamylamine, but the response to a spot moved in the preferred direction was rarely reduced by more than 50%. Physostigmine, on the other hand, had a dramatic effect on directionally sensitive cells: the cells responded to movement in both preferred and null directions. The effect of physostigmine was indistinguishable from the effect of picrotoxin, and consistently occurred at one-fifth the concentration. Physostigmine and picrotoxin potentiated each other's effects. Mecamylamine reduced the effects of both physostigmine and picrotoxin on DS cells.

1966 MEMBRANE PROPERTIES OF SOLITARY ROD PHOTORECEPTORS. Charles R. Bader*, Peter R. MacLeish* and Eric A. Schwartz* (SPON: Ann E. Stuart). Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115 and Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60647.

Single, isolated (solitary) rod photoreceptors were obtained by enzymatic dissociation of the tiger salamander (Ambystoma tigrinum) retina. These solitary rods retained the morphological features of rods of the intact retina and could be maintained in vitro for several days. Solitary rods could be penetrated by one or two micropipettes under observation using infra-red illumination and an infra-red to visible light image converter. The dark-adapted solitary rods were isopotential, had a resting potential of approximately -45 mV and a steady-state slope resistance of 500 MΩ measured at the resting potential. Solitary rods responded to light with hyperpolarizations graded with light intensity; the change in potential produced by light often exceeded 25 mV. The time-course of the responses from solitary rods was similar to that from rods in the intact retina stimulated with large diameter spots of light. The current-voltage relationship, obtained by injecting extrinsic current through an impalling pipette, showed both inward- and outward-going rectification. The amplitude of the light response could be altered by changing the membrane potential of solitary rods. Depolarization, produced by injecting current, reduced the amplitude of the response; polarization beyond 0 mV reversed the polarity of the response. The same reversal potential was observed in both inner and outer segments.

The kinetics of the light-induced current and the voltage dependence of the light insensitive currents are being studied by the voltage-clamp technique.

1967 VISUAL DEFECTS IN THE MUTANT MOUSE PEARL. G. W. Balkema, Jr., U. C. Dräger, L. H. Pinto and J. W. Vanable, Jr.*. Dept. of Biol. Sci., Purdue Univ., West Lafayette, IN 47907 and Dept. of Neurobiol., Harvard Med. Sch., Boston, MA 02115.

When exposed to moving visual patterns normal mice display optokinetic nystagmus (OKN). By testing OKN we screened 52 mouse mutant stocks for visual defects. In pearl mice, a recessive coat color mutant, it was not possible to elicit an OKN either under low or high illumination test conditions. However, pearl's postrotatory nystagmus was normal. Furthermore, at the light microscope level we have not detected any morphological abnormalities, either in the retinas or the brains of pearl mice. The electroretinographic light response of pearl was similar to that of +/+; there were no detectable differences in either the waveforms or the illuminations for half-maximal amplitudes of a- and b-waves. Thus, pearl mice do not show morphological or electroretinographic signs of retinal degeneration.

We recorded the responses of cells in the primary visual cortex of pearl and +/+ mice to light stimuli projected onto the receptive fields; background illumination was in the upper scotopic/low photopic range. For equivalent responses the stimulus had to be 1-2 log units brighter for pearl cells than for cells of +/+ mice.

In recording from single cells in the superficial layers of the superior colliculus we determined intensity-response curves and plotted incremental threshold. At dim background levels (-5 to -3 log cd/m^2) the thresholds for pearl were on the average 2 log units higher than for +/+. For increasingly brighter backgrounds the mean thresholds for pearl approached the mean thresholds for +/+ (0.25 log units difference at 2.5 log cd/m^2). This threshold elevation in the dark for pearl does not seem to be caused by a prolonged time course of dark adaptation, since the rates of dark adaptation were similar in pearl and +/+: the mean time for 50% threshold recovery following 3 min light exposure at 3.8 log cd/m^2 was 16 min for pearl and 21 min for +/+.

Thus, we have found that pearl mutants show a decreased light sensitivity when measured in the primary visual cortex and the superior colliculus, but so far we are unable to explain how this defect is related to pearl's lack of OKN.

1968 ELECTRON MICROSCOPIC INVESTIGATION OF THE CAT SUPERIOR COLLICULUS. M. Behan*, J.T. Weber, M.F. Huerta and J.K. Harting. Department of Anatomy, University of Wisconsin, Madison, Wisc. 53706. (SPON: G.J. Royce).

Our findings to date relate primarily to the normal ultrastructural features of the stratum zonale (SZ) and the stratum griseum superficiale (SGS). Throughout the synapses examined, terminals with round or pleomorphic vesicles are clearly distinguished. There appears to be no correlation between pale or dark mitochondria and vesicle shape. In general, round and pleomorphic vesicles are associated with asymmetric and symmetric thickenings respectively. While most of the synapses within the SZ and the SGS are axodendritic, a few dendrodendritic contacts were most apparent in serial synapses.

The stratum zonale (20-25μm thick) contains numerous astrocytes arranged parallel to the pial surface. Astrocytic processes extend into the upper portion of the SGS. Many small myelinated axons (.4-4μm in diameter), are found in the SZ. The ratio of terminals containing round vesicles to those with pleomorphic vesicles is approximately 1:1.

Transition from the SZ to the SGS is well defined. The SGS can be subdivided into three layers. In the SGS1 (70-100μm thick) the number of myelinated profiles is greatly reduced. There is also an increase in the number of synapses in the dense neuropil. The ratio of round to pleomorphic vesicles in terminals is closer to 2:1. Frequently terminals end on dendritic spines.

The transition from the SGS1 to the SGS2, clearly seen in 1μm toluidine blue stained sections, is less apparent in the electron microscope. In contrast to the SGS1, the SGS2 (150-200μm thick) shows an increase in the number and size of myelinated axons, has larger dendritic profiles and fewer glial elements. The number of synapses in any measured area is approximately the same as the SGS1.

The SGS3 (150-200μm thick) is distinguished from the SGS2 by having larger cells (up to 30μm diameter) with very large dendritic profiles and a further increase in the number of myelinated axons. The ratio of terminals with round or pleomorphic vesicles is still approximately 1:1. The SGS3 merges gradually and almost indistinguishably into the stratum opticum.

Supported by Grants EY01277 and BMS76-81882. J.T. Weber is supported by NIMH Fellowship MH05601.

1969 THALAMO-CORTICAL PROJECTIONS AND HISTOCHEMICAL IDENTIFICATION OF SUBDIVISIONS OF THE LP-PULVINAR COMPLEX IN THE CAT. David M. Berson* and Ann M. Graybiel, Dept. of Psychology, Mass. Institute of Psychology, Cambridge, MA 02139. (SPON: R.L.M. Faull).

The LP-pulvinar complex of the cat's thalamus contains a distinct medial zone receiving input from the superficial layers of the superior colliculus (LPm), a second zone receiving input from the striate cortex (LPℓ) and a third, lateral zone in the pulvinar (Pul) receiving input from the pretectal region. In an effort to identify these three zones in normal material, we studied the distribution of acetylcholinesterase in the caudal thalamus and found the LPm sharply defined by high AchE activity, the pretectorecipient Pul also rich in AchE, and LPℓ a pale AchE-poor band between the two.

Since it has been suggested that these vision-related subdivisions of the LP-pulvinar complex may also be dissociable on the basis of their patterns of thalamo-cortical projection (Brain Res.44, '72 & 147,'78), the ascending projections of LPm, LPℓ and Pul were studied autoradiographically. The distribution of cortical labelling in these autoradiographic experiments varied systematically with the location of the center of the thalamic injection site. Injections centered in the pretectorecipient zone (Pul) elicited heavy labelling in the cortex of the middle suprasylvian crown and in the fundus of the splenial sulcus, and variably labelled parts of area 19. Deposits centered in the striate corticorecipient zone (LPℓ) heavily labelled area 19, the medial bank of the middle suprasylvian sulcus and the caudo-medial bank of the posterior suprasylvian sulcus and produced sparse labelling of areas 17 and 18. Injections centered in the tectorecipient zone (LPm) labelled the ventral bank of the anterior ectosylvian sulcus; the fundus and, caudally, the lateral bank of the middle suprasylvian sulcus; and those parts of the posterior suprasylvian sulcus not labelled by LPℓ injections.

The finding that particular regions of the posterior neocortex receive projections from certain of the subdivisions of the caudal thalamus but not from others was confirmed in many cases by the fact that injections of HRP into circumscribed cortical areas produced one, or in some cases two bands of retrograde thalamic cell-labelling which respected the boundaries of LPm, LPℓ and Pul. While some degree of overlap in the thalamo-cortical projections of these thalamic zones thus seems likely, LPm, LPℓ and Pul nevertheless appear to be associated with distinct sets of cortical areas. Inasmuch as the inputs to these thalamic zones from cortical or subcortical elements of the visual mechanism are clearly distinct, it is concluded that the subdivisions of the LP-pulvinar complex may serve to forward different types of visual information to distinct targets within the posterior association cortex.

Supported by an NSF graduate fellowship and NSF BNS 75-18758.

1970 CHANGING-DISPARITY AND CHANGING-SIZE INPUTS FEED THE SAME MOTION-IN-DEPTH STAGE. Kenneth I. Beverley and David Regan*. Psychology Dept., Dalhousie Univ., Halifax, N.S. B3H 4J1.

We have previously reported psychophysical evidence for the existence of neural filters specifically sensitive to changing object size (Regan & Beverley, Vision Res., 1978a,b, in press). We now report that these changing-size filters feed the same motion-in-depth stage that is fed by changing-disparity inputs.

Subjects viewed a bright adapting square, or mean side length 1°, with both eyes. All four sides (of the square) moved towards each other at a constant velocity of 0.3°/sec for 1.0 sec, then the square disappeared for 0.25 sec and the cycle repeated. After 20 min adaptation subjects reported the illusion that a static test square of 1° side appeared to move towards the head, whether the test square was viewed monocularly or binocularly, or was of positive or negative contrast. Our aftereffect data suggests that this motion-in-depth aftereffect can be attributed to unbalanced activity at a motion-in-depth stage fed by changing-size filters.

We then arranged that the test squares seen by the left and right eyes remained constant in size, but moved towards each other (i.e. the disparity was changed). Subjects found that they could cancel the motion-in-depth aftereffect by adjusting the rate of change of disparity of the squares. This can be explained if the changing-disparity signal feeds the motion-in-depth stage that mediated the aftereffect.

Further evidence that changing-disparity and changing-size inputs converge onto the same motion-in-depth stage is: (1) The time constant for the exponential decay of the motion-in-depth aftereffect was not significantly different whether measured by cancelling with a binocularly-viewed changing-size test square (24 sec, SE=2 sec) or by a changing-disparity test square (28 sec, SE=3 sec). (2) We were able to combine changing-size stimulation and changing-disparity stimulation so as to evoked antagonistic motion-in-depth responses. Subjects viewed identical 1° squares with the left and right eyes in binocular fusion. The two squares moved away from each other with a ramping waveform producing the compelling illusion of a single square moving in depth towards the head. We then arranged that the size of the squares decreased in the same manner as the disparity increased. Subjects reported that for small rates of size change the binocularly-fused square appeared to move towards the head, but for large rates of size change the square moved away from the head. For an intermediate rate of change the square did not move in depth at all.

We compared the effectiveness of changing-disparity and changing-size inputs by normalizing them in terms of the inputs that would be produced by a real square moving in depth. On this basis the changing-disparity input was 9 times more efficient than the changing-size input at our viewing distance of 145 cm.

1971 ADAPTATION TO A GRATING REVEALS SECOND HARMONIC COMPONENTS OF THE HUMAN VEP TO CONTRAST MODULATION. I. Bodis-Wollner and C.D. Hendley*. Dept. Neurology, Mt. Sinai Sch. Med., New York, N.Y. 10029.

Contrast of a 6 cycles/degree grating with sinusoidal luminance profile was modulated at the rate of 8 Hertz. Contrast modulation is the successive presentation of the same grating at higher and lower contrast. Analytically, a contrast modulated grating may be regarded as the sum of counterphase flicker and steady grating of equal spatial frequencies. 100% contrast modulation is the successive presentation of a grating and a blank field with the same mean luminance as the pattern--or simply it is pattern-on, pattern-off presentation. Analytically it is a pure counterphase grating having the same frequency but half the spatial contrast of the on-off grating.

Evoked potentials to contrast modulated gratings were recorded for four observers whose response was a single hump for a complete cycle of contrast modulation. Frequency analysis of the evoked potential of these observers showed that most of the response energy was concentrated at the fundamental frequency of modulation (8 Hertz). When stimulus presentation was interspersed for 3 second periods with an adapting high contrast steady grating of the same spatial frequency as the contrast modulated grating, its effect on the evoked potential was two-fold. While there was some reduction of peak to trough amplitude of the adapted EP to on-off presentation, its waveshape appeared as a double hump during the modulation period. Frequency analysis showed that most of the energy of the evoked potential was now concentrated at the second harmonic component. Apparently pattern adaptation caused a frequency shift in the EP spectrum of these observers. These results suggest that 1) a predominant first harmonic EP to on-off presentation of a grating is not simply a response to local luminance change (flicker), and 2) frequency doubling in the human VEP to counterphase grating presentation may be the response of visual pathways which are resilient to pattern adaptation.

This work was supported in part by Grant No. EY 01708 of the National Eye Institute.

1972 BEHAVIORAL MEASURES OF VISUAL RECOVERY IN MONOCULARLY DEPRIVED MONKEYS (MACACA NEMESTRINA) REARED WITH AND WITHOUT RETINAL LESIONS. John Boles, James R. Wilson and Anita Hendrickson. Dept. of Ophthalmology, Univ. Washington, Seattle, WA 98195.

Ten monkeys were deprived of binocular vision by suturing shut the lids of one eye (EYE 1) prior to the 24th postnatal day. EYE 1 was opened and EYE 2 closed (reverse suture) when the monkeys were between 10 & 12 mo old. The Early Lesion (EL,n=2) group received a laser lesion of the central 10° of the retina in EYE 2 when EYE 1 was sutured. Late Lesion (LL,n=4) animals received the same type of lesion in EYE 2 at the time of reverse suture. No Lesion (NL,n=4) animals had the initial and reverse suture only. Grating visual acuity was tested in a modified Wisconsin General Test Apparatus before and after reverse suture, using a two-alternative, forced choice procedure and food reinforcement. Each grating of a set of four was paired randomly with a control card (30cy/deg). The set of gratings was chosen to bracket a monkey's threshold between 50% and 90% correct response. Visual perimetry, visuo-motor tasks like reaching for objects and placing, and play behavior were assessed before and after reverse suture. The normal grating acuity threshold (72% correct) reached by Nl and LL using EYE 2 was about 10cy/deg. The thresholds of EL monkeys using EYE 2 was about 3.8cy/deg, which we take to be a measure of peripheral retinal acuity.

Continued testing for at least 6 mo after reverse suture has indicated:

1. EYE 1 of all animals showed improved visual acuity. The acuity threshold of the EL and LL monkeys reached 7.5-10cy/deg by 4 mo after reversal. The acuity of NL monkeys was as good as 2-4 cy/deg by 5-6 mo after reverse suture.
2. The onset of recovery occurred at different rates. LL and EL monkeys started to recover about 1 mo after reversal. NL animals did not begin until at least 4 mo after reverse suture.
3. The rate of recovery for all animals proceeded similarly once it began. Recovery of the EL monkeys was confounded by their having to overcome an "eccentric fixation" caused by the central lesion in EYE 2 during the critical period.
4. The perimetry tests showed that it was unlikely that only the monocular segment of EYE 1 was responsible for this recovery. Visuo-motor behavioral tests indicated that the EL and especially LL groups were indistinguishable from normals while NL animals were more handicapped in visual behavioral tests.

We conclude that laser lesions of an experiencing (EL) and experienced (LL) retina promote recovery of behaviorally useful vision in the central retina of a visually deprived eye.

Supported by PHS grants EY01208, EY07013, RR00166 and RCDA EY39039 to A.H.

1973 A ROLE FOR CYCLIC GMP IN VERTEBRATE PHOTORECEPTORS. M.D. Bownds, M.L. Woodruff, M.S. Biernbaum*, A.S. Polans, J. Hermolin*, P.R. Robinson and B.P. Abramson*. Department of Zoology and Laboratory of Molecular Biology, and Neurosciences Training Program, University of Wisconsin, Madison, Wisconsin 53706.

The molecular events that link light absorption to a sodium conductance decrease in vertebrate photoreceptors may involve a cyclic nucleotide system. Frog retinal rod outer segment (ROS) cyclic GMP levels (but not cyclic AMP levels) are reversibly decreased by illumination over the same range of light intensity that decreases ROS membrane permeability. The latency of the cyclic GMP decrease is less than 50 msec and $t_{\frac{1}{2}}$ for the decrease is about 125 msec. Cyclic GMP levels are reduced by 10^4 to 10^5 molecules per photopigment molecule bleached at illumination bleaching only a few photopigment molecules in each ROS. The light-induced decrease in cyclic GMP occurs on the same time scale and shows the same sensitivity as the light-induced conductance decrease that underlies the electrophysiological response of vertebrate rod cells.

The decrease in cyclic GMP levels is caused by an enhanced hydrolysis of cyclic GMP (phosphodiesterase: cyclic GMP→5'GMP), rather than a decrease in the rate of cyclic GMP synthesis (guanylate cyclase: GTP→cyclic GMP). Control of cyclic GMP levels is complex: GTP, both the precursor to cyclic GMP and a cofactor for the light activation of ROS phosphodiesterase, can be decreased 70% ($t_{\frac{1}{2}}$ = 7 sec) by low levels of illumination. ATP levels are not influenced by light.

Cyclic nucleotides have been shown to regulate cell physiology by stimulating the level of specific protein phosphorylations (protein kinase activity). Two small molecular weight proteins (M.W. 12,000 and 13,000) associated with ROS are phosphorylated in the dark and dephosphorylated in the light. Dephosphorylation of both proteins is graded with light intensity over the same range of intensity that decreases cyclic GMP levels and suppresses ROS permeability. The phosphorylation of both proteins is increased by cyclic nucleotide addition.

Several pharmacological agents affect cyclic GMP, the phosphorylation of the two small molecular weight proteins and outer segment permeability similarly. For example, phosphodiesterase inhibitors, that increase the level of cyclic GMP in ROS, increase both the level of protein phosphorylation and permeability. Calcium ions decrease the level of cyclic GMP (via inhibition of guanylate cyclase), the level of protein phosphorylation and permeability.

This work was supported by grant EY-00463 from the National Institutes of Health (to M.D.B.).

1974 A VISUAL CLIMBING FIBER INPUT TO THE VESTIBULOCEREBELLUM: AN ACCESSORY OPTIC-INFERIOR OLIVARY-VESTIBULOCEREBELLAR PROJECTION SYSTEM. N. Brecha and H.J. Karten. Dept. of Psychiatry, S.U.N.Y. Stony Brook, N.Y. 11794.

The avian accessory optic nucleus, the nucleus of the basal optic root (nBOR), receives a specfic projection from the displaced retinal ganglion cells. The nBOR has recently been reported to project bilaterally directly upon the oculomotor complex, trochlear nucleus and vestibulocerebellum. The cerebellar projection terminates as a mossy fiber system within a horizontal band upon the superficial regions of the granule cell layer adjacent to the Purkinje cell layer. These findings suggest this system is responsible for a fast visual input to the oculomotor neurons and the vestibulocerebellum.

Unilateral injections of ^{3}H-proline/^{3}H-leucine into the nBOR resulted in labeled fibers which join the brachium conjunctivum cerebellopetale(BCP). At the level of the nucleus semilunaris labeled fibers leave the BCP and either turn ventrally to join the ipsilateral ventral tegmental tract or to cross the midline and then turn ventrally to join the contralateral ventral tegmental tract. Labeled fibers course caudally to terminate bilaterally upon the medial division of the inferior olivary complex(OIm) and the labeling was somewhat heavier over the ipsilateral OIm. Unilateral HRP injections into the olivay complex have confirmed this projection system. Unilateral HRP injections into folia IXc, IXd or the paraflocculus of the vestibulocerebellum resulted in retrograde labeling of cells only within the contrlateral OIm.

These results thus demonstrate the OIm which receives a bilateral nBOR projection, gives rise to a climbing fiber projection upon those cerebellar regions which also receive a mossy fiber projection from the nBOR. Moreover, the OIm is likely to give rise to a visual climbing fiber input in view of its afferentation from the accessory optic system. The observations of the displaced ganglion cells of the retina giving rise to a specfic projection upon the avain accessory optic nucleus (nBOR) and the accessory optic nucleus projecting directly upon oculomotor neurons and both directly and indirectly upon the vestibulocerebellum further empasizes the specfic role this system may play in visual processes including oculomotor behavior.

Supported by NEI 2146 to H.J.K.

1975 MIMICKING OF DARK ADAPTATION BY Ba^{2+} RECORDED IN OUTER SEGMENTS OF TOAD RODS. Kenneth T. Brown and Dale G. Flaming*. Dept. Physiol., Sch. Med., UCSF, San Francisco, CA 94143.

Intracellular recording was conducted in outer segments of red rods in the isolated, inverted and superfused retina of the toad, Bufo marinus. Recently developed microelectrode techniques were used to readily obtain high quality recordings in which the membrane potential and light response could be maintained stably for 2-3 hr. Effects of changing the extracellular concentration of both Ca^{2+} and Ba^{2+} were studied. Increasing $[Ba^{2+}]_o$ in the range from 0.0 - 2.0 mM was shown to mimic dark adaptation by depolarizing the cell, increasing the amplitude of the light response, increasing sensitivity, increasing the time-to-peak of the light response, and slowing decay of the light response. Lipton, Ostroy and Dowling (J. gen. Physiol. 70: 747, 1977) have reported that adaptation changes the values of both V_{max} and σ in the V-logI curves of red rods in the toad retina. The value of σ is the light intensity that elicits a light response of 1/2 the saturated amplitude, V_{max}. They also found that although changes of $[Ca^{2+}]_o$ altered V_{max}, the value of σ was unaffected. We maintained adaptation constant at slightly above the completely dark adapted level, and changed $[Ba^{2+}]_o$ from 0.0 to 2.0 mM. This increased V_{max} more than 50% and decreased the value of σ by about 0.7 log units. Thus Ba^{2+} can mimic adaptation even better than Ca^{2+}, since Ba^{2+} can control the value of σ. This makes Ba^{2+} of great interest in the ionic control of adaptation, expecially since barium has been reported by Bellhorn and Lewis (Exp. Eye Res. 22: 505, 1976) to be concentrated in the outer and inner segments of rods in the cat retina.

(Supported by NIH grant No. EY 00468)

1976 DISSOCIATION OF MOVEMENT AND ATTENTION: NEURONAL CORRELATES IN POSTERIOR PARIETAL CORTEX. M. Catherine Bushnell*, David Lee Robinson, and Michael E. Goldberg. Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20014.

Cells in monkey superior colliculus (SC), frontal cortex, and posterior parietal cortex (PP) have an enhanced response to a spot of light in their receptive field (RF) when the monkey uses the light as a target for a saccadic eye movement. This response facilitation is spatially selective; it does not occur when the animal makes a saccade to a stimulus outside of the RF. Thus this enhancement may be part of the neuronal mechanism of attention. Since attention can be dissociated from movement, we attempted to determine if the effect could likewise be separated. The facilitation in SC is dependent on an eye movement (Wurtz and Mohler, 1976).

Rhesus monkeys were trained to fixate a spot of light and release a lever when the spot dimmed. Peripheral visual stimuli were presented both within and without the RF, and the monkeys were trained to make one of several responses: continue to fixate the spot and ignore peripheral stimuli; make a saccade to a peripheral target; continue to fixate the spot, attend to the peripheral stimulus and release the level when it dimmed. Extracellular single-unit recordings were made while the animals performed their tasks.

Nearly half of the visually responsive neurons in both PP and frontal cortex showed more vigorous responses when the monkey was preparing to make a saccade to the RF stimulus. In PP all neurons tested showed similar enhancement when the monkey was attending to the RF stimulus but not preparing to make a saccade to it. This no-saccade enhancement was spatially specific; attention to stimuli outside of the RF did not result in enhancement. In contrast to PP data, frontal cortex neurons showed little or no enhancement when the monkey attended to a RF stimulus without making a saccade to it. In all cases, enhancement required the presence of a stimulus in the RF.

The enhancement phenomenon in PP is fundamentally different from that in SC and frontal cortex. SC and frontal cortex seem to participate only when the stimulus is the target for an eye movement and may be important in transferring visual information to the oculomotor system. In PP the enhancement seems to function as a general attention system when the stimulus is important to the animal regardless of the motor strategy the animal uses to handle the stimulus.

1977 THE ROLE OF THE MIDDLE TEMPORAL AREA (MT) IN THE RESIDUAL VISUAL CAPACITY OF THE STRIATE LESIONED BUSHBABY. Ruth B. Caldwell and Jeannette P. Ward. Dept. Psychol., MSU, Memphis, TN 38152

Striate cortex lesions were once thought to produce "cortical blindness", but destriate vision has now been demonstrated in many species, including man. Dual visual system hypotheses have suggested that such residual visual capacity is mediated through the tecto-cortical visual system, and while this hypothesis has not been tested directly, the effects of large occipital lobe lesions in tree shrew and monkey support this idea. The bushbaby is an ideal subject for a test of this hypothesis because its tecto-cortical projection is limited to a cytoarchitecturally distinct area in the temporal lobe (MT).

The present experiments were intended to determine the limits of destriate vision in the bushbaby and to evaluate the role of the MT area in this capacity. Bushbabies were trained on a variety of visual tasks, including two-choice discrimination problems, visuospatial localization and food localization tasks. After pretraining, six bushbabies were given extensive striate lesions and were retested. Then four animals received a second lesion, either of the MT area (17-MT) or of an intrinsic pulvinar projection area (17-VT), and were retested. A third group of animals with MT area lesions, which had been tested on similar tasks, received extensive striate cortex lesions and were retested (MT-17).

The results of these experiments were: (1) After extensive retraining, the final level of performance of five of the six striate lesioned animals was similar on most tasks to their preoperative performance. Acuity was decreased, however, and the animals could not learn a form discrimination. (2) The addition of a second lesion to the initial striate lesion did not increase the deficit on most tasks. (3) The 17-MT group was equivalent to the 17-VT group and both were better than the MT-17 group. (4) Although the MT-17 group had shown no deficit on any task before their striate lesions, they did poorly on most tasks attempted after the striate lesion. (5) The difference between the MT-17 group and the other groups could be explained on the basis of lesion size and by the fact that the groups receiving striate lesions first received more extensive training. (6) The hypothesis that the MT area is necessary for destriate visual capacity was not supported, rather the results of this experiment suggest that the striate lesion deficit depends on the extent of damage to circumstriate cortex and the amount of training the animals receive.

1978 PHOTORECEPTOR STRUCTURE AND FUNCTION EXHIBIT CIRCADIAN RHYTHMS IN THE *LIMULUS* EYE. Steven C. Chamberlain* and Robert B. Barlow, Jr. Institute for Sensory Research, Syracuse University, Syracuse, NY 13210.

Efferent fibers in the optic nerve trunk mediate circadian rhythms in the structure and response of the *Limulus* lateral eye. At night, synchronous bursts of efferent impulses increase the aperture between the cuticular cone and the light sensitive rhabdome, increase the area of photoreceptive membrane, and move the rhabdome closer to the cornea. The efferent input to the lateral eye at night also increases the electroretinographic and optic nerve responses, increases the acceptance angle of a single ommatidium, and decreases the spontaneous discharge of the optic nerve. During the day, these structural and functional effects can be produced by shocking the optic nerve or by perfusing the retina with serotonin.

The acceptance angle of a dark-adapted ommatidium increases from 6° during the day to about 13° at night. This is consistent with the observed changes in the structure of the ommatidium and the idea that the cuticular cones function primarily as light collectors and not as imaging lenses.

Visual sensitivity at night exceeds daytime levels by as much as 10^5. Intracellular records from single photoreceptor cells indicate that the endogenous circadian clock enhances visual sensitivity by elevating photoreceptor response (receptor potential) and lowering photoreceptor noise (spontaneous quantum bumps).

Photoreceptor rhabdome breaks down and renews daily. These transient structural changes appear to be controlled by efferent activity and light. Within 15 min after light onset at dawn the rhabdome is reduced in volume by at least 50%. 30 min after light onset the rhabdome is restored. This daily renewal may be analogous to disc shedding in vertebrates.

Photoreceptors are dynamic components of the *Limulus* visual system. They undergo circadian changes in structure and sensitivity presumably to compensate for diurnal changes in ambient illumination.

Supported by the Grass Foundation and NIH grant EY 00667.

1979 RESPONSES OF RETINAL GANGLION CELLS IN SIAMESE CATS TO MOVING SLITS. Yuzo M. Chino*, Michael S. Shansky*, (SPON: E. Kicliter). Division of Visual Science, Illinois College of Optometry, Chicago, Ill. 60616, and D. I. Hamasaki, William L. McKnight Vision Research Center, Bascom Palmer Eye Institute, University of Miami, Miami, Fla. 33136.

It has been reported that optic tract recordings in Siamese cats reveal reduced encounter rates for Y-type retinal ganglion cells (Chino, Shansky, Hamasaki, Science, 197: 173-174, 1977) and this reduction is directly related to the degree of convergent squint exhibited by individual animals (Chino, Shansky, Hamasaki, Brain Res. 143: 459-473, 1978). In addition, responses to temporal and intensity variations in the stimulus as well as to a contrast reversal stimulus are significantly weaker than in common cats. These anomalies suggest that the receptive field organization of Siamese units may be different from that of common cats. In this study, we employed a moving slit (2^o/sec.) to investigate the interaction of center and surround in Siamese retinal ganglion cells.

We recorded optic tract responses from 96 units in 3 Siamese two of which exhibited a convergent squint, and 106 units in 3 common cats for purposes of comparison. Our results indicate that:

1) The significant reduction in Y-cell encounter rate is confirmed for this sample.
2) The RFC sizes of X-type units near the area centralis are significantly larger in Siamese.
3) Responses to moving slits are weaker among Siamese units.
4) Surround influences in Siamese neurons are notably weaker than in common cat units.

These results confirm the anomalous responses reported earlier and further imply an abnormal receptive field organization in Siamese retinal ganglion cells.

(Supported by NIH grants EY01444 and EY00701)

1980 **ULTRASTRUCTURAL EVIDENCE OF EARLY RETINAL GANGLION CELL DIFFERENTIATION IN XENOPUS LEAVIS.** **Charles Cima* and Philip Grant*(SPON: D. P. Kimble). Dept. of Biology, University of Oregon, Eugene, OR 97403.**

Ultrastructural evidence indicates that Xenopus retinal ganglion cell axons differentiate when retinal polarity is specified, that is between Stages 28 and 32. Light microscope studies indicated the presence of argyrophilic material in the ventral retina and optic stalk of early embryos. Ultrastructural analysis of this region confirmed the presence of axons in the stalk and interstices of ventral retinal cells. Axons containing aligned microtubules and neurofilaments and elongated mitochondria with a paucity of other cell inclusions are found with increasing frequency in the ventral retina from Stages 28 through 33/34. Central and dorsal regions of the retinae examined show little or no evidence of axons. A discrete, small bundle of axons is found in the optic stalk of Stage 28 embryos and by Stage 30/31 the number of axons in bundles has increased, suggesting early fasiculation.

Between Stages 28 and 33/34 (± 12 hours) extracellular space surrounding early axons diminishes and processes from neuroretinal cells in contact with axons 'wrap' developing axon bundles.

The evidence presented suggests that axon initiation occurs in stages much earlier than previously reported. Other investigators have failed to detect ganglion cell differentiation prior to Stage 32 possibly because they examined regions of the retina with few axons. The precocious differentiation of ganglion cell axons also suggests the appearance of fibers may be correlated with the events involved in stabilization of retinal positional information. Further, experiments which rotate the retina in the orbit may therefore have to be re-evaluated since regenerating axons may use previously established pathways to organize and 'home' in on tectal target cells.

1981 ROD-CONE INPUT TO LEMON SHARK GANGLION CELLS. Joel L. Cohen and Samuel H. Gruber. School of Marine and Atmospheric Sciences, University of Miami, Miami, Florida 33149.

Single unit responses were recorded from ganglion cells of the lemon shark, Negaprion brevirostris, retina, using the eyecup preparation suffused with 95% O_2 and 5% CO_2. Three cell types were distinguished: on, off, and on-off. Most responses were recorded from off-center cells. Receptive field center (RFC) diameters were found in the dark, using the area-summation method, with white light. RFC were found to be 1.25mm to 1.5mm in diameter. This corresponds to 7.5 to 9.0 degrees of visual angle. Spectral sensitivities were measured by recording a criterion response (1-2 spikes) to light of various wavelengths. After correcting for an equal quantum spectrum the data were plotted on a wavenumber basis. Three spectral inputs were found. Off-center cells receive a short (485nm) and medium (535nm) wavelength input. On-center cells receive an intermediate (506) and medium (535nm) wavelength input. Upon light adaptation with white light, the intermediate (506nm) wavelength component dissappears. We hypothesize that the short (485nm) and medium (535nm) wavelength spectral sensitivities arise from the cones, while the intermediate (506nm) wavelength peak arises from the rods. No color opponent cells were observed.

Supported by Sigma Xi Grant in Aid to J.L.C. and O.N.R. Contract # N00014-77-C-0173 to S.H.G.

1982 RELATIVE USE OF VISUAL PATHWAYS IN ALBINO RAT: AUTORADIOGRAPHY WITH 14-C-DEOXYGLUCOSE COMPARED TO 14-C-PROLINE. Robert C. Collins, Torris V. Caston* and Edward F. Vastola*. Neurol., Wash. Univ. Med. Sch., St. Louis, Mo., 63130.

14-C-Proline was injected into the eye or visual cortex of albino rats. Autoradiography was performed on SB-5 X-ray film on 20 μm coronal slices of brains of animals killed 24-72 hours later. The relative distribution of retinal and corticofugal pathways was measured with a densitometer. Retinal projections to the superior colliculus were 2.5 times more dense than to the dorsal lateral geniculate nucleus, but the cortico-geniculate pathway was 2.3 times more prominent than the cortico-collicular pathway. These findings were compared with relative changes in 14-C-deoxyglucose utilization in visual pathways in additional groups of animals who were stimulated with strobe light, rotating vertical stripes, or bipolar electrical stimulation of the cortex. Compared to blind rats (enucleation), visual stimulation increased glucose utilization 68% in geniculate, 123% in superior colliculus, and 28% in visual cortex. The retinal influence on metabolism in the superior colliculus was thus 1.8 times greater than on the lateral geniculate. Electrical stimulation of visual cortex increased local cortical metabolism 100%, dorsal lateral geniculate 100%, and superior colliculus 58%. Thus the cortical influence on geniculate metabolism can be at least 1.7 times greater than on the superior collicus. These findings suggest that the differences in glucose metabolism at different sites in visual pathways in response to these stimuli are the result of differences in the distribution of first order retinal and corticofugal connections. The findings also indicate anatomical and metabolic differences between the tectal and geniculo-cortical systems that may be correlated with differences in their contribution to the animal's behaviour.

1983 CONGRUENT BINOCULAR PREFERRED DIRECTIONS IN THE SUPERIOR COLLICULUS OF KITTENS REARED WITH ONE EYE ROTATED. Max Cynader Colin Blakemore and R.C. Van Sluyters. Dalhousie University, Halifax, N. S.; Physiological Laboratory, Cambridge, England; U.C. Berkeley, Berkeley, California.

In the last decade several workers have shown that it is possible to bias the neuronal distribution of preferred orientations and/or directions in the visual system of specially-reared kittens. Alterations in the incidence of neurons with particular trigger properties have at least two possible causes: (1) atrophy (or death, or loss of responsivity) among unstimulated neurons so that only neurons which have been adequately stimulated during development are recorded (2) modification of the properties of individual neurons so that their trigger properties are now matched to their visual input. It has proved difficult to separate these two mechanisms with the deprivation procedures commonly employed.

One possible approach to this problem is to attempt to experimentally create a large number of neurons with trigger properties which do not occur in nature. The existance of such neurons would provide compelling evidence in favour of the modification hypothesis outlined above. We attempted to induce the formation of neurons with unusual response properties by surgically rotating one eye of five kittens through 90° while leaving the other eye unoperated. The kittens were then allowed at least 6 months of exposure with both eyes open in a normal environment before we recorded in the superior colliculus. We concentrated on the superior colliculus contralateral to the rotated eye, and furthermore on that part of the colliculus in which receptive fields from the two eyes were in rough topographic register in visual space despite the eye rotation. We searched for binocularly-driven collicular neurons and compared the preferred directions for these neurons as determined through each eye separately. Computer methods were used for stimulus presentation and data collection. In the absence of adaptive modification, one would expect that the preferred directions in visual space for a given cell would be separated by 90° when the stimulus was presented through either eye. Our results indicate however that the large majority of such binocularly-driven units show the same or similar preferred direction in visual space tested through either eye. This corresponds to orthogonal preferred directions on the two retinae. Since cells with these properties are not observed either in young kittens or in normally-reared adult cats, their existance provides evidence against the notion that atrophy of unstimulated cells underlies the effects of selected visual exposure.

1984 PERCEPTION OF MIRROR IMAGES BY CHILDREN. Nancy A. Dahl and Jean L. Pyfer*, Dept. Physiology & Cell Biology; Dept. Health, Physical Ed. & Recreation, U. Kansas, Lawrence, Ks. 66045.

Distinguishing mirror images from one another is impossible for most animals, difficult for some children, and even a problem for some adults. This confusion becomes critical in reading and writing and is believed to play a role in some dyslexia problems. Until perceptual strategies of identifying the difference between such letters as d and b can be ascertained, teaching techniques for helping children deal with reversal problems are, at best, based on guesswork. Left-right discrimination experiments that require symbolic responses could force left hemisphere participation and hence, confound results. This series of studies attempted to overcome that procedural weakness by requiring subjects to manually turn a hidden, raised arrow to match the visual stimulus of a tilted arrow. One hundred and ten children were tested from kindergarten (K), first (1st) and second (2nd) grades of local schools. During training and testing the subjects looked at a target circle on a screen as the stimulus was flashed for 200 msec. As a face appeared in the circle an arrow also appeared 5° to the right or left of the fixation point and tilted 45° from vertical (left or right, up or down). The arrow placement and tilt were in random order. The subject responded by imitating the face (with a smile, frown, or open mouth) and then rotating a hidden, raised arrow to match the stimulus arrow. The subject was required to turn the arrow with his/her right or left hand; the arrow was placed either to the right or left of his/her midline. Responses were classified as correct, mirror images across the vertical (m), reflect across the horizontal (i), or 180° different from the stimulus (p).

The K subjects made about twice (23%) as many total errors than did 1st (10%) or 2nd (9%). However, all subjects made more m errors than any other kind. To eliminate the random component of m errors that could have resulted from inattentiveness or confusion, the following formula was designed:

$$\text{Mirror Index (MI)} = \left[m - \frac{i+p}{2}\right]\frac{100}{n \text{ trials}}$$

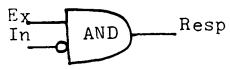
Ten subjects were found to have an MI greater than 20. Examination of the individual responses made by the high MI subjects revealed that their mirror responses were made at random. There were no differences between motor responses ipsilateral or contralateral to the visual field stimulated; there was no difference whether or not the hand crossed the midline of the body; left and right visual fields also elicited equal numbers of m errors implying that neither hemisphere was superior at this spatial perception task; and handedness conferred no advantage.

This work was partially supported by NIH Grant RR 07037.

1985 INHIBITION IN VISUAL CORTEX: A LOGIC PROCESSING MODEL TO ACCOUNT FOR SOME EXPERIMENTAL FINDINGS. J.D. Daniels. Div. of Engineering and Center for Neurosciences, Brown University, Providence, RI 02912.

A simple definition, shown by the truth table and logic gate below, is proposed for postsynaptic inhibition.

Ex	In	Resp
0	0	0
0	1	0
1	0	1
1	1	0



It is further proposed that logic processing, such as that for inhibition, may take place in the dendritic tree of a neuron. The outcome of the logic processing will regulate the spiking frequency of the neuron.

In order to incorporate inhibition realistically into a model,a connection rule, which specifies that that no afferent inhibition is allowed, is enforced.

Using the connection rule and inhibition definition a model for orientation specificity in visual cortical neurons is developed. No Inhibition from LGN fibers is allowed. In order to create a model with a minimum of connections an inhibitory neuron, the M-cell, is utilized to veto responses from afferent fibers to P- (pyramidal) cells which exceed pattern specifications for a particular orientation.

The M-cells themselves have non-specific responses, fan out to several neighboring P-cells, and have connectivity relationships similar to those of basket cells seen in cerebellar, hippocampal and cerebral cortex.

Aspects of the model as it relates to orientation specificity have recently been published (Biological Cybernetics, in press).

The logic processing model has now been extended to matters of binocular vision, including development under monocular occlusion. The responses of a binocular neuron are seen to be influenced by three factors--input from the left eye, from the right eye, and simultaneous input from both eyes. Effects of the third factor serve to create responses which,in binocular vision, are not the sum of the single-eye responses. Based on this, a model with modifiable inhibitory connections is presented.

1986 MICROCIRCUITRY OF LAYER IVab IN CAT AREA 17: NEURON TYPES AND PATTERNS OF INPUT. T.L.Davis* and P.Sterling (SPON: L.Palmer). Dept.Anat., Univ.Pa., Phila.19174.

Cell classes in cortex have been defined on the basis of differences in morphology which are believed to reflect differences in connectivity. Here we define classes by identifying directly differences in connectivity. From an animal whose lateral geniculate nucleus (lgn) had been destroyed (4 day survival), we reconstructed 32 adjacent neurons from electron micrographs of 150 serial sections through layer IVab. The cells were divided into seven classes based on differences in size, shape, dendritic branching pattern, and synaptic input.

Class I: pyramidal soma (at III-IV border); apical and basilar dendrites; dendritic spines; flat-vesicle terminals on soma (13/100μm^2); lgn terminals on basilar dendrites.

Class II: large stellate (20μm); dark cytoplasm; many flat-vesicle and round-vesicle terminals on soma (77/100μm^2, F/R=2); lgn terminals on cell body, primary, secondary, and tertiary dendrites.

Class III: stellate; varicose dendrites; medium distribution of terminals on soma (13/100μm^2); lgn and round-vesicle terminals almost completely restricted to secondary dendrites.

Class IV: radially-elongated soma; apical dendrites sharply tapered; dendritic spines; flat-vesicle terminals on soma (18/100μm^2); lgn terminals restricted to shafts of secondary dendrites.

Class V: multipolar soma; flat-vesicle terminals on soma (11/100μm^2); few lgn inputs on dendrites.

Class VI: small soma (7μm); dark cytoplasm; sparse distribution of flat-vesicle and round-vesicle terminals on soma (7/100μm^2, F/R=0.2); lgn terminals on soma.

Class VII: pyramidal soma; apical dendrites sharply tapered; dendritic spines; medium distribution of flat-vesicle and round-vesicle terminals on soma (14/100μm^2, F/R=2.1); no geniculate terminals.

No geniculate terminals were found on the spines or shafts of apical dendrites from deeper layers. We conclude that lgn input is distributed in specific patterns to at least 6 classes of neurons in layer IVab and anticipate that the classes defined here by their differences in connectivity will show corresponding physiological differences. (NIH EY00828)

1987 DENSITY AND CENTRAL PROJECTIONS OF RETINAL GANGLION CELLS IN THE TREE SHREW. Edward DeBruyn* and V. A. Casagrande. (SPON: Lucille H. Aulsebrook). Dept. Anat., Sch. Med., Vanderbilt Univ., Nashville, TN. 37232.

To investigate the normal morphological parameters of retinal ganglion cells in the tree shrew, adult retinae were isolated and whole-mounted on slides. Following staining with methylene blue, the numbers of ganglion cells were counted and relative densities measured. Results show a series of concentric elliptical isodensity lines with their long axes oriented approximately along the horizontal meridian. The ganglion cell densities ranged from 5,000 per mm^2 in the periphery to 13,000 per mm^2 in a central area. The central band of high density is reminiscent of the "visual streak" reported in a number of mammals.

Frequency histograms of cell areas indicate a skewed curve with the majority of cells (86%) ranging in size from 10μ^2-110μ^2, and the remaining large cells ranging in size from 110μ^2-302μ^2. A suggestion of bimodality was consistently observed, with a major peak occurring at 25μ^2 and a minor peak at 45μ^2. There is a progressive increase in the average cell area as eccentricity from the central area increases (from a mean of 32.8μ^2 at 28° eccentricity to a mean of 63μ^2 at 89°), and also an increase in the percentage of large cells (2.1% at 13° eccentricity, 14.2% at 77°).

Injections of horseradish peroxidase into either the dorsal lateral geniculate nucleus (LGN) or the superior colliculus (SC) resulted in slightly different distributions of labelled cells and a large group of small-sized unlabelled cells. Following the LGN injection (eccentricity = 40°), 55% of the cells (size range: 30-175μ^2) in the area of densest label contained reaction product. The frequency distribution appeared bimodal with a major peak at 75μ^2 and a minor peak at 115μ^2. The unlabelled cells (10-75μ^2) showed a unimodal curve with a peak at 25μ^2. The collicular injection (eccentricity = 45°) resulted in label in 52% of the cells (10-175μ^2), distributed with peak frequencies at 15μ^2, 90μ^2, and 115μ^2. Unlabelled cells (15-60μ^2) were again unimodally distributed with a peak at 25μ^2. In both cases, all large (>110μ^2) cells were labelled, indicating that this population contained cells whose axons innervated both the LGN and the SC. In contrast, the smallest cells (<20μ^2) contained label only after the SC injection. In both cases the majority of small cells were unlabelled indicating that they are either interneurons, or that they project to other visual centers, such as the ventral lateral geniculate nucleus or the pretectal nuclei. These results are consistent with earlier reports demonstrating differences in retinal distributions and central connections of ganglion cells based on size.

(Supported by EY 01778 and 1 K07 EY 0061).

1988 LOCAL RATES OF BRAIN GLUCOSE CONSUMPTION IN PIGEON DURING REST, OPTOKINETIC STIMULATION AND UNILATERAL VISUAL DEPRIVATION. Dale G. Deutsch and Anton Reiner. Depts. of Biochem. and Psych. SUNY at Stony Brook, Stony Brook, N.Y. 11794.

The autoradiographic 2-[^{14}C]deoxyglucose (2-DG) technique (L. Sokoloff et.al., J. Neurochem 28, 897, 1977) was employed to qualitatively measure the local rates of glucose utilization in the White Carneaux pigeon brain. Pigeons received an injection of 25μCi 2-DG in the brachial vein. Following an hour in the home cage, birds were sacrificed and the brains rapidly removed and frozen in liquid Freon at -60°C. Ten micron sections were cut on a cryostat, picked up on cover slips, dried at 60°C for 5 min and placed in x-ray cassettes with Kodak X-Omat XR-1 film and exposed for 10 days. In these resting birds, high rates of glucose consumption were evident in a variety of regions, many of which have been anatomically shown to be components of avian central sensory lemniscal pathways. For example: the tectofugal visual pathway, the tectum to nucleus rotundus of the diencephalon to the ectostriatum of the telencephalon; the thalamofugal visual pathway, opticus principalis thalami of the diencephalon to the intercalated nucleus of the hyperstriatum accessorium of the telencephalon; and the auditory pathway, the cochlear nuclei and the superior olive to the nucleus mesencephali lateralis, pars dorsalis of the mesencephalon to nucleus ovalis of the diencephalon to neostriatal Field L of the telencephalon. Of these three systems, the components of the auditory pathway had the highest level of glucose consumption, with the tectofugal visual pathway having the second highest. Additional structures also showed higher than background levels of glucose uptake: the nucleus of the basal optic root, several pretectal nuclei, the vestibular nuclei, the anterior lobe of the cerebellum and the vestibulo-cerebellum. The results for resting birds are comparable to those for resting rats (Schwartz and Sharp., J. Comp.Neur. 177, 335, 1978).

Several experimental manipulations were attempted to determine whether glucose utilization could be altered by stimulation. With optokinetic stimulation for one hour, autoradiographs were not notably different from those of normal resting birds. However, with monocular occlusion 12 hours prior to 2-DG injection, and one hour survival, the tectum, nucleus rotundus and ectostriatum contralateral to the occluded eye showed somewhat decreased glucose consumption compared to the same structures on the normal side of the brain. We thank Harvey J. Karten and Melvin V. Simpson for their help and support during these studies. Supported by USPHS 1 F32 NS 05682-01(A.R) and New York State Health Research Council #855 (D.G.D)

1989 EFFECTS OF VISUAL DEPRIVATION ON THE DEVELOPMENTAL ANATOMY OF VISUAL CORTEX IN RATS. Timothy J. DeVoogd, James N. Cohn*, Mitchel Lichtenstein*, Thomas H. Burnstine*, and William T. Greenough. Dept. Psychology and Neural and Behavioral Biology Program, University of Illinois at Urbana-Champaign, IL 61820.

Valverde and others have shown that dark rearing in rodents results in reduced numbers of dendritic spines in visual cortex. These studies have generally not controlled either for the variance between litters of such anatomical measures or for the sensory deprivation imposed on the mother.

In the present study, litters of hooded rats were divided into three groups at birth. One group was raised in normal diurnal lighting, one group was raised in darkness, and one group was blinded. Tunnels allowed all mother rats to visit another cage which was in diurnal light. The rats were killed at 12, 15, 18, and 30 days of age. Their brains were removed, photographed, and stained by a rapid Golgi procedure. No consistent differences in gross brain dimensions (length and width) were associated with the dark rearing. Enucleated animals, however, had narrower posterior cortex than diurnal animals at 12 days and continued to grow more slowly than the diurnal animals. Cortical thickness was measured at precisely defined loci using a computer-assisted microscope. Cortex at the center of area 17 increased in thickness over time for all groups. Here, too, the enucleated rats were consistently lower than the diurnal rats and no consistent differences were seen between the diurnal and dark reared rats. This contrasts with the deficits in cortical dimensions obtained by Gyllensten and co-workers with dark rearing and may reflect differences in maternal cyclicity allowed by the access to diurnal lighting in this study. Spine counts in striate and parastriate cortex are in progress. Supported by Grant NSF BNS77-23660.

1990 CAT VISUAL CORTEX LACKS THE LUXOTONIC UNITS FOUND IN MONKEY. Edgar A. deYoe* and John R. Bartlett. Center for Brain Research, University of Rochester, Rochester, New York 14642.

The intensity of steady, diffuse, "background" illumination, determines the maintained discharge rate of at least 25% of the units in macaque and squirrel monkey striate cortex. (Proc. Intl. Union Physiol. Sci. 13: 55, 1977, J. Neurophysiol. 37: 421, 1974). Unlike the "sustained" units identified in cat visual system, these "luxotonic" units do not require that light be limited to the center of the receptive field and in fact, the "receptive field" of some such units appears to occupy all or most of the visual field. For luxotonic units, activity is either increased (photergic units) or decreased (scotergic units) by increasing light intensity and, again unlike the cat, such changes remain clearly identifiable for minutes or hours rather than seconds, even in the face of some adaptation. However, these long-duration effects are all but obliterated by low doses of either barbituates, nitrous oxide or even diazepam (Valium-Roche) and this could explain why diffuse illumination has long been considered an inadequate stimulus for cells of cat visual cortex. On the other hand, if cat visual cortex truly lacks luxotonic activity then, for cat vs monkey, there must exist a very basic and puzzling difference in the mechanism for visual analysis. To examine this possibility the activity of units in Areas 17, 18, 19 and Clare-Bishop of unanesthetized but painlessly immobilized cats is being recorded in relation to changes in diffuse illumination of the entire visual field. Of the over 90 units so far investigated, all of which responded to flashed or moving light, not one has exhibited anything approaching luxotonic activity although in a few cases increases or decreases in activity were "sustained" for 2-3 seconds. Most of the 90 units (66) have been from Area 17 and, while it is logically impossible to prove that this area is totally devoid of luxotonic activity, the statistical statement can now be made that the chances of as few as 5% of cat Area 17 units being luxotonic is less than 5 in 100. Whether the same will be true for the other areas under investigation remains to be seen but it now seems clear that in addition to the already known differences in binocular organization, Area 17 of cat and monkey also differ either 1), in their ability to differentiate between levels of ambient illumination or 2), in the mechanisms by which this is accomplished.

Supported by NINCDS Grant NS03606.

1991 ELECTRON MICROSCOPIC EVIDENCE OF REGIONAL VARIATION IN FIBER SIZE IN PIGEON OPTIC CHIASM AND TRACT. Thomas A. Duff, Grayson Scott* and Regina Mai*. Depts. of Surgery (Neurosurgery) and Anat., Univ. Wisc., Madison, WI 53706

Electron-micropic study of pigeon optic chiasm and tract revealed a regional variation in mean fiber size which closely parallels the gradient previously found in optic nerve (Soc. Neurosci. Absts: 3:558, 1977).

As fibers from one optic nerve enter the chiasm, they collect into a series of horizontal bands which interdigitate with similar bands containing axons from the other eye. Measurement of fiber size from sample electron-micrographs taken along bands located in dorsal, mid, and ventral chiasm revealed an overall mean axonal diameter value similar to that of optic nerve. Mean fiber size along each band formed a gradient, the dimension of which was related to band location in the dorsoventral axis. Uniform sampling of optic tract likewise demonstrated a gradient in mean fiber size, with relatively homogeneous, small axons located in the ventromedial region and more diversified fibers in the dorsolateral area. The overall fiber diameter spectrum in chiasm and tract appeared to be unimodal, but the histogram of samples possessing a large mean diameter value revealed a bimodal distribution similar to that found for the same population of fibers in optic nerve.

	Optic nerve	Optic chiasm	Optic tract
Modal diameter	0.75 μ	0.77 μ	0.77 μ
Mean diameter	1.04 μ	0.96 μ	1.06 μ
S.D.	0.44	0.48	0.53
Small mean diameter population (<0.99 μ)	temporal	ventromedial	ventromedial
Large mean diameter population (>1.24 μ)	nasal	dorsolateral	dorsolateral

These findings indicate that foveal and dorsotemporal retina convey impulses to anterior and mid optic tectum (McGill, et al., J. Anat. 100: 5, 1966) via a relatively homogeneous, small fiber system whereas the projection from nasal retina to dorsocaudal tectum is composed of fibers possessing a broader spectrum in size. These results also reveal that regional variation is a consistent feature of the retinotectal pathway, and, coupled with evidence of variation in both retinal and tectal ultrastructure, suggest regional specialization in processing of information arriving from different visual fields.

1992 EXPERIMENTAL AMBLYOPIA - PRODUCTION BY RANDOM MONOCULAR SHIFTS OF VISUAL INPUT. Frank H. Duffy, George D. Mower*, and James L. Burchfiel*. Seizure Unit and Neurophysiology Lab, Dept. of Neurology, Childrens Hosp. Med Ctr. & Harvard Med. Sch., Boston, Mass. 02115.

From the age of 4 to 12 weeks, kittens received 1 to 2 hours per day of specialized visual input but were otherwise raised in darkness. They wore goggles in which a 12 diopter wedge prism was placed before one eye. The prism was randomly rotated every 5 minutes producing unpredictable changes in the position of otherwise normal visual input. The other eye received normal visual stimulation.

Recordings in visual cortex revealed suppression of input from the treated eye. 60% of the cells had mappable receptive fields in the normal eye only, and these receptive fields had normal characteristics. 19% of the cells had binocular receptive fields. These receptive fields were abnormal in that they showed weak directional characteristics to both eyes and had larger receptive areas in the treated eye. 17% of the cells were mappable only from the treated eye. These cells had either simple or omnidirectional receptive field properties. 4% of the cells were visually unresponsive.

Thus, shifts in ocular dominance and other findings usually associated with the production of experimental amblyopia, were produced by unpredictable monocular shifts of visual input. Anatomical studies are in progress.

1993 CONTRAST SENSITIVITY OF THE FLY VISUAL SYSTEM. David Dvorak* (SPON. D.M. Snodderly) Dept. Neurobiology, Research School of Biological Sciences, Australian National University, Canberra, Australia.

Spatial contrast sensitivity functions were measured for the fly, Lucilia sericata, by recording extracellularly in the fourth optic ganglion the response of a wide field direction sensitive motion detecting neuron to a drifting sinewave grating displayed on a C.R.T. screen. At high light levels (0.1-4.0 cd/m^2) contrast sensitivity was maximal in the middle frequency range of 0.04-0.10 cycles/degree with marked high frequency attenuation and less steep but obvious low frequency roll off as well. As adaptation levels were lowered the high frequency cutoff point and peak sensitivity shifted towards lower frequencies. At the lowest light levels (10^{-2}-10^{-4} cd/m^2) low frequency attenuation disappeared altogether. A visual acuity curve (highest resolvable spatial frequency at different light levels) for the fly was compared with similar curves for the human derived from the psychophysical studies of Daitch and Green (1969) and DeValois, Morgan and Snodderly (1974). The variation in acuity with illumination in fly most closely follows that of the human peripheral retina. The results point to the notion that, as with the human, the retinal mosaic of the fly becomes functionally coarser as luminance drops. Possible physiological mechanisms underlying this behavior will be discussed.

Daitch, J.M. & Green, D.G. (1969). Contrast sensitivity of the human peripheral retina. Vision Res. 9 : 947-952.

DeValois, R.L., Morgan, H. & Snodderly, D.M. (1974). Psychophysical studies of monkey vision. III. Spatial luminance contrast sensitivity tests of macaque and human observers. Vision Res. 14 : 75-81.

1994 CORPUS CALLOSUM INFLUENCE ON DEVELOPING DEPTH PERCEPTION IN YOUNG CATS. Andrea J. Elberger* (SPON: J. M. Sprague). Department of Anatomy, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

The visual cliff apparatus (Walk and Gibson, 1961) was used to measure the development of depth perception in 4 normal cats (N), 4 cats that had received a surgical section of the posterior corpus callosum at 15-19 days old (E), and an older surgical subject, at 29 days old (E-29). Testing consisted of 10 - 2 minute trials per day starting at 20 and continuing through 40 days old. For each trial, the subject was placed on a red and white, 6 x 7mm checked centerboard and allowed to descend to one of two glass surfaces equidistant from the centerboard. One glass surface had the same checked pattern immediately underneath it (shallow, or safe, side), while the other glass surface had the checked pattern placed 25 inches below it (deep, or "cliff", side). Subjects were scored per trial as to whether they descended onto the deep side, the shallow side, or not at all.

The results show significant differences in the pattern of development of depth perception between the E and the N groups. The N group ceased almost all responses to the deep side by 28 days old, and after 33 days old, descended from the centerboard on every trial. The E group chose the deep side more often and persisted in doing so through 38 days old ($F=25.95$; $df=1,20$; $p< .001$). The E group also refused to descend from the centerboard more often and continued doing so through 39 days old ($F=7.08$; $df=1, 20$; $p< .025$). Examination of E-29's data indicate that these abnormalities occur even if the posterior callosum is sectioned at a time when depth perception is becoming accurate. The overall E group data suggest that there are two components to the development of depth perception of the visual cliff - avoidance of the deep side, and positive preference for the shallow side. For the N group, these two components develop simultaneously. Previous experiments have shown that cats in the E group have a tendency to have divergent strabismus and loss of binocular overlap of the visual field through at least 1 year old (Elberger, 1977). In view of this, early posterior corpus callosum section alters and retards the development of depth perception probably by altering early visual input so that depth cues that are present are ignored.

1995 COBALT LABELING OF THE GANGLION CELLS OF THE FROG FRONTAL ORGAN. William D. Eldred* and John F. Nolte. Dept. Anat., Univ. Colo. Sch. Med., Denver, CO 80262.

The frog's frontal organ is a photoreceptive system capable of discriminating between stimuli of different wavelengths. Previous physiological models have suggested that simple photoreceptor-ganglion cell interactions could account for these discriminations, but anatomical knowledge of the cell types involved, and their interconnections, is scanty. We examined the morphology and synaptic connections of the ganglion cells using axonal iontophoresis of $CoCl_2$. A suction electrode filled with 10% $CoCl_2$ solution was used to fill the frontal nerve axons, and the ganglion cells from which they originate, in the isolated frontal organ. The preparation was maintained in tissue culture medium during the 18-36 hours necessary for labeling. Subsequent fixation and precipitation with ammonium sulfide produced clear labeling of the ganglion cell bodies. The results indicate that, contrary to previous models, there is extensive synaptic interaction between ganglion cells. Small numbers of ganglion cells form clusters around areas of neuropil where some make ribbon-type synaptic contacts on each other. The pseudounipolar shape of the majority of labeled cell bodies corresponds to the shape of previously reported cells in the frontal organ of Rana esculenta stained to demonstrate acetylcholinesterase. The number of cells labeled with $CoCl_2$ varied from 40-105 which correlates well with our electron microscopic counts of axon numbers in the frontal nerve, and with previously published results from Rana esculenta. These results indicate that future physiological models should take into account the fact that synaptic interactions in addition to receptor-ganglion cell synapses occur in the frontal organ. In addition, the fact that at least some axons in the frontal nerve originate from cells which make ribbon-type synapses indicates that not all ganglion cells of the frontal organ are directly comparable to ganglion cells of the lateral eye retina.

1996 A COMPARISON OF THE HORIZONTAL AND VERTICAL OPTOKINETIC RESPONSES OF THE RABBIT. R. G. Erickson* and N. H. Barmack, (SPON: C. C. Bell). Neurological Sciences Inst., Good Samaritan Hosp. & Med. Cntr., Portland, OR.

Because of an otolithic input, the vertical vestibuloocular reflex (VVOR) of the rabbit has a higher gain (eye velocity/head velocity) and different phase characteristics than the horizontal vestibuloocular reflex (HVOR) for low angular accelerations. Since both vestibuloocular reflexes and optokinetic reflexes must combine to provide the rabbit with a relatively fixed spatial reference, it would appear that the optokinetic input would be of greater importance for providing this reference for the horizontal system. The present experiment examines this prediction by comparing the closed-loop gain (eye velocity/stimulus velocity) and open-loop gain (eye velocity/retinal slip velocity) for both horizontal and vertical systems. A random noise, optokinetic stimulus was presented monocularly to rabbits placed in front of a rear-projection tangent screen subtending 72 x 72 deg. Eye position of the non-stimulated eye was measured using an infra-red light projection technique. The closed-loop gain of optokinetically evoked horizontal eye movements evinced a directional selectivity, with a much higher gain for stimulation in the posterior —>anterior direction (P—>A), with reference to the stimulated eye. The P—>A gain was .8 at a stimulus velocity of .25 deg/sec, declined to .7 at 10 deg/sec and was .2 at 50 deg/sec. The A—>P gain was .55 at a stimulus velocity of .1 deg/sec declined to .02 at 10 deg/sec, and was 0 at 20 deg/sec. The vertical optokinetic gain was nearly equal for stimuli moving in either the vertical up or vertical down direction. At a stimulus velocity of .25 deg/sec the gain was .5, declined to .15 at 10 deg/sec, and was .02 at 50 deg/sec. The open-loop gain in the horizontal P—>A direction was 2.3 at a retinal slip velocity of .1 deg/sec, and declined to .15 at 50 deg/sec. The vertical open-loop gain at a retinal slip velocity of .1 deg/sec was 1.3, and declined to .03 at 50 deg/sec. The dynamics of eye movements evoked in the vertical and horizontal planes were also different. In the horizontal plane the eye never exceeded a deviation of 15 deg before being interrupted by a resetting saccade. Vertical deviations greater than 20 deg were often maintained for intervals exceeding 10 sec without resetting. These data suggest that the higher horizontal optokinetic gain may compensate for the reduced gain of the HVOR at lower angular accelerations of the head. An equivalent vertical optokinetic gain would be obviated by the contribution of the otoliths to the VVOR at these lower angular accelerations. (Supported by PHS Grant EY-00848 and The Oregon Lions Sight Foundation).

1997 THE GANGLION CELLS OF RABBIT RETINA. E.V.Famiglietti,Jr. and E.C.Siegfried*. Dept. Physiol. and Biophys., Wash. Univ. Sch. Med., St. Louis, MO 63110.

Retinal ganglion cells of rabbit, like those of cat, may be divided into 4 classes, based upon analysis of Golgi preparations. Class I cells have medium-to-large size cell bodies, thick axons and dendrites, "radiate" dendritic branching pattern (DBP), narrow dendritic stratification (DS), and large dendritic field diameter (DFD). Class II cells have small cell bodies, thin axons, a "tufted" DBP with a profusion of dendritic appendages, broad DS, and small DFDs. Class III cells have small-to-medium size cell bodies, relatively thin axons and dendrites, narrow DS, a variety of DBPs, and a wide range of DFDs at a single retinal locus. Class IV cells, with medium size cell bodies and either multistratified or diffuse dendritic branching, include at least 2 kinds of bistratified cell, and 2 kinds of "E-type" cell (Lettvin et al., 1961). Dendritic field, cell body and axonal diameters of all but a few ganglion cells of classes III and IV increase with increasing distance from the visual streak. Most class I and class III cells can easily be assigned to a or to b types, according to the level of the inner plexiform layer (IPL) at which their dendrites stratify. Class II cells are somewhat more heterogeneous than in cat and more difficult to type.

A fifth class of "intrinsic" ganglion cell has been identified. Its small-to-medium size cell body usually lies in the IPL, and it gives rise to long, wavy, somewhat spiny, and relatively unbranched dendrites which form a large dendritic field. Typically, 2 "axons" emerge from 1st or 2nd order dendritic branches as tapering "initial segments". Slender and beaded, the "axons" of class V cells ramify in rectilinear fashion, narrowly stratified together with the wavy dendrites of these cells in mid-IPL. These "axons", closely resembling the axons of Golgi type II neurons of the CNS, do not leave the IPL. (This work does not support the assertion of Marenghi, 1901, that some retinal ganglion cells have axon collaterals.) Neither in their overall morphology, nor in their "axonal" ramifications, do intrinsic ganglion cells of rabbit retina resemble the "giant associational nerve cells" of Gallego and Cruz, 1965. At present attempts are being made to relate the morphology and synaptic connections of rabbit ganglion cells to their neurophysiology and pharmacology.

1998 THE AXONAL ARBORIZATION WITHIN AREA 17 OF DIFFERENT CLASSES OF LATERAL GENICULATE NEURONS. David Ferster* and Simon LeVay, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115

The cat's lateral geniculate nucleus (LGN) contains three morphological types of neurons: types 1,2 and 4 (Guillery, JCN 128:21). We have previously suggested that these correspond to the three physiological types: Y-, X- and W-cells (LeVay and Ferster, JCN 172:536).

Do the axons of each cell type have a distinctive pattern of arborization within area 17? To answer this question injections of horseradish peroxidase were made into a cut in the optic radiations of adult cats. Golgi-like staining of neurons in the LGN, and of axonal arborizations in the visual cortex were obtained. In laminae A, A1 and C, examples of type 1 and type 2 neurons were reconstructed and their axon diameters measured. Type 1 neurons possessed large diameter axons (2.0-3.3μm, exclusive of myelin) while type 2 neurons had medium sized axons (1.0-1.7μm). Both types gave off collaterals to the perigeniculate nucleus. In laminae C1-C3, neurons with very fine axons (0.5-1.0μm), probably type 4 neurons, were found. Identification of type 1 and type 2 neurons as Y- and X-cells is strengthened by the determination of their axon diameters, since these are appropriate for the conduction velocities reported for Y- and X-cells.

In the cortex, the terminals of filled axonal arborizations were restricted to 3 levels: (i) the upper half of layer I, (ii) a band extending from the bottom of layer III to the top of layer V and (iii) the upper half of layer VI. This distribution matches the distribution of geniculo-cortical afferents observed after ^{3}H-proline injections into the LGN (LeVay and Gilbert, Brain Res. 113:1) Camera lucida reconstructions of 30 complete axonal arborizations revealed 3 types. Axons of one type terminated primarily in layer IVab and the lower part of layer III. Their diameters, measured in the white matter, matched those of the type 1 geniculate relay cells. Their arborizations spread laterally for up to 2mm in layer IVab with their boutons grouped into several clumps, similar in size and arrangement to ocular dominance columns. Axons of the second type terminated primarily in layer IVc. Their diameters matched those of the type 2 geniculate relay cells. They had much smaller arborizations, usually with only a single clump of boutons. Most afferents of both these types gave off collaterals in the upper half of layer VI. The third type of afferent innervated the upper half of layer I. Their axon diameters matched those of the small cells in laminae C1-C3. They sometimes gave off collaterals to layer V or the lower half of layer III. We conclude that the different classes of relay cells, though partially intermixed in the LGN, have different laminar projections in area 17. (NIH grants R01 EY01960 and T01 EY00082)

1999 RETINAL AFFERENTS TO THE MIDBRAIN RAPHE OF THE CAT. Warren E. Foote and Elizabeth Taber-Pierce. Depts. of Psychiatry and Anatomy, Harvard Med. Sch.-Mass. Gen. Hosp., Boston, Mass. 02114.

During the course of recent work exploring the effects of midbrain raphe stimulation on principal cells in the dorsal lateral geniculate of the cat it was noted that a small number of optic tract fibers were antidromically driven by single shocks to this midbrain area. This observation suggested that ganglion cell axons might be traversing areas of the midbrain not known to receive visual information. In an attempt to provide anatomical verification of this possibility a series of animals were utilized in studies employing techniques of orthograde and retrograde transport.

Orthograde transport was accomplished by injecting a mixture of tritiated leucine and proline into one eye in each of five animals. After survival times ranging from 2 to 14 days, the animals were perfused with formalin and the tissue embedded, cut, and dipped in Kodak NTB-2 emulsion. Microscopic examination revealed the presence of light label in the nucleus raphe dorsalis and the central superior nucleus. Grain counts were performed to confirm this observation.

Retrograde transport was accomplished by iontophoretic application of a 4% solution of horseradish peroxidase (Sigma Type VI or Boehringer Grade I) dissolved in .05 M Tris-HCl buffer and 0.2 M KCl pH 8.6. Applications were made into either the dorsal raphe nucleus or the nucleus central superior. After 24 hours survival the animals were perfused with a 2.5% glutaraldehyde 0.5% paraformaldehyde mixture and the brains and retinas removed and reacted with Hanker-Yates solution. Under oil immersion reaction product was observed in the peripherally located, large ganglion cells of all retinas. These neurons appear to correspond to the alpha ganglion cells of Boycott and Wassle (J. Physiol. 240: 397, 1974). Since not all of the large ganglion cells contained reaction product and those that did were found in all retinal quadrants, these data coupled with autoradiographic material indicate a small binocular projection from peripheral retina to the midbrain.

2000 SURROUND ANTAGONISM AND OPPONENT PROCESSES IN DIRECTIONALLY SPECIFIC PIGEON TECTAL NEURONS. B.J. Frost, S.C.P. Wong*, and P.L. Brooks*, Dept. Psych., Queen's Univ., Kingston, Ontario, Canada.

It is now generally accepted that directional selectivity in neurons is produced by the asymmetrical propagation of inhibition in the null direction. We have confirmed these findings in directionally selective neurons in the pigeon tectum and have used a variety of techniques to reveal that these units also have a centre-surround structure where the directional characteristics of the surrounding region are the reverse of the central area. Conventional extracellular recording methods were used to study the motion characteristics of 126 neurons located in all tectal laminae of white Carneaux pigeons. A dual channel optical system permitted the independent manipulation of the size, direction, velocity and pattern of two stimuli.

Central Inhibition in the null direction was demonstrated when the normal excitatory response produced by movement of the test stimulus in the preferred direction through the centre of the receptive field, was inhibited by a second spot moving along the same path in the null direction. Additionally PSTHs produced by sweeping a single stimulus forward and back in the preferred and null direction of spontaneously active units directly shows inhibition in the null direction, which arises from the same area of the receptive field as excitation in the preferred direction. Surround inhibition has been demonstrated by increasing the size of a test stimulus past the optimal size, which results in a reduction and sometimes abolition of the response. Surround inhibition has been more directly revealed by moving a second stimulus synchronously (in same direction & velocity) with the test spot moving in the preferred direction through the centre of the receptive field. The excitatory response produced by the test spot alone is completely abolished when the second stimulus moves in the same direction through the surround. Finally surround facilitation has been demonstrated by moving a large textured pattern in the surround area only (centre-masked), in the opposite direction to the central test stimulus which is moved in the preferred direction. The receptive field structure of directionally specific neurons that emerges from these observations is a spatially organised, antagonistic centre-surround organisation, in which the central region exhibits excitation in the preferred direction and inhibition in the null direction, while the surround is arranged in an opponent fashion whereby "preferred" direction motion produced inhibition and "null" direction motion produces facilitation of the centrally generated response.

2001 THE ANATOMY OF THE OPTIC NERVE OF THE TURTLE, PSEUDEMYS SCRIPTA ELEGANS. J.E. Fulbrook and A.M. Granda. Inst. for Neuroscience, University of Delaware, Newark, DE 19711.

The optic nerve of the turtle was studied by both light and electron microscopy. The great majority (70-80%) of the fibers are myelinated. Myelinated fibers ranged in size from about 0.8 to 4.0 microns in diameter. Unmyelinated fibers ranged in size from 0.2 microns to about 1.0 microns in diameter. Fiber counts from different regions of optic nerve yielded a total estimate of 724,000 axons. The larger myelinated fibers were located more in the periphery of the optic nerve. Smaller myelinated fibers were located predominantly in the central region. Unmyelinated fibers were found throughout the nerve with no apparent differential distribution. Only one type of neuroglial cell was observed.

A well-defined neural groove was identified in the optic nerve preparations. The neural groove appeared as an invagination extending dorsally from the ventral surface halfway into the nerve. The groove originates at the optic disk and extends back to the chiasm where it disappears in the crossing fibers. The neural groove is bordered on both sides by discrete collagenous sheaths similar to the pial sheath surrounding the optic nerve. Blood vessels of varying sizes were found predominantly within the neural groove and pial sheath. Only a few blood vessels were found within the nerve proximal to additional small nerve invaginations and within regions of high neuroglial cell density. Comparative data on axon fibers and vascular organization will be discussed.

2002 RESPONSE LATENCIES IN CAT VISUAL CORTEX. Jill C. Gardner and Max S. Cynader. Dept. Psych., Dalhousie University, Halifax, N.S., Canada.

Responses to flashed stimuli were measured in a sample of over 500 neurons in cat areas 17 and 18. Unless otherwise indicated, the stimulus was a bright bar (2.74 cd/m^2 against a background of .086 cd/m^2) of 50 msec. duration.

Of the parameters which were examined, changes in stimulus luminance had the greatest effect on response latencies. Over a 2 log unit range, decreases in luminance resulted in latency increases of 40-50 msec. Other manipulations produced relatively small effects. Varying the orientation of the stimulus or its position on the receptive field caused changes of less than 8 msec. although these manipulations could produce substantial changes in firing rate. In general however, shorter latencies resulted from stimuli which evoked more spikes. An exception to this rule was seen with increases in stimulus size. Increasing the length or the width of the stimulus usually resulted in a decrease in the number of spikes and a decrease in latency. In part, this latency shift was attributable to the longer latency of surround inhibition.

The observations noted above held in both cortical areas. In other respects, responses in areas 17 and 18 showed marked differences.

Area 18 units were more responsive to flashed stimuli. Most cells responded well to even brief flashes (5 msec.) while this stimulus proved to be quite ineffective for area 17 units. With only rare exceptions, units in area 18 responded with a transient burst of impulses to a prolonged flash (250 msec.). Many area 17 cells gave sustained responses or showed both a sustained and transient component in their response. Within each area, simple and complex cells had similiar latencies. Responses in area 18 were very homogeneous, with almost all cells responding within a 20 msec. range, while area 17 showed a much broader latency spread. In area 17, a special class of hypercomplex cell found in the superficial layers was characterized by extremely long latencies. Response latencies averaged 10-15 msec. shorter in area 18 than in area 17.

The results indicate clear differences in the intracortical organization of areas 17 and 18. In area 17, strong inhibitory interactions and long latency responses contrast with the homogeneous area 18 response. Responses in area 18 reflect a simpler network, well designed to encode temporal information.

2003 PERIMETRIC DEFICITS ASSOCIATED WITH CHRONIC MONOCULAR PARALYSIS. P.E. Garraghty*, W.L. Salinger and M.G. MacAvoy*. Dept. Psych., University of North Carolina at Greensboro, Greensboro, NC 27412.

Single-unit recordings in adult cats which have undergone chronic monocular paralysis (14 days or more) reveal profound alterations in the cell populations of lateral geniculate nucleus and visual cortex (Salinger, W.L., Schwartz, M.A. and Wilkerson, P.R., Brain Research, 1977; Fiorentini, A. and Maffei, L., Vision Research, 1974). Little, however, is known regarding the behavioral significance of these physiological changes. In this study a behavioral perimetry technique was used to detect visual field deficiencies which may have developed concomitantly with the previously reported physiological changes. In this study a behavioral perimetry technique was used to detect visual field deficiencies which may have developed concomitantly with the previously reported physiological changes. Monocular paralysis was accomplished by surgical transection of cranial nerves III, IV, and VI. The width of the visual field for each eye was repeatedly measured before monocular paralysis surgery, and perimetric testing continued daily for a period of two weeks after surgery. Chronic monocular paralysis results in a peripheral visual field compression divided between nasal and temporal fields, and totaling slightly less than 20° in the paralyzed eye. A small part of the ultimate field compressions were present at the time of the first post-surgical tests, while the balance appeared progressively during the two weeks of monocular paralysis. The small initial compression could perhaps be accounted for by optical defects caused by transection of nerve III. The progressively appearing field compression, however, appears physiological in origin. The progressively appearing component of the perimetric deficit seems to be a behavioral correlate of the physiological changes which are found following monocular paralysis.

2004 RETINOTOPIC ORGANIZATION AND SINGLE UNIT RESPONSES IN THE PULVINAR OF THE CEBUS MONKEY. Ricardo Gattass*, Eduardo Oswaldo-Cruz* and Aglai P. B. Sousa* (SPON: Charles Gross) Institute of Biophysics, U.F.R.J., Rio de Janeiro, RJ 20.000, BRASIL.

Single and multi-unit recording from the pulvinar of 24 Cebus monkeys revealed two retinotopically organized areas. The largest was located in the ventro-lateral part of the pulvinar and included the pulvinar inferior and the ventral third of pulvinar lateralis. The second area was dorsal to the first, in the middle third of pulvinar lateralis. Both representations were restricted to the contralateral hemifield but had different magnification factors for the center of gaze. The ventro-lateral representation had a larger foveal representation than the dorsal representation.

The visual properties of single units in the ventro-lateral (N=134) and the dorsal areas (N=39) were similar. 74% gave clear time-locked responses to visual stimuli and had properties similar to those reported in the geniculo-striate system or the superior colliculus. Of these units, 76% were binocular, 75% preferred moving stimuli, 75% showed directional specificity and 47% showed orientation specificity. In addition, the activity of virtually all the units could be modified by somesthetic, auditory, olfactory or visual stimulation but the responses had long and variable latencies, habituated easily and often continued after the stimulus. A given unit often gave different patterns of responses to different modalities.

2005 THE PROJECTIONS OF THE LATERAL GENICULATE NUCLEUS TO AREAS 17 AND 18 IN THE CAT. E. E. Geisert, Jr. The Neuroscience Training Program and the Department of Anatomy, The University of Wisconsin, Madison, Wisconsin 53706.

Both areas 17 and 18 receive input from the lateral geniculate nucleus. The extent to which these two areas share the geniculate inputs is basic to an understanding of visual function. I have developed a new application of the retrograde transport method designed to demonstrate neurons which project to several cortical areas by axons that branch, and have applied this method to a study of the geniculo-cortical system of the cat. This method depends on the retrograde axonal transport of two markers, each of which is uniquely detectable by histological methods. In this study, horseradish peroxidase (detectable by the enzyme reaction product only) and tritiated proteins (either enzymatically inactivated tritiated horseradish peroxidase or tritiated bovine serum albumin, both of which are detectable by the tritium label only) were used. One of these markers was injected into area 17 and the other was injected into area 18. The animals were perfused with 4% glutaraldehyde. The tissue was sectioned at 200μm and processed to reveal the active horseradish peroxidase. Then these sections were embedded in methacrylate, resectioned at 4μm and processed by the autoradiographic method. In eight animals, individual neurons were seen which contained both the brown reaction product and the tritium label. These neurons project to both area 17 and area 18 by axons that branch. In layers A and A_1 of the lateral geniculate nucleus, 10% of the cells project to both areas 17 and 18, 70% of the cells project to area 17 only, less than 1% of the neurons project to area 18 only, and approximately 20% of the cells are interneurons. In the C laminae, 50% of the cells project to both areas 17 and 18, 20% of the neurons project to area 17 only, 10% of the cells project to area 18 only and 20% of the cells do not project to either area 17 or area 18. In the medial interlaminar nucleus, neurons also project to both area 17 and area 18 by axons that branch. (Supported by grants RO1 NS 06662 and RO1 EY 00962.)

2006 BIFURCATION OF THE CORTICOPONINTE PATHWAY IN THE CAT. Alan Gibson*, James Baker*, George Mower*, Farrel Robinson*, and Mitchell Glickstein. Dept. Psych., Brown University, Providence, RI 02912

There is a visual input to the rostro-medial pons which arises from layer V cells of several visual areas of the cerebral cortex. Layer V cells of the same cortical areas also provide an input to the superior colliculus. Do the axons of the corticopontine cells bifurcate and give off one branch to the superior colliculus and another to the pontine nuclei? To answer this question, we first recorded cells in the medial pontine visual area and then placed an array of stimulating electrodes at the same location. We next placed an array of stimulating electrodes in the superior colliculus. We recorded from cortical visual cells and tested to see if they could be activated antidromically from the pons. We then tested to see if such corticopontine cells could also be activated antidromically from the colliculus. Antidromic latencies from the stimulating sites and collision between collicular and pontine-elicited spikes were used to establish bifurcation.

68% (N=35) of the corticopontine cells in area 18 were activated from both the pons and colliculus. No consistent differences in antidromic latencies between the pons (mean latency= 3 ms) and colliculus (mean latency=3.2 ms) were seen. 54% (N=11) of lateral suprasylvian corticopontine cells had bifurcated axons (pons latency=3.4 ms, colliculus latency=2.6 ms). The posterior middle-suprasylvian area (MSS), a visual association area, has a heavy output to medial pons and superior colliculus. 42% (N=12) of MSS corticopontine cells bifurcated (MSS latency=5.5 ms, SC latency=6.4 ms). All of our estimates of the percentage of bifurcated axons must be minimal, since the exact location of the collicular stimulating electrodes would strongly affect the probability of detecting bifurcation.

We conclude that a high percentage of cortical fibers carrying visual information to the pons also sends a copy of that information to the superior colliculus. The cortex supplies visual input to the cerebellar hemispheres via the medial pontine nuclei, while the colliculus provides visual input to the vermis of the cerebellum via the dorsolateral pontine nuclei. The paravermian cortex and the cerebellar hemispheres have been associated with control of distal musculature, and the vermis with the control of proximal musculature (Chambers & Sprague, J. Comp. Neurol. 102: 105-129, 1955). Perhaps the cortical visual information carried by the bifurcated axons is useful for visuomotor coordination of both proximal and distal muscles.

2007 CLASSIFICATION OF CONCENTRIC CELLS IN THE RABBIT LGN AS X OR Y BASED UPON LINEARITY OF SPATIAL SUMMATION. David L. Glanzman* and Loraine G. Miller* (SPON: K. L. Chow). Dept. of Psychology, Stanford Univ. and Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305.

Recently, Hochstein and Shapley (J. Physiol. 262: 237, 1976) have argued that the fundamental basis for a distinction between X and Y cells in the cat visual system is reflected in the property of linearity of spatial summation; other classificatory criteria which have been employed in studies of X and Y cells, e.g., whether a cell gives a sustained or transient response to standing contrast, reflect non-essential properties. Thus, these latter criteria yield overlapping categories, whereas the criterion of linearity of spatial summation yields discrete categories of X and Y cells in the cat retina and LGN. We now report the first instance in which LGN cells in a mammal other than the cat have been classified as X or Y by means of the rigorous criterion of spatial summation linearity. Using sinusoidal grating stimuli similar to those employed by Hochstein and Shapley, we have found that concentric neurons in the rabbit LGN can be classified into two non-overlapping groups depending upon whether they exhibit linear or nonlinear spatial summation over their receptive fields. We have also found that properties such as size of receptive field center and type of response to standing contrast cannot be used to discretely classify rabbit LGN cells as X or Y.

The rabbit LGN, unlike that of the cat, contains a significant proportion of cells with uniform receptive fields. Uniform cells, like concentric cells, give either a sustained or transient response to standing contrast. We have tested uniform cells with sinusoidal grating patterns in order to determine whether they may also be grouped into X and Y categories. (Supported by NIH Grant EY 00691 and NASA Grant NGR 05-020-435.)

2008 NEURONAL ACTIVITY FROM MOTOR CORTEX IN VISUOBEHAVIORAL DEPRIVATION. Jay D. Glass. Dept. Pharmacol., U. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261

Visuobehavioral deprivation by monocular eyelid closure yields valuable insights into the development of the afferent visual pathways and into the relationship between the development of those pathways and the animal's capacity for visually guided behaviors. We have previously shown that the deprivation effects visually evoked activity not only from visual cortex as reported by others, but also from frontal cortex in man and motor cortex in cats. We now wish to report further observations on visually evoked activity recorded from motor cortex in chloralose anesthetized and awake cats following extended periods (11-18 months) of monocular closure.

In two cats under deep chloralose anesthesia, no differences were found between the single-unit or slow-wave response evoked from the deprived eye (DE) and nondeprived eye (NDE). However, as anesthesia level decreased, the response evoked from the NDE became robust whereas the response evoked from the DE retained its "depressed" form. In two cats with implanted electrodes studied during light, nonsurgical anesthesia, the slow-wave and single-unit response evoked from DE and NDE were dramatically different.

Four cats with implanted electrodes were studied while awake. A reliable sequence of slow waves was evoked from the NDE and an increase in unit activity usually occurred in phase with certain components of the slow wave. No reliable responses were evoked from the DE nor did an increase in unit activity occur. In addition, when only the DE was open the level of unit activity was lower than with the NDE, at times specific cells would cease firing.

In the chloralose anesthetized preparation, the response evoked from the DE always resembled the response evoked from a deeply anesthetized cat. In the awake cat utilizing only the DE, unit activity was at a lower rate than when utilizing only the NDE. A previous report about humans with visuobehavioral deprivation showed intense alpha rhythm which is not blocked by a photic stimulus to the DE, even though a response is recorded from visual cortex. Reports of behavioral impairments in deprived cats indicate a deficit in attentional mechanisms. These lines of evidence suggest that the deprivation has altered the relationship between visual input to the deprived eye and nonspecific arousal mechanisms.

2009 THE ROLE OF THE NUCLEUS ISTHMUS IN THE IPSILATERAL VISUAL PROJECTION TO THE FROG'S OPTIC TECTUM. Steven Glasser and David J. Ingle, Dept. Psych., Brandeis Univ., Waltham, MA. 02154.

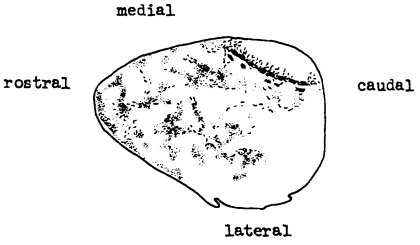
We investigated the effects of unilateral electrolytic lesions of nucleus isthmus in adult frogs (Rana pipiens), using single and multiple unit mapping of the optic tecta. While our lesions had no effect upon density or response properties of retinal fiber terminals activated from the contralateral eye, units of the ipsilateral projection could not be found over most of the dorsal tectum. No such loss was obtained in frogs where we intentionally missed the nucleus isthmus. Our data support the hypothesis (Gruberg, E.R. and Udin, S. E., J.C.N.179: 487, 1978) that the nucleus isthmi provide relay stations for the ipsilateral projection to frog tectum, and thereby provide one basis for binocular vision in Anuran amphibians. However, projections for nucleus isthmi to tectum may provide other functions besides binocular integration. Following large isthmus lesions, we often noticed two abnormal phenomena: 1) an increase in spontaneous activity throughout both tecta and 2) units in the superficial tectum which gave a prolonged discharge to the dimming of light. Thus, we suggest that removal of a modulating input from nucleus isthmus produces a kind of global disinhibition on both sides.

2010 The role of symmetrical and asymmetrical binocular input in the development of orientation specificity. Barbara Gordon, Joelle Presson*, James Packwood*, and Robert Scheer*. Department of Psychology, University of Oregon, Eugene, OR 97403.

In normal cats the orientation preferences of single units in the visual cortex are uniformly distributed. Previous investigations have used two procedures in attempts to alter this distribution. In some experiments kittens have been reared in striped drums which allow both eyes to see the same orientation (Blakemore and Cooper, 1970). Other workers have reared kittens wearing striped goggles so that the eyes receive asymmetrical binocular input; that is, one eye sees horizontal stripes and the other eye sees vertical stripes (Hirsch and Spinelli, 1971). While there is general agreement that the goggle procedure is effective, Stryker and Sherk (1975) failed to replicate the effect of drum rearing. In an attempt to resolve this controversy, we have reared kittens in four conditions that separate the drum vs. goggle variable from the symmetrical vs. asymmetrical binocular input variable. The conditions were: 1) drum reared, both eyes seeing horizontal (HH drum), 2) drum reared, left eye seeing horizontal, right eye seeing vertical (HV drum), 3) goggle reared, both eyes seeing horizontal (HH goggle), 4) goggle reared, left eye seeing horizontal, right eye seeing vertical (HV goggle). We recorded from at least 65 oriented cells from at least 2 cats in each condition. In contrast normal cats in which almost all visual cortex cells have orientation preferences, only 55% of the cells in drum and goggle reared cats showed orientation preferences. Of the remaining 45% of the cells, 21% were unoriented and 24% were visually unresponsive. For each condition we calculated the ratio of the number of cells preferring stimuli within 30° of the experienced orientation to the number preferring orientations within 30° of the perpendicular to the experienced orientation. In the HV goggle condition, which produced the most dramatic effect, this ratio was 9. For the other 3 conditions this ratio varied from 1.5 to 2.5. We conclude that, while both goggle and drum rearing are effective, the combination of goggles and asymmetrical orientations is the most effective way to alter the distribution of preferred orientations.

2011 PERIODICITY IN THE VISUAL COMMISSURAL PROJECTIONS OF THE GREY SQUIRREL. Harry J. Gould, III. Dept. Anat., University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

The topographic relationships of interhemispheric visual connections are demonstrated in the grey squirrel using the Fink-Heimer technique on tissue that was flattened by the method of Welker and Woolsey. Sectioning the corpus callosum reveals degeneration that is organized into three parallel bands on the lateral surface of the occipital cortex. Although cytoarchitectonic landmarks are difficult to determine on the flattened tissue, these bands apparently correspond to the 17-18 border, the 18-19 border and the 19-temporal border. As shown in the figure provided, the most prominent or major commissural band corresponds to the 17-18 border. It is characterized by alternating areas of dense and sparse degeneration. It continues rostromedially and caudally onto the medial wall of the hemisphere; these rostral and caudal extentions end before meeting. A second, narrow band of dense degeneration corresponds to the 18-19 border. Rostrally, on the lateral surface of the hemisphere, it is continuous with the major commissural band. It extends caudally to reach the medial wall of the hemisphere. A third, wide band of sparse degeneration corresponds to the 19-temporal border. Rostrally, this band is indistinguishable from the degeneration observed in the parietal cortex. Caudally, it ends before reaching the medial wall of the hemisphere. Both the second and third commissural bands are characterized by alternating regions that contain or are free of degeneration. The functional significance of the periodicity in these projections is unclear, yet the pattern is reminiscent of that observed for the geniculostriate contribution to ocular dominance columns in the cat and monkey. This suggests that ocular dominance may be important in interhemispheric integrative mechanisms. (Supported by URC 8-19101-1311-39)



2012 A LIGHTMICROSCOPIC AND ELECTRONMICROSCOPIC STUDY OF THE SUPERFICIAL LAYERS OF THE SUPERIOR COLLICULUS OF THE TREE SHREW (TUPAIA GLIS). J. Graham* and V.A. Casagrande. Depts. of Anat. and Psych., Vanderbilt Univ., Nashville, TN 37232.

Histochemical, Golgi and electronmicroscopic methods were used to study the superficial layers of the superior colliculus of tree shrews. Zones of retinal and cortical input were compared with the locations of efferent cells projecting to the dorsal lateral geniculate nucleus (dLGN) and the pulvinar (Pul). Following horseradish peroxidase injections of the dLGN and the Pul, retrogradely labeled cells were found in the upper 2/3rds and lower 1/3rd of the stratum griseum superficiale (sgs), respectively, as has been described by Albano, et al. (1977). The impression given by this material that the orientation of processes and size of the labeled cells varied according to their somata's location and extrinsic connections was born out by correlating this material with tissue prepared by various Golgi techniques. Cells located in the upper sgs had narrow dendritic fields, both the dendrites, often ascending to the sgs-stratum zonale border but confined to the upper sgs, and the somata being strikingly vertically oriented. By comparison, cells located in the lower sgs had larger somata and wider dendritic fields, both the dendrites, usually confined to the lower sgs, and the somata giving more of a multipolar appearance.

For electronmicroscopy, following enucleation and/or decortication (usually only of the striate cortex), the animals were allowed to survive from 2 days to several months. Differences between normal and lesioned material suggested that size of terminals and morphology of the (round) synaptic vesicles did not vary according to lesion type. However, pale mitochondria with irregularly organized cristae and scalloped profiles with multiple postsynaptic processes were associated with optic terminals, and dark mitochondria with more regularly organized cristae and profiles with fewer postsynaptic processes were associated with cortical terminals. Enucleation caused considerably greater number of terminals to degenerate than decortication. Although in both cases degenerating terminals were observed throughout the full depth of the sgs and into the upper stratum opticum, the majority of the degenerating optic terminals were located in the upper sgs, whereas degenerating cortical terminals were evenly distributed throughout the sgs. These results indicate that cells that project to the dLGN and those that project to the Pul differ in two ways, besides location and size. First, these cells exhibit different dendritic patterns. Second, the substrata in which their somata and dendrites lie receive different proportions of retinal and cortical afferents.

Supported by EY 07007 and EY 01778.

2013 EFFECTS OF CHOLINERGIC AGENTS ON RETINAL FIELD POTENTIALS IN NECTURUS. Steven Grant. Dept. Psych., Univ. of Georgia, Athens, GA 30602.

The effects of acetylcholine (ACh), eserine, nicotine, oxotremorine, atropine, and mecamylamine (0.25-10mM) were examined in the perfused mudpuppy eyecup. None of the agents abolished the ERG a-wave. ACh produced a reduction of all of the potentials; however, the b-wave and M-wave were abolished at lower concentrations than the PNR.

Eserine and nicotine enhanced the amplitudes of all of the potentials. Oxotremorine enhanced the PNR, reduced the M-wave, and had a weak and variable effect of the b-wave.

Atropine and mecamylamine reduced the b-wave over all concentrations. At low levels of illumination, atropine enhanced the off-response of the ERG. The effects of atropine and mecamylamine on the PNR were biphasic at high concentrations (5-10mM): a short period of enhancement preceded the eventual abolishment of the PNR. At lower concentrations (0.5-1.0mM), only the enhancing action was observed. The M-wave was reduced or abolished at all concentrations.

In conclusion, there are either seperate nicotinic and muscarinic receptors in this retina, or there is a single type of receptor with mixed pharmacological properties.

Supported by NIH grant EY00973 to L.M. Proenza.

2014 THE DISTRIBUTION AND SHAPES OF TECTAL CELLS PROJECTING TO THE NUCLEUS ISTHMI IN FROGS. Edward R. Gruberg*and Jerome Y. Lettvin* (Spon: S.A. Raymond). M.I.T., Cambridge, Ma 02139

The path that carries information from one eye to the ipsilateral tectum goes in this way: The optic nerve is connected to the neurons of the contralateral tectum. These neurons in turn project topographically to the nucleus isthmi (NI) ipsilateral to them and are its exclusive input. Finally, the neurons of NI send axons to the tectal lobe contralateral to them (as well as ipsilateral). The receptive fields of NI units have small and large centers. The small centers are about 3-5° in diameter, about the size of a type II optic nerve fiber. Their response is to much the same geometric features of stimulus as excite type II fibers. The large centers are variable, but about 7-10° minimum, and respond to much the same features as excite types III and IV optic nerve fibers. This responsiveness is in strong contrast to that of tectal cells which have much larger receptive fields, show far less vivacious response, adapt extremely rapidly to repeated stimuli, and are hard to describe in terms of characteristic stimuli. It appears, by recording, that the cell firing of tectal neurons is a subset of the axonal firing, so that soma spike information does not represent properly the tectal output.

To find the location of tectal neurons projecting to NI, horseradish peroxidase was injected into NI by ionotophoresis and stained with the benzidine blue reaction after 1,2 or 4 days survival. The somata lie almost exclusively below layer G of Potter, i.e., below the tectal layers containing optic nerve fibers. A majority of the stained cells were in layer 6, while others were scattered through deeper layers. Many cells were colored throughout the extent of their dendrites into the fine rami, giving the appearance of a Golgi stain. Every type of cell previously described for frog tectum was seen, including ganglionic, pyramidal and bipolar varieties. Most common were cells in layer 6, of pyramidal type with a stout, unbranched apical dendrite of constant diameter extending up to layer A, to terminate there in a tight bush.

2015 ANATOMY AND PHYSIOLOGY OF OCULAR DOMINANCE IN THE STRIATE CORTEX OF MONOCULARLY DEPRIVED MACACA NEMESTRINA MONKEYS REARED WITH AND WITHOUT RETINAL LESIONS. Anita Hendrickson, James R. Wilson and John Boles. Dept. Ophthal., Univ. Washington, Seattle, WA 98195.

Ten monkeys were deprived of binocular vision by suturing shut the lids of one eye (EYE 1) prior to the 24th postnatal day. Between 10 & 12 mo of age EYE 1 was opened and EYE 2 closed (reverse suture) in all animals. The Early Lesion (EL,n=2) group had the central 10^{o} of EYE 2 retina ablated when EYE 1 was closed. Late Lesion (LL,n=4) animals had the same retinal lesion but at the time of reverse suture. No Lesion (NL,n=4) animals had only the initial and reverse sutures. At 6-12 mo after reverse suture the distribution and size of ocular dominance columns (ODC) in striate cortex was studied anatomically and electrophysiologically.

Electrophysiology was done in nitrous oxide anesthetized, paralyzed monkeys using standard techniques. Tungsten microelectrodes were passed tangentially to the cortical surface and the ocular dominance and orientation specificity of some single units and all multiunit background activity was determined. In all animals the deprived eye drove some cortical units. NL cortex showed an ODC repeat in layer IV of 350uEYE2/150uEYE1, but above or below IV only a few units and no background was driven by EYE 1. EYE 1 of EL drove the cells encountered in central cortex, but most were abnormal and often were widely separated ; no ODC could be found in IV. In peripheral cortex of EL, EYE 2 was dominant with recordings resembling NL animals.

The anatomy of ODC was studied using either transynaptic autoradiography (TrAr) after H^{3} proline&fucose injection into one eye which labeled dorsal lateral geniculate terminals in layer IV or C^{14}-deoxyglucose (dG) tracing after stimulation of EYE 1 which marks "active" ODC in all striate layers. TrAr in the NL group showed narrow ODC from EYE 1 and wide ODC from EYE 2; the dG activity from NL-EYE 1 was very light but some faint ODC extended from I to VI. The EL group after TrAr from EYE 1 showed clear narrowed ODC in peripheral cortex while central cortex had continuous label in IV; dG label from NL-EYE 1 had faint ODC in peripheral cortex but well labeled ODC from I to VI (1000u repeat) with a continuous label in IVC in central cortex. The LL-dG animal showed good labeling of ODC driven from EYE 1 in both central and peripheral cortex with central slightly more active than peripheral cortex.

We conclude that removing the EYE 2 central retina either during or after the critical period restores some functional capacity to central striate cortex as judged by both electrophysiology and dG marking. This effect seems to be due to changes above and below layer IV rather than within IV itself. Supported by EY01208, EY07013, RR00166 and RCDA EY39039 to A.H.

2016 MULTIPLICATIVE INTERACTION OF AFFERENT INFORMATION AT THE RETINO-GENICULATE SYNAPSE. R. Hess and B.B. Lee. Dept. of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, P.O. Box 968, D-3400 Göttingen, GFR

Responses of retinal ganglion cells and of neurones in the lateral geniculate nucleus (LGN) of the cat to the velocity of moving visual stimuli were recorded with microelectrodes. The relationship between average response frequency and stimulus velocity were adequately described by power functions, and the slopes of the regression lines were extracted as characterizing parameter.

In the retina, the slopes varied between 0.2 and 0.55, with maxima for X-cells of 0.25-0.3, and for Y-cells of 0.35-0.4. In the LGN, the variability was larger ranging from 0.15 to 1.0. The maximum of the distribution was between 0.45 and 0.5. The difference of the slope distributions was significant at the 0.01 level. This average shift of the slopes indicates multiplicative interaction of inputs at the geniculate level.

The slopes found most frequently in X- and Y-cells in the retina occur relatively frequently in the distribution of slopes for LGN-neurones, and there is a conspicuously frequent occurence in the LGN of twice the slope of retinal Y-cells. This may indicate that in these cases there is a direct 1:1 transmission or a 2:1 excitatory convergence with multiplicative interaction. For the majority of cases, however, it seems more likely that the multiplication of the input functions is achieved through the lateral inhibitory network of the LGN. Further experiments to clarify the nature of this multiplicative interaction are in progress.

2017 LAMINAR ORIGINS OF TWO DESCENDING PATHWAYS FROM THE SUPERIOR COLLICULUS IN THE GREY SQUIRREL (Sciurus carolinensis). Virginia Holcombe* and William C. Hall. Departments of Anatomy and Psychology, Duke University, Durham, N.C. 27710.

While the superior colliculus is known to project to a variety of structures in the lower brainstem, the laminar distribution of the cells which project to these structures is not known. The goal of our experiments is to determine the distribution of these cells by labeling them with retrogradely transported horseradish peroxidase (HRP).

HRP (30% in saline) was injected electrophoretically into two major pathways from the superior colliculus: the predorsal bundle and the ipsilateral tectobulbar pathway. After survival times ranging from 24 to 48 hours, the brains were fixed by aldehyde perfusion, frozen-sectioned at 48μm, and processed according to modified LaVail and LaVail (DAB) and/or Mesulam (BDHC) protocols.

Following midline injections of HRP into the predorsal bundle as it crosses in the dorsal tegmental decussation, cells containing reaction product were found bilaterally in the superior colliculus. The vast majority of labeled cells (95%) were in stratum griseum intermediale while only a few scattered labeled cells (3%) were present in stratum griseum profundum.

Very small unilateral injections restricted to the target of the predorsal bundle in the medial reticular formation of the mid-pons resulted in labeled cells located predominantly (85-100%) in the contralateral superior colliculus. Once again, most of these cells (83-88%) were located in stratum griseum intermediale.

In contrast, an injection into the lateral reticular formation of the mid-pons resulted in labeled cells located predominantly (82%) in the ipsilateral superior colliculus. Most of these cells (86%) were located in stratum griseum profundum while only a few cells (3%) were present in stratum griseum intermediale.

We conclude from these experiments that the majority of axons in the predorsal bundle arise from cells located in stratum griseum intermediale of the contralateral superior colliculus, whereas the majority of axons in the ipsilateral tectobulbar pathway arise in the stratum griseum profundum. (Supported by NIH grant #NS-09623 and NIMH RSDA #MH-25734).

2018 DISTRIBUTION OF INPUTS FROM THE TWO EYES TO STRIATE CORTEX OF SQUIRREL MONKEYS D.H. Hubel and T.N. Wiesel. Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115

In the macaque monkey the striate cortex is subdivided into well defined ocular dominance slabs, and input to layer IV c is correspondingly segregated into alternating left-eye and right-eye stripes. A similar segregation has recently been reported in the spider monkey (Florence and Casagrande, ARVO Abstracts, Inv. Ophthal. & Vis. Sci. suppl. April 1978, p. 291). In the squirrel monkey, following an injection of tritiated amino acid into one eye, the transneuronally transported label as seen in autoradiographs is distributed not in discrete stripes along IV c but uniformly (Hubel, Wiesel and LeVay 1976 Cold Spring Harbor Symp. XL, pp. 581-589); this has since been confirmed in several other laboratories. On searching carefully, however, one can see some suggestion of mild periodic variations in density of label, especially in the upper part of layer IV c (IV c α). Opposite the more densely labelled regions we found faint aggregations of label situated above the upper tier of label in IV a, occupying much of layer III.

In the squirrel monkey the lateral geniculate layers are less clearly demarcated than in old-world monkeys. There is thus a greater risk that label may diffuse from the layers corresponding to the injected eye to the other layers; this could tend to produce an apparent blurring of any segregation of left-eye and right-eye regions in the cortex. To check the autoradiographic findings we recorded responses in area 17 of several squirrel monkeys, in oblique and tangential microelectrode penetrations. Compared with macaque monkeys, squirrel monkeys indeed showed much less segregation of inputs from the two eyes. The upper layers had fewer monocular cells and more cells that could be driven about equally from the two eyes (dominance groups 3-5). As in the macaque, cells of layer IV c of squirrel monkey were difficult to resolve and there was no obvious orientation selectivity. In the macaque, multiunit activity at any point along IV c is almost entirely influenced from one eye, with abrupt alternations from one eye to the other roughly every 400 μm. In the squirrel monkey there was input from both eyes along the entire length of IV c that we explored except for occasional small patches with monocular input. Nevertheless both in IV c and in the upper layers there was a periodic fluctuation in dominance from one eye to the other at intervals of about 250 μm. The squirrel monkey thus seems to have ocular dominance columns, though they are probably narrower and certainly less well defined than those of the adult macaque.

Supported by grants from NIH EY00605 and EY00606, and from the Rowland Foundation, Inc.

2019 PATTERNS OF RETINAL TERMINATIONS AND LAMINAR ORGANIZATION IN THE LATERAL GENICULATE NUCLEUS OF PRIMATES. M.F. Huerta, J.H. Kaas, J.T. Weber and J.K. Harting. Dept. Anat., Univ. of Wisconsin, Madison, WI 53706 and Dept. Psychology, Vanderbilt Univ., Nashville, TN 37240.

Autoradiographic tracing procedures have been used to study the organization of retinogeniculate axons in seven primates, i.e. four species of New World monkeys, one species of Old World monkeys and two species of prosimians. These data suggest that the basic primate pattern of geniculate lamination consists of two parvocellular layers, two magnocellular layers, and two poorly developed, and highly variable, ventrally located superficial (S) layers. Ocular input to each member of the three pairs differs. In the macaque, the squirrel, and the saki monkey, the parvocellular layers subdivide and interdigitate into four leaflets so as to give the appearance of four parvocellular "layers". These leaflets are much less extensive in owl monkeys and marmosets. In some individual macaque monkeys there is further splitting of the parvocellular leaflets into subleaflets, giving the appearance of six parvocellular "layers" within this portion of the nucleus. The prosimians, galago and slow loris, have two additional layers that are not found in pithecoid primates, and only one superficial S layer is apparent. The additional layers are termed "koniocellular" since they consist of very small cells. Finally, New and Old World monkeys have both ipsilateral and contralateral retinal input to the interlaminar zones.

We conclude that the basic pattern of lateral geniculate organization is six layers, but not the traditional six. Prosimians have evolved two additional layers, the koniocellular layers, and have possibly lost one superficial layer. Both New World and Old World monkeys have elaborated the parvocellular layers by forming leaflets to varying extents. With the possible exception of the single S layer in prosimians, layers form pairs that are similar in cell types, but different in ocular input.

Supported by Grant NS 12377 to J.H. Kaas and EY01277 and BMS76-81882 to J.K. Harting. J.T. Weber is supported by NIMH Fellowship MH05601.

2020 MAPPING THE PRIMATE VISUAL SYSTEM WITH THE [^{14}C] 2-DEOXYGLUCOSE TECHNIQUE. C. D. Jarvis, M. Mishkin, M. Shinohara*, O. Sakurada*, M. Miyaoka* and C. Kennedy. NIMH, Bethesda, Maryland 20014.

Converging evidence from studies of lesion effects, anatomy, and single units indicates that, in the primate, the system for processing visual stimuli extends beyond the striate cortex to include the circumstriate and inferior temporal areas. From the inferior temporal area, in turn, visual information appears to be transmitted to subcortical structures in the temporal lobe. We attempted to map this functional system by means of the 2-deoxyglucose technique, an autoradiographic method for measuring local cerebral glucose utilization (Kennedy et. al., Science 187: 850, 1975). To achieve this, we prepared monkeys with a unilateral optic tract section combined with section of the forebrain commissures, thus visually deafferenting one hemisphere while leaving the other intact. This made it possible to compare values for local glucose utilization in a "seeing" and a "blind" hemisphere within the same animal, and thereby map the visually related areas. The studies were carried out in awake monkeys presented with visual patterns either in a rotating drum or in a discrimination apparatus. In the latter case, the animal performed the discrimination for water reward using the hand opposite the blind hemisphere.

In both situations, reduced glucose utilization in the blind as compared with the seeing hemisphere was seen cortically not only in the geniculostriate system, but throughout the entire expanse of circumstriate and inferior temporal cortex as far forward as the temporal pole. The functionally depressed zone included tissue adjacent to the inferior temporal cortex in the upper bank of the superior temporal sulcus and in the fusiform and perirhinal areas. Subcortically in the temporal lobe, side-to-side differences were seen in lateral and dorsal amygdala, ventral putamen, ventral claustrum, and tail of caudate. Outside this stimulus-processing system and its subcortical projections, asymmetries were also seen in the superior colliculus, inferior and lateral divisions of the pulvinar, and lower bank of intraparietal sulcus and exposed surface of the inferior parietal lobule. Performance on the discrimination task led to an asymmetrical increase of glucose utilization in structures associated with the active hand and to a symmetrical increase in structures associated with the act of drinking: within the visually related areas, however, the effects of the two different testing situations were substantially the same.

The results not only provide striking confirmation of an occipitotemporal stimulus-processing system, but also help to reveal its full extent and to localize its points of contact with the limbic and striatal systems.

2021 VISUAL FIELD DEFECTS AND MORPHOLOGICAL CHANGES RESULTING FROM MONOCULAR DEPRIVATION IN PROSIMIAN PRIMATES. Rhawn Joseph* and V. A. Casagrande (SPON: Oakley Ray). Departments of Psychology and Anatomy, Vanderbilt University, Nashville, TN. 37232

One normal galago senegalensis and 10 galago crassicaudatus, 7 of which were reared with monocular lid suture, served as either anatomical and/or behavioral subjects. We examined the visual field of 8 monocularly deprived and 2 normally reared subjects using a perimetry technique previously described (Sherman, Brain Res. 49: 25, 1973). When tested with the left (non-deprived) eye, all subjects exhibited 135° of vision, i.e., from 90° into the ipsilateral hemifield to 45° into the contralateral hemifield. When tested with the right (deprived) eye alone, the deprived subjects responded initially as if blind. After 1 week deprived subjects began to respond to stimuli presented from 45° - 60° within the ipsilateral monocular segment. Over the next 1 - 2 months, the vision in the deprived eye increased to 30° - 90° and stabilized. Thus, with the exception of the monocular-binocular borders, vision remained restricted to the monocular segment. With both eyes open the deprived and normal subjects responded to stimuli over the entire 180° of the visual field. Surprisingly, it was found that when stimuli were introduced simultaneously into both monocular segments at equal distance from each eye, 75% - 85% of the responses of deprived subjects favored the deprived right eye, whereas the responses of the 2 normal subjects favored both eyes about equally.

After testing was completed, 4 of the deprived behavioral galagos and 3 normally reared animals received an eye injection of ^{3}H proline. After survival periods of 2 days to 2 weeks the animals were sacrificed, their brains cut, and alternate sections through the lateral geniculate nucleus processed for autoradiography. This procedure aided in laminar and monocular segment identification of adjacent nissl-stained sections. One hundred-fifty cells from the binocular region of each LGN (25 cells per layer) and 25 cells from each monocular segment were measured according to previously described criteria (Guillery & Stelzner, J. Comp. Neur. 139: 413, 1970). Consistent with our earlier experiments (Casagrande et al., Soc. Neuroscience, III: 555, 1977) we found that mean cell areas in both the deprived binocular (except laminae 5) and monocular segments were significantly smaller than their non-deprived counterparts ($p<.001$), with the greater net change occurring in the binocular segment. As in cats (Sherman, '70), we conclude that the main effect of monocular deprivation can best be explained by some form of binocular competition.

(Supported by Grants EY 01778 and 1 K07 EY0061 from the National Eye Institute.)

2022 PROJECTION OF THE RETINA TO THE LATERAL GENICULATE NUCLEUS IN THE CAT FOLLOWING NEONATAL ABLATION OF VISUAL CORTEX. R.E. Kalil. Dept. Anat., Univ. Wis., Madison, Wis. 53706.

When visual cortex is damaged in the newborn cat, severe retrograde cell degeneration takes place in the dorsal lateral geniculate nucleus(LGN) within a few days. Despite the cell death which ensues, the basic laminar organization of the LGN is maintained, and many neurons survive in the nucleus. In particular, large isolated neurons can be seen scattered throughout laminae A and A1, and they are especially prominent ventral to A1 in the region of the C laminae.

Retrograde degeneration is not confined to the LGN, but also occurs transsynaptically in the retina. Thus in the ipsilateral temporal and contralateral nasal hemiretinae there is a marked loss of ganglion cells, especially those which are small and medium sized.

To study the projection of the retina to the LGN in cats that had received unilateral visual cortex damage on the day of birth, Fink-Heimer and autoradiographic methods for tracing axonal pathways were employed. Six months to 1 year after visual cortex removal, the cats were enucleated or injected intraocularly with tritium labelled proline. Following survival periods of 7 to 14 days, the animals were perfused with 10% formol-saline and frozen sections through the LGN were stained for degenerating axons or prepared as autoradiographs.

In the silver stained material, axonal debris in the LGN ipsilateral to the early visual cortex lesion is very dense, but does not show the clear organization of the normal retinogeniculate projection. Thus in the contralateral LGN degenerating optic axons tend to run along the lines of projection, but in the degenerate LGN axons from the retina form a feltwork which is largely disorganized, and contains many fibers which cross the lines of projection. In the autoradiographs, the normal retinogeniculate projection is characterized by an even distribution of silver grains in the appropriate lamina. By contrast, the projection to the degenerate LGN is marked by patches of label indicating a nonuniform pattern of innervation.

Taken together these results indicate that ganglion cells in the retina maintain a projection to the degenerate LGN. Although the projection is massive, it lacks the orderly arrangement of the normal retinogeniculate pathway, and suggests that axons from spared retinal ganglion cells may converge on surviving lateral geniculate neurons.

(Supported by N.I.H. grant EYO 1331)

2023 TRANSIENT RESPONSE ENHANCEMENT IN MUDPUPPY RETINA. Chester J. Karwoski and Luis M. Proenza. Dept. of Psychology, Univ. Georgia, Athens, GA 30602

A variety of responses in the retina of normal mudpuppy eyecup preparations show a transient response enhancement effect (TRE): that is, when series of small flashed spots are alternated with series of large flashed spots, the first small spot response following the large spot sequence is greater than the preceding small spot responses. TRE is strong in the proximal negative response and M-wave, the proximal K^{+}-increase, Müller cell responses obtained in the proximal retina, spike responses of OFF and ON/OFF ganglion cells, and intracellular responses of hyperpolarizing on/off neurons. TRE is weak or absent in responses of horizontal and bipolar cells, spike responses of ON ganglion cells, and the b-wave.

Psychophysically, it is known that threshold for detection of a small, centered, flashed spot is lower for up to several seconds after offset of a large adapting field, than after offset of a small adapting field (Teller et al, Vision Res., 11: 1445, 1971). The results presented here suggest that this phenomenon may have a component of retinal origin and that, like Werblin's windmill effect, it may arise from interactions in the proximal retina.

Supported by NIH Grant EY-00973 to LMP.

2024 A BEHAVIORAL ANALYSIS OF THREE VISUAL SYSTEMS IN THE LESSER BUSHBABY (GALAGO SENEGALENSIS). William Keys* (SPON: M. Wilson). Univ. Connecticut, Dept. Psych. U-20, Storrs, CT 06268.

Three regions of the bushbaby's visual cortex receive nonoverlapping projections from three thalamic nuclei which in turn receive different kinds of visual afferents (Glendenning, Hall, Diamond, and Hall, 1975). Earlier evidence has suggested that the geniculostriate, tectopulvinar-middle temporal area (MT) and superior pulvinar-extrastriate systems function in different ways, and resemble higher primates' systems for stimulus-feature analysis, visuospatial localization and visual learning, respectively.

In order to determine the degree to which these anatomically discrete systems are functionally independent, 12 bushbabies were divided equally among one normal control and three operated groups having bilateral cortical lesions in area 17 (S), MT, or areas 18 and 19 (XS). The performance of these groups on three tasks emphasizing different aspects of visual behavior was then measured for 9 months postoperatively. The first task consisted of a series of stripe orientation and size discriminations and provided measures of simple discrimination learning and sensory capacity. The second task required an animal to enter a tunnel and move to a goal box whose position in both horizontal and vertical planes was changed from trial to trial. This provided a measure of visuospatial behavior. The final task consisted of a series of stripe orientation discrimination reversals which tested the ability to learn complex visual tasks. Histological analyses were performed on all brains at the end of behavioral testing.

Group S showed major deficits in complex visual learning, yet was unimpaired in learning a simple visual discrimination and showed no long-lasting deficits in visuospatial localization. Group MT was impaired on all three visual problems, but had the most difficulty with the tasks requiring visuospatial localization and complex learning. Group XS was also impaired in the three tasks but showed less severe deficits in visual learning and sensory capacity than Group MT.

These results suggest that each of the three thalamocortical visual systems of the bushbaby play a major role in the mediation of different aspects of visual behavior. The geniculostriate system may function as a major source of visual information for advanced analyses in extrastriate cortices, while the tectopulvinar-middle temporal and superior pulvinar-extrastriate systems are important in both visual learning and visuospatial analyses.

2025 DEVELOPMENT OF KITTEN EYES AFTER MONOCULAR VISUAL DEPRIVATION: MYOPIC OR NOT? Albert W. Kirby and Harold Weiss*. Ophthalmology Dept., Kresge Eye Institute, Wayne State Univ. School of Medicine, Detroit, Michigan.

The effect of monocular deprivation on the central visual system of young cats and monkeys is well known, however the development of the non-neural elements is reportedly quite different. Wiesel & Raviola (1977) reported that neonatal lid fusion in the rhesus monkey resulted in considerable changes in the development of the sutured eye. Among those was elongation of up to 21% with resulting axial myopia (-13.5 diopters). Gollender et.al. (1976) reported that in the cat the eyes develop normally following lid suture with no axial elongation.

We followed the growth of the eyes on kittens whose eyes were sutured either before or just entering the critical period. A-scan ultra-sonography revealed a difference in axial length between the sutured and normal eye on each animal with the sutured eye ranging from 1.5 - 2.0 mm longer in the adult. Although the axial length of the deprived eye was greater, the eye was not myopic. In fact, the sutured eye was often hyperopic.

While the kitten study was in progress, we obtained a 4 month old rhesus monkey and immediately sutured one eye closed. Since our cat data was apparently differing from earlier reports, we wanted to use the monkey as a control and see if it also differed. Although the lid closure was not complete and the monkey was somewhat advanced in age at the time of suture, the partially deprived eye was 1.5 mm longer than the control in the adult and was in fact myopic (-3.5 diopters). Development of the deprived monkey eye therefore was in agreement with the report of Wiesel & Raviola.

To determine why the sutured eye of the cat was not myopic even though its axial length was greater than the control eye, keratometry was done to determine the refractive power of the cornea. It was found that the sutured eye in the cat had less refractive power in its cornea than the control eye. Our results indicate that the cornea of the sutured eye in the cat compensates for the increase in axial length so the eye does not become myopic. We do not know the mechanism for eye elongation in either the cat or the monkey or why the cornea apparently compensates for axial length differences in the cat.

Wiesel, T.N. & Raviola, E. 1977. Nature 266:66-68.

Gollender, M., Erickson, P., & Thorn, F. 1976. Meeting, Assoc. for Research in Vision and Ophthalmology, Sarasota, Fla. (ARVO)

This work was supported by N.I.H. Grant Number 05384-16.

2026 GOLGI STUDY OF THE TREE SHREW SUPERIOR COLLICULUS. Thomas P. Langer* and Thomas T. Norton, Dept. of Psychol., MIT, Cambridge, MA 02139 and Dept. of Psychol., Duke Univ., Durham, NC 27706.

The marked lamination and comparatively large volume of the tree shrew superior colliculus suggest that its neuronal elements may be well differentiated, strictly organized, and possibly specialized. Therefore, it is of interest to compare its detailed anatomy with that of other, apparently less differentiated, colliculi.

Neuron profiles were collected from a series of tree shrew superior colliculi stained by the Golgi-Rapid or Golgi-Kopsch methods, sectioned either transversely or parasagittally.

The tree shrew superior colliculus has a variety of well differentiated cell types in each of the broad classes of neurons characteristic of the superior colliculus. The cells of the various types appear to be confined to definite laminae.

Based on the Golgi impregnated profiles, the tree shrew superior colliculus is divisible into a superficial and a deep division, with a transitional region in the stratum opticum. The superficial division is characterized by a preponderance of neurons with their dendritic fields elongated or eccentric about the cell body while the neurons of the deep division are predominantly stellate cells.

The superficial division may be partitioned into at least three subdivisions. The superficial subdivision (0-250 μ below the surface) has marginal cells in its zonal portion, horizontal cells throughout and piriform cells along its deep margin. The middle subdivision (250-600 μ) contains predominantly narrow field vertical cells of a number of varieties along with wide field vertical cells in its deep portion. The deep subdivision (600-900 μ) contains a mixture of narrow field vertical cells and stellate cells with occasional wide field vertical cells.

The deep division may be resolved into several subdivisions based upon the cell types seen, but the cells throughout this region are predominantly small and intermediate sized stellate cells with thin, smooth dendrites which branch sparsely. Large spiny, densely arborized neurons and massive stellate cells occur in characteristic patterns in the superficial and deep portions of the deep division.

Comparison of the neurons in the tree shrew superior colliculus with data from other studies indicate that the tree shrew colliculus is probably more differentiated in that it contains a broader spectrum of well defined cell types somewhat more strictly ordered than is generally observed. At the same time its basic organization is the same as occurs in other superior colliculi.

Supported by grants NIH 1 F32 NS05527-01, NINCDS 5 PO1 NS12336-02 and NSF BNS 76-18334.

2027 PHASE RELATIONSHIPS OF RESPONSES TO MOVING SINUSOIDAL GRATINGS OF SIMPLE CELLS IN CAT STRIATE CORTEX B.B.Lee, A.Elepfandt†, and V.Virsu.‡ Max Planck Institute for Biophysical Chemistry, D-3400 Göttingen, FRG

Sinusoidal gratings have often been used for the determination of the modulation transfer function and visual acuity of single cells in the cat visual system but little attention has been paid to phase relationships of responses in relation to receptive field location. We have studied these relationships by placing a photocell near a cell's receptive field and by recording unit responses in relation to photocell readings at various spatial and temporal frequencies and directions of movement. An estimate of the phase relation of the cell's response could then be made independent of photocell position. We have shown elsewhere (Lee, Elepfandt & Virsu, ENA meeting 1978) that retinal ganglion cell and lateral geniculate nucleus (LGN) neurone responses have the same phase relation to moving gratings irrespective of spatial frequency except for some deviations at low spatial frequencies in some LGN neurones. For on- and off-centre cells response peaks were about 40 to 50° ahead of the maximum and minimum luminances in the grating respectively (when latency is taken into account). We report here the application of this technique to simple cells in the striate cortex. Most simple cells show about the same phase relation to moving gratings irrespective of spatial frequency and could be divided into two groups responding 130° out of phase with one another. The one group displayed behaviour consistent with input from on-centre LGN neurones, the other with input from off-centre LGN neurones. This is consistent with the hypothesis that most simple cells receive input predominantly from on- or off-centre LGN neurones.

2028 SPATIAL AND TEMPORAL CONTRAST SENSITIVITY FUNCTIONS OF DORSAL LATERAL GENICULATE CELLS IN CATS RAISED WITH MONOCULAR LID SUTURE. Stephen Lehmkuhle*, Kenneth E. Kratz, Stuart C. Mangel*, and S. Murray Sherman. Dept. Physiol., Sch. Med., UVA, Charlottesville, VA 22901.

After neonate monocular lid-suture, the proportion of functional Y-cells in the cat's dorsal lateral geniculate nucleus appears dramatically reduced from normal. However, no effects of lid-suture have been reported for X-cells. We re-evaluated this by plotting spatial and temporal contrast sensitivity functions (CSFs) of geniculate cells in normal and monocularly deprived cats. Conventional single unit, extracellular recording methods were applied. The eyes were refracted and provided with 3 mm diameter artificial pupils to optimize the optics. CSFs were obtained with counterphased, sine-wave gratings for which contrast, spatial frequency, and temporal frequency (counterphase rate) were independently varied. The stimuli were generated on an oscilloscope and had a mean luminance of 33 cd/m^2. The CSFs plot contrast threshold for evoked neuronal discharge as a function of spatial and/or temporal frequency. Data were taken only from laminae A and A1 representing the central 25° of visual field.

No differences for X-cells were found among normal and non-deprived laminae A and A1; likewise, effects in deprived laminae A and A1 seemed identical. We found a significant reduction in spatial resolution of deprived X-cells (i.e., in deprived laminae). Whereas the highest spatial frequency which evoked responses from normal and nondeprived X-cells (at a contrast of 0.60) had a mean of 2.6 c/deg, the value for deprived X-cells was only 1.4 c/deg ($p<0.001$ on a Mann-Whitney U-test). At lower spatial frequencies, however, the CSFs of deprived X-cells were normal. For instance, at lower spatial frequencies, deprived, normal and nondeprived X-cells displayed equal sensitivity, and they all showed equivalent low frequency attenuation in contrast sensitivity. Although deficits appeared in spatial CSFs, the temporal CSFs of deprived X-cells seemed normal at spatial frequencies for which the cells were maximally sensitive.

As expected, few deprived Y-cells were found, but their CSFs were not obviously abnormal.

These data indicate that both X- and Y-cells are affected in deprived geniculate laminae, but that the effect on X-cells seems much less severe. Whereas few normal Y-cells can be found in deprived laminae, deprived X-cells seem to suffer only a reduction in sensitivity to high spatial frequencies.

(Supported by NIH Grant EY01565 and NSF Grant BNS77-06785.)

2029 BEHAVIORAL DEMONSTRATION OF McCOLLOUGH EFFECT IN THE MONKEY.

William M. Maguire*, Glenn E. Meyer*, and Joan S. Baizer, Division of Neurobiology, Department of Physiology, School of Medicine, SUNY/BUFFALO.

In 1965 McCollough showed that if observers are exposed to red vertical stripes and green horizontal stripes, subsequently viewed white vertical stripes will appear greenish, and white horizontal stripes pinkish. The "McCollough Effect" is thus an aftereffect dependent on both color and orientation of the adapting stimuli.

There has been considerable speculation about the neural basis of this aftereffect. The most popular hypothesis is still that suggested by McCollough, that long term adaptation of cortical cells tuned for both color and orientation gives rise to the phenomenon. Cells tuned for orientation and color have been described in monkey striate cortex (Hubel and Wiesel, 1968). In order to test the hypothesis that changes in cells studied in monkey underlie a perceptual effect studied, until now, only in man, it is important to demonstrate that monkeys, like man, experience a McCollough effect.

We have obtained evidence that the monkey does show a McCollough effect. Monkeys were adapted by requiring them to fixate a spot moving slowly across alternating horizontal and vertical gratings of complementary color. Following adaptation a test grating whose color changed from red to green or green to red was presented. Animals were trained to release a response lever during the interval that the grating was white. After adaptation, there were orientation specific changes in the monkeys' responses as would be predicted if the animals were experiencing a McCollough effect. This work was supported by NIH grants 5 T32 EY 07019-03 and 1 R01 EY 02230-01 and NIH grant 5 SO7 RR 05400-16.

2030 CONTRIBUTIONS OF INDIVIDUAL LATERAL GENICULATE LAMINAE TO SINGLE CELL ACTIVITY IN THE CORTEX OF THE RHESUS MONKEY. Joseph G. Malpeli, Peter H. Schiller* and Stanley J. Schein. Dept. Psychol., M.I.T., Cambridge, MA 02139.

In this study we have made use of a technique of selective inactivation of precisely localized regions of brain tissue by means of microinjections of the local anesthetic lidocaine. Injections were made through a micropipette onto which had been plated a platinum recording surface for monitoring the extent and duration of the block. Injections of 20 nanoliters of 2% lidocaine into the lateral geniculate nucleus (LGN) reversibly inactivate a region approximately 300 microns in diameter for 3 to 8 minutes. By precise retinotopic alignment of an injection site in the LGN with a recording site in the visual cortex it was possible to block the input from a single LGN layer to cortex. Previous work has shown that parvocellular layers (3-6) are comprised mainly of color-opponent cells, while the magnocellular layers (1 and 2) are made up of broad-band cells. We have studied the effects of inactivation in a magnocellular layer (1), a parvocellular layer (4 or 6) and combined inactivations in magnocellular and parvocellular layers (1 and 6) on the visually driven activity of single cells in the parafoveal region of the monkey striate cortex. Control experiments demonstrated that the injections were restricted to the target lamina and did not interrupt retinal or geniculo-cortical fibers for the retinotopically corresponding parts of other laminae. The results are as follows:

1. Simple cells with a single edge subfield (S_1 cells of Schiller et al. J. Neurophysiol., 39:1281-1374, 1976) depend either on magnocellular input alone, or on parvocellular input alone.

2. Some complex cells are driven exclusively by either parvocellular or magnocellular input, while others receive excitatory input from both kinds of layers.

3. The effects of blocking individual parvocellular layers on the responses evoked by light and dark edges support the view that the upper and lower pairs of parvocellular laminae consist mainly of ON- and OFF-center cells, respectively.

Our results do not support the idea that some classes of LGN cells drive simple cells while other classes drive complex cells. We have seen no evidence that magnocellular input contributes selectively to orientation or direction specificity. (Supported by NSF grant #BNS76-82543, NIH grants #5 R01 EY00676 and NS05477-01A1, and NINCDS grant #5 P01 12336-03.)

2031 SPATIAL AND TEMPORAL CONTRAST SENSITIVITY OF X- AND Y-CELLS IN THE LATERAL GENICULATE NUCLEUS OF CATS. Stuart C. Mangel*, Kenneth E. Kratz, Stephen Lehmkuhle*, and S. Murray Sherman. Dept. Physiol., Sch. Med., UVA, Charlottesville, VA 22901.

We plotted the spatial and temporal contrast sensitivity functions (CSFs) for individual X- and Y-cells in the cat lateral geniculate nucleus. Standard single unit, extracellular recording techniques were used. The optics were optimized by refracting the eyes and providing them with 3 mm diameter artificial pupils. CSFs were derived with counterphased, sine-wave gratings for which contrast, spatial frequency, and temporal frequency (counterphase rate) were independently varied. The stimuli were generated on an oscilloscope and had a mean luminance of 33 cd/m^2. The CSFs represent contrast threshold for evoked neuronal discharge as a function of spatial or temporal frequency. Spatial CSFs were taken at a temporal frequency of 2 Hz, and temporal CSFs were taken at the spatial frequencies for which the cells were most sensitive. Data were limited to laminae A and A1 representing the central 25° of visual field.

Three differences between X- and Y-cells were seen in the spatial CSFs. First, at a contrast of 0.60, X-cells tended to respond to slightly higher frequencies than did Y-cells. The highest spatial frequencies to which X-cells responded ranged from 1.0-5.5 c/deg; for Y-cells, from 0.6-4.5 c/deg. Second, X-cells showed decreased sensitivity to low spatial frequencies whereas Y-cells did not. Finally, the receptive field center diameter of X-cells, as determined by hand plotting, was negatively correlated with the highest spatial frequency to which the cells responded. No such correlation was noted for Y-cells. Only one difference between X- and Y-cells was evident from the temporal CSFs. At 0.60 contrast, Y-cells clearly responded to higher temporal frequencies than did X-cells. The highest temporal frequencies to which Y-cells responded ranged from 10-27 Hz; for X-cells, from 3-18 Hz. Also, neither X- nor Y-cells displayed decreased sensitivity at low temporal frequencies. These findings indicate that X-cells, compared to Y-cells, on average are more sensitive to higher spatial frequencies and less sensitive to higher temporal frequencies. However, considerable overlap between the X- and Y-cell populations was seen both for spatial and temporal parameters.

(Supported by NIH Grant EY01565 and NSF Grant BNS77-06785.)

2032 **NEURAL MECHANISM OF ORIENTATION-SPECIFIC ADAPTATION IN PRIMATE VISION.** **R. J. W. Mansfield and John G. Daugman* Psychology Department, Harvard University, Cambridge, Mass. 02138**

A central problem in determining the neural bases of vision is that of characterizing the relevant population of neurons activated by a stimulus. Attempts to relate perceptual processes to neural events have frequently relied upon apparent trigger features of single neurons. The simplified concept of a trigger feature is difficult to reconcile, for example, with the broad pattern of activation evoked by an oriented visual target in Area 17 of the macaque monkey as revealed by 2-deoxyglucose localization (Hubel, Wiesel and Stryker, J. Comp. Neurol., 177, 361-380, 1978) or by single unit recording. On the other hand, by examining the population response profile, we have identified a candidate neural mechanism for the orientation channels defined psychophysically in human vision using pattern-specific adaptation.

In separate experiments but under similar stimulus conditions, both the receptive field characteristics of striate neurons in the macaque monkey and human performance were determined. High contrast grating targets under computer control were superimposed upon otherwise stabilized retinal images to produce adaptation. Our neurophysiological results suggest that (1) the majority of orientation-selective neurons in the supragranular layers (II-IVb) of striate cortex adapt to repeated stimulation; (2) in general the degree of adaptation decreased with increasing angular difference from the optimal orientation for the cell; (3) the decrement in discharge frequency during adaptation followed an exponential time course with a mean time constant on the order of 10 sec.

Our analysis focused on the supragranular layers as they are known to contain the efferent neurons that form the initial segment of the cortical pathway subserving fine pattern discrimination. Reconstruction of the activation pattern of the efferent neurons was accomplished by means of a mathematical model incorporating the distribution of orientation tuning curves convolved with the function describing the decrement induced by adaptation. Orientation channels, defined by the elevation in threshold produced by adaptation for probe stimuli spanning 180° centered about the orientation of the adapting grating, were measured in human observers. Threshold elevation was found to be proportional, to a first approximation, to the calculated decrement in neural activation produced by adaptation.

(Supported in part by NSF grant BNS75-08437)

2033 ROLES OF CENTRAL STRIATE CORTEX IN FORM DISCRIMINATION. Ronald R. Marcotte* and Jeannette P. Ward. Dept. Psych., Memphis State Univ., Memphis, TN 38152.

Bushbabies (Galago senegalensis), trained on a form discrimination task in a two-choice apparatus prior to disruption of the central representations of vision in striate cortex, were found to be protected from postoperative deficit. All animals were found to be deficient on novel form discriminations following partial central striate lesions. Pre- and postoperative testing of these same subjects on discriminations of fine stripe patterns and of small food objects revealed no evidence of deterioration of epicritic visual capacities. It is suggested that preoperative training allows the assignment of behavioral significance to a stimulus such that, during postoperative testing, the remaining parts of the visual field are capable of mediating recognition of the preoperatively learned form item.

Thus, there seem to be two levels of functioning within the central visual pathways: on one level central vision is involved in the sensory processing and extraction of features from visual stimuli; on another level, central vision seems to have an important and a unique role in the assignment of meaning to novel form stimuli. In this study it was shown that stimuli which acquire behavioral significance prior to central field disruption are readily recognized postoperatively while discrimination of novel stimuli which have acquired no behavioral significance prior to central field disruption is impaired.

2034 AUTORADIOGRAPHIC LOCALIZATION OF ACETYLCHOLINE IN THE RABBIT RETINA. Richard H. Masland and John W. Mills*, Massachusetts General Hospital and Harvard Medical School, Boston MA 02114.

We have attempted to locate the sites at which the rabbit retina incorporates extracellular choline into acetylcholine (ACh), and to distinguish them from sites where choline serves primarily as a lipid precursor. All retinas were incubated in vitro for 15 min in a medium containing 0.3 μM 3H-choline. Some were then incubated for a subsequent 1 h under conditions that promote the release of 3H-ACh and 3H-choline (flashing light, 1 mM unlabeled choline). Others were incubated under ACh-protecting conditions (20 mM Mg++, 0.2 mM Ca++, 30 μM eserine, 1 mM unlabeled choline). Half of each retina was analyzed chemically. A small piece was fixed with osmium tetroxide. The remainder was frozen and processed dry for autoradiography. The first group of retinas was found to contain radioactivity almost exclusively in the lipid pathway (phosphorylcholine and phosphatidylcholine, 93%). Radioactivity of the second group was distributed between ACh (28%) and the lipid pathway (67%).

All retinas showed silver grains over the photoreceptors and faint labeling of most ganglion cells. Radioactivity at these sites survived fixation with aqueous osmium tetroxide. The photoreceptor outer segments were progressively more labeled as the duration of incubation increased. Apparently the photoreceptor and ganglion cells are distinguished by a relatively great synthesis of membrane phosphatidylcholine.

The retinas that contained ACh showed dense grains, superimposed on the background described above, in two bands over the inner plexiform layer and over a few somas located at both of its margins. That the radioactivity responsible for these grains represented ACh was confirmed by its loss from freeze-dried sections exposed to water or from tissue initially fixed with aqueous osmium tetroxide. It was further confirmed by direct chemical analysis of the 3H-compounds eluted from sections divided at the level of the outer plexiform layer under visual control: more than 96% of the retina's total 3H-ACh was found in the proximal half-retina.

These results indicate that the inner plexiform layer contains cholinergic synapses, a finding consistent with conclusions reached earlier on the basis of chemical and electrophysiological experiments (J. Neurophysiol. 39:1210-1235, 1976). The inner nuclear layer cells that contain ACh have the size and position of conventional amacrine cells. Those of the ganglion cell layer could be either ganglion cells or displaced amacrines. Assuming that ganglion cells make no retinal synapses, this suggests that the only neurons of the rabbit retina to release ACh are a small group of amacrine cells.

2035 TOWARD A FUNCTIONAL ARCHITECTURE OF THE RETINA. B.A. McGuire*, J.K. Stevens and P. Sterling. (SPON: P. Liebman). Dept. Anat., Sch. of Med., Univ. of Pa., Philadelphia, PA 19104.

At least 12 classes of ganglion cells, 12 classes of amacrines, and 5 classes of bipolars can be distinguished in mammalian retina, according to Cajal, based on differences in size and dendritic morphology. Each cellular layer can be viewed as a mosaic of repeating subunits in which every cell class is represented at least once. In order to visualize such a subunit it would be necessary to accurately classify a substantial number of adjacent cells. As a start, we reconstructed from electron micrographs of 150 serial sections portions of 15 ganglion cells and 30 amacrine cells in a small patch (50 μm x 15 μm) of cat retina located 1-2° from the area centralis. We distinguished 5 classes of ganglion cells by differences in cell volume, dendritic morphology and pattern of synaptic contacts:

(I) Large soma (>45x10^3 μm^3); several thick primary dendrites to inner 1/3 of inner plexiform layer (IPL) (near the ganglion cells); proximal dendrites receive contacts (1 cell).

(II) Medium soma (14-19x10^3 μm^3); one or more dendrites to middle 1/3 of IPL; distal branches receive contacts (2 cells).

(III) Medium soma 14x10^3 μm^3); single stout dendrite to outer 1/3 of IPL (near the amacrine cells); distal branches receive contacts (3 cells).

(IV) Small soma (2-5x10^3 μm^3); single dendrite to middle IPL; distal branches receive contacts (2 cells).

(V) Small soma (1-2x10^3 μm^3); single fine dendrite in inner 1/3 of IPL; proximal dendrite receives contacts (2 cells).

The ganglion cells segregated by soma volume into 3 groups, but the ratios of surface area to volume (a measure related to input resistance) distributed unimodally.

Four classes of amacrines were distinguished in a similar manner and certain classes of both ganglion and amacrine cells were represented more than once. We anticipate that reconstructing a larger area will reveal additional classes and additional sublayers of the IPL. This should clarify the dimensions and full composition of the subunit, as well as the synaptic interconnections within it. (supported by NIH EY00828)

2036 UNITARY W-CELL SYNAPTIC ACTIONS IN THE CAT'S SUPERIOR COLLICULUS: EXTRACELLULAR MANIFESTATIONS. James T. McIlwain. Neuroscience Section, Div. Biol. & Med., Brown University, Providence, RI 02912.

The bulk of the cat's retino-collicular projection crosses in the chiasm and terminates just beneath the stratum zonale in the superficial gray layer. In this region, extracellular microelectrodes recorded .1-1 mV negative waves, 5-8 ms in duration, commonly occurring .3-.5 ms after a small biphasic prepotential. The large negative waves, observed only in a narrow zone just beneath the stratum zonale, were named juxtazonal potentials or JZPs. JZPs and their prepotentials were evoked by light stimulation of the contralateral but not the ipsilateral retina. The underlying projection was highly ordered retinotopically. Stimuli moving faster than 10-15°/s were ineffective but stimuli moving less than 1°/s evoked vigorous responses. Electrical stimulation of the optic tract evoked JZPs and their prepotentials in an all-or-none fashion. Shocks of increasing intensity recruited discrete JZPs of different latencies which summed to form the large negative field potential characteristic of this region of the colliculus. Prepotentials were driven reliably by optic tract stimulation of 100 Hz, whereas such stimulation produced a rapid but reversible decline in JZP amplitude. There was only a weak temporal correlation between JZPs and cellular action potentials recorded concurrently, but optic tract stimulation evoked JZPs at the same time that deeper cells were excited by the direct W-cell pathway to the colliculus. Individual JZPs and their prepotentials also exhibited latencies typical of the direct W-cell projection. Optic tract fibers conducting at W-cell velocities were found to mediate the negative field potential produced by summated JZPs. Extensive chronic ablation of occipital cortex, including areas 17, 18 and 19, produced no detectable effects on JZPs and prepotentials recorded ipsilateral to the ablated cortex.

These results, together with available morphological evidence, support the hypothesis that the prepotential arises when an action potential invades the elaborate terminal spray of a single W-cell axon. Extracellular currents, resulting from the subsequent synchronous activation of multiple excitatory contacts, produce the JZP. (Supported by PHS NS 09997 and EY 02505)

2037 DEVELOPMENT AND DISTRIBUTION OF A GANGLION CELL SUBPOPULATION IN THE CHICK RETINA. Steven C. McLoon and W. Franklin Hughes*. Dept's. Ophthal. and Anat., Rush Medical School, Chicago, Il. 60612

Unilateral destruction of the primordial optic tectum in the chick embryo at three days of incubation results in degeneration of a majority (>60%) of the retinal ganglion cells in the contralateral eye. This degeneration occurs largely between the 11th and 14th days of incubation. Those residual cells in the ganglion cell layer which do not degenerate are the topic of this report. These cells were studied in retinal whole mounts and in material sectioned for light and electron microscopy. At 15 days of incubation they were arranged in lines having a radial orientation. By 17 days they were more dispersed and showed a regular distribution throughout the retina. Measurements of these cells revealed large and amsll-size classes but an absence of the medium-size cells which are also present in the normal retina. The large cells were confined to the more peripheral retina. The small cells, however, showed a regular distribution throughout the retina. The residual cells showed the characteristics of neurons, and it was presumed that they had sustaining connections in non-tectal areas. To test this hypothesis, the eyes contralateral to tectal lesions were injected with 3H-proline at 17 days, and the central projections mapped by autoradiography. These retinas from experimental eyes showed projections to the non-tectal nuclei comparable to those in normal animals, e.g. lateral anterior nucleus, nucleus externus, and the ectomammillary nucleus. These results demonstrate that at least some of the cells which remain in the ganglion cell layer following tectal ablation have sustaining connections in the brain. Several characteristics of these cells will be presented in regard to their production and distribution in the ganglion cell mosaic.

Supported by PHS-EY01477 and the Helen Regenstein Fellowship.

2038 AN INTERESTING SYSTEM OF SMOOTH ENDOPLASMIC RETICULUM (ER) IN ROD CELLS.
Arthur M. Mercurio* and Eric Holtzman. Department of Biological Sciences, Columbia University, N.Y., N.Y. 10027

An extensive system of organized smooth ER is located at the base of the ellipsoid in rod cells of the frog retina. This system consists of sacs and tubules with a moderately electron dense content. In serial sections, it shows direct continuities with rough ER. Often it is located close to the plasma membrane. The sacs and tubules form elaborate stacked arrays oriented, in different cells, either parallel or perpendicular to the long axis of the myoid. Vesicles and tubules, similar in morphology to elements of this system, are found throughout the ellipsoid region. They are particularly prevalent in the filament-rich peripheral zones and at the tip of the inner segment near the basal body. Initial EM autoradiographic experiments utilizing isolated retinas have demonstrated the incorporation of ^{3}H-leucine into this subellipsoid smooth ER. This occurs at about the same time label appears in the Golgi apparatus but well before the outer segment discs are labelled.

This smooth membrane system is separate from and distinct from the Golgi apparatus which is often close by. It does not stain for the Golgi marker enzyme, thiamine pyrophosphatase. It is also distinct from the smooth-surfaced sacs and tubules, probably smooth ER, found in the myoid near the nucleus, in terminals, and in rod axons. This latter membrane can bind Pb^{++} when incubated in the appropriate cytochemical medium, a property it shares with the synaptic vesicles, of which it may be a source. The subellipsoid system differs from the ER of the paraboloid regions, prominent in some cones, in appearance and by the fact that no glycogen granules are associated with it.

Given the location and extent of the subellipsoid system, the relatively large amount of membrane it contains, and the reported abilities of ER to transport proteins and synthesize lipids, we advance the possibility that this smooth ER plays a role in the biogenesis of outer segment membrane. (Electron micrographs of this system have been published in Holtzman et al, Cell Surface Reviews IV, p. 192 and will appear in Holtzman et al, Biomembranes 10.)

Supported by NINCDS Grant 09475 and NIH Training Grant IT32GM0721603.

2039 NEUROTOXIC AMBLYOPIA IN THE MACAQUE. William H. Merigan. Dept. Rad. Biol. & Biophysics., Univ. Rochester Med. Ctr., Rochester, N. Y. 14642.

Two pigtail macaques were exposed to low levels of methylmercuric chloride for extended periods without developing obvious clinical symptoms. Previous studies in our laboratory have shown that the distinctive pattern of cell loss in the calcarine fissure of the visual cortex which results from such exposures is very similar to that found in human poisoning. Of the two monkeys, the one exposed to the higher dose of methylmercury (blood level = 3ppm, 300 days) developed the visual field constriction typical of human methylmercury poisoning. The sensitivity of this monkey to sine wave flicker (temporal MTF) was tested behaviorally at three adapting luminances several months after termination of the exposure. At high luminance, the high frequency limb of the temporal MTF was shifted about 1/2 octave toward lower frequencies. Low luminance sensitivity to flicker was profoundly reduced (over 1/2 log unit) at all rates of flicker. In the minimally poisoned monkey (blood levels = 2ppm 1000 days) the visual field appeared normal and flicker thresholds were identical to those of normal macaques.

This syndrome of visual changes in experimental methylmercury poisoning is consistent with an impairment of the peripheral visual field. The constricted visual field seen in perimetry is one index of peripheral impairment. Loss of peripheral vision should also degrade sensitivity to high luminance-high frequency flicker and low luminance flicker since the periphery is especially sensitive to these stimuli. Since these are the changes we find, it is likely that the residual flicker sensitivity reported here represents the sensitivity of the intact central region of the visual field.

DOE report No. UR-3490-1386, supported by grants ES-01248, and ES-01885.

2040 SYNAPTIC ORGANIZATION OF THE INNER RETINA BASED ON GANGLION CELL PSP ANALYSIS AND PHARMACOLOGICAL STUDIES. Robert F. Miller, Thomas E. Frumkes, Ramon F. Dacheux,* and Malcolm Slaughter.* Dept. Physiol., SUNY at Buffalo, Buffalo, NY 14214.

A PSP analysis of retinal ganglion cells was carried out in the perfused retinal eyecup preparation of rabbit and mudpuppy. Intracellular current injection, conductance measurements and the selective blocking action of GABA-Glycine antagonists (bicuculline, picrotoxin, strychnine) were used to evaluate excitatory, inhibitory, and dysfacilitatory PSP components. Ganglion cells were classified according to the presence of "on," "off," or "on-off" EPSPs. Each of the 3 types were further classified according to the degree and type of inhibition received. Sustained on and sustained off cells show very little evidence of inhibition and resemble in waveform bipolar cell responses. Transient on and transient off cells show inhibitory inputs of 2 kinds. Type I on cells receive a sustained inhibitory input after an initial EPSP: Type I off cells receive a phasic IPSP at light off. Presumably, these responses reflect a strong inhibitory input from sustained type amacrine cells. Some evidence suggests that the sustained inhibition of on cells is GABA mediated, while that for off cells is Glycinergic. Type II on and type II off cells receive inhibition from on-off amacrine cells and show prominent on and off IPSPs. When the IPSPs are fully blocked with bicuculline or strychnine, both on and off EPSPs are observed in both types suggesting that they receive some excitatory input from both bipolar cell types, but under normal conditions, one of the EPSPs is obliterated by the powerful inhibitory action. On-off ganglion cells receive excitation from both bipolar cell types and are strongly inhibited by on-off amacrine cells. These on-off IPSPs are blocked by either bicuculline or strychnine. Very few cells show a selective block of the off IPSP with strychnine, suggesting that a small proportion of on-off cells receive inhibition from separate neurons at on and off.

These data suggest that there are 4 types of amacrine cells: an on sustained type releasing GABA, an off sustained type releasing Glycine, and on-off types releasing either GABA or Glycine. The complexity of a ganglion cell's receptive field is partially dependent on the amount and type of amacrine inhibition they receive. Sustained on and sustained off ganglion cells receive little if any direct inhibition, but other ganglion cell types receive inhibition from amacrine cells according to several somewhat stereotyped patterns.

Supported by NIH Grants EY00844 and EY01802.

2041 ROLE OF VISUAL CORTEX IN VISUAL FEEDBACK FOR POSTURAL CONTROL. Alar Mirka*, Richard E. Talbott and John M. Brookhart. Dept. of Physiology, Sch. of Med., UOHSC, Portland, OR 97201.

The participation of vision in the execution of a predictable postural control task by dogs was quantitatively evaluated before and after bilateral visual cortex lesions. The dogs maintained a trained stance during sinusoidal movement of the supporting platform, either alone or in combination with the striped central and /or peripheral visual scene. The frequency ratio of platform to visual field oscillation was fixed at 2.875. The conditions imposed on vision included: normal sight (N); blindfolding (B); vision restricted to central 70^0 of visual field only (CO); vision restricted to only peripheral visual field $>50^0$ (PO). The behavioral response was described in terms of Fourier coefficients for longitudinal pelvis movement at the forcing frequencies of platform and visual field movements. This permitted differentiation of behavioral response components induced by visual field movement from those induced by platform movement. Most or all of areas 17, 18 and 19 were ablated by subpial suction. At this time, complete data are available from three dogs; three others are under study.

Preoperatively, B increased the dog's body motion during platform oscillation alone. Thus, system performance is altered by the loss of visual input even in this predictable postural task. Postural responses were induced by visual field motion during CO, PO and N. Movement of the platform and peripheral visual scene during N provided conflicting visual input from the oscillated peripheral and stationary central visual scenes. Removal of the input from central visual field (PO) augmented the response induced by peripheral movement as compared to N. Thus, during N, the conflict between peripheral and central visual field inputs reduces peripheral field influences on postural control. These data indicate that postural control processes are subject to influences from both peripheral and central visual fields.

Postoperatively, the response to platform oscillation alone during N and B was unchanged. The differences between responses in the N and B conditions persisted in the absence of the visual cortex. Peripheral visual field movement induced a similar response during either N or PO; and no postural response could be induced by visual field movement during CO. Thus, postural influences from the central visual field are eliminated by ablation of the visual cortex. Conversely, the visual cortex is not essential for the influences on postural control that originate from the peripheral visual field. It follows that peripheral visual field feedback alone can support quantitatively unchanged postural responses to platform oscillation.

(Supported by NIH Grants NS04744 and K04-NS-70021)

2042 INFLUENCE OF THE VISUAL CORTEX UPON RECEPTIVE FIELD ORGANIZATION OF LATERAL GENICULATE NUCLEUS NEURONS, IN RABBITS. S. Molotchnikoff, D. Richard and G. Baron. Dept des Sciences biologiques, Université de Montréal, Montréal, Qué., Canada H3C 3J7.

A previous report (Molotchnikoff and Lachapelle, Exp. Brain Res. 29: 1977) has shown that inactivating the Visual Cortex (V.C.) by an application of 3M KCl., produces suppression or enhancement of ON and/or OFF Lateral Geniculate cells discharges in response to diffuse light. These results led us to investigate receptive fields spatial organization following cortical depression. Experiments were performed on adult nembutal anesthetized and paralyzed rabbits. Eye movements were prevented surgically. The abolition of evoked potentials recorded from the V.C. indicated cortical inactivation. Receptive fields (R.F.) were mapped with spots onto a tangent screen at a distance of 57 cm. For the purpose of this study, cells (N = 41) were divided into two groups: 1) cells with center-surround organization 2) cells which presented an homogenous R.F. In the first group two subclasses were distinguished: ON-center (24%) and OFF-center (33%) neurons.

The most consistent effect of cortical depression, in all classes of cells, was a significant *reduction* of the responsive area in the periphery of the R.F. The outside diameter of the surround annulus dimished up to 50%. Rates of spontaneous activity were unmodified. This surround area from which no response could be elicited was identified as the distal periphery. ON and OFF responses were elicited from the intermediate area which then expanded centripetally in the majority of tested cells. Thus, the center area from which pure center response was evoked also declined in its surface. The excitatory center response remained unchanged or increased by cortical inactivation in all neurons analyzed but two ON-center cells, where the excitation has been replaced by an inhibitory pause.

These results demonstrate that cortex exerts a powerful action upon receptive field spatial organization. It is the surround which is mostly sensitive to corticofugal influences. It is suggested that cortical functions reduce center-surround inhibitory processes within the Lateral Geniculate Nucleus.

Supported by N.R.C.C. A6943 and Université de Montréal

2043 A SPATIAL FOURIER ANALYZER BY ANY OTHER NAME IS AN EDGE DETECTOR. J. Anthony Movshon, Dept. Psychol., New York Univ., New York, NY 10003.

Models of pattern vision which postulate some form of spatial Fourier analysis have recently received considerable attention. The claim has been presented that descriptions of visual neuronal responses in terms of the spatial frequency and orientation of sinusoidal gratings are in some sense more powerful than other plausible characterizations, such as those based on spots, lines or edges. Since any spatially linear analyzing system should in principle be equally well described by any orthogonal set of basis functions, and since frequency analysis methods have no obvious a priori advantages in dealing with spatially nonlinear systems, I have examined the superiority of grating stimuli in experiments on single neurons recorded from the lateral geniculate nucleus and visual cortex of cats.

For LGN X cells and simple cortical cells, whose behavior is to a first approximation linear, quantitatively equivalent descriptions may be obtained using either grating or line stimuli. The results obtained using either kind of stimulus may be directly related to those obtained using the other by Fourier analysis. Moreover, both spatial frequency response and line weighting data can be used to make accurate predictions of neuronal responses to broad bars of rectangular or Gaussian luminance profile and to complex gratings.

For spatially nonlinear neurons, such as cortical complex cells, results from grating and line experiments may not be so easily related. Furthermore, neither set of data may be used alone to make accurate predictions of responses to broad bars and to complex gratings.

In no strong sense, then, may grating stimuli be considered "better" than other sorts of stimuli. Practical considerations do make some kinds of data easier to obtain and analyze when gratings are used, but the fact that these methods provide a convenient level of description does not provide strong evidence about their functional significance in visual processing. It is not possible on the basis of single neuron response data alone to define a unique "trigger feature" for a visual neuron, be it a bar, an edge, a spot -- or a spatial Fourier component.

Supported by NSF (BNS 76-18904) and NIH (EY 2017).

2044 RAPID RECOVERY FROM VISUAL DEPRIVATION IN NEURONS OF CAT PARASTRIATE CORTEX. Michael J. Mustari and Max Cynader. Dalhousie University, Halifax, NS B3H 4J1.

Fourteen kittens were reared from birth until 4 months of age in total darkness, following which, visual responses of area 18 units were examined. Single units were recorded from cats at 2 weeks intervals up to 8 weeks and also at 3-6 months after removal from the dark. A total of 340 single units were studied in these experiments, about one half with computer methods for stimulus presentation and data collection. Particular attention was directed towards orientation and direction selectivity and peak firing rates of neurons. A unit was considered orientation biased (OB) or direction selective (DS) if the ratio between number of spikes in the best orientation or direction to number of spikes in the orthogonal orientation or opposite direction was 0.5 or less. Some OB cells were further classified as orientation selected (OS) if they failed to respond to stimuli oriented perpendicular to the optimal stimulus orientation.

Initially many cells in recently-deprived kittens were visually unresponsive; others proved difficult to drive, habituated rapidly and generally lacked specificity in receptivefield properties. Only 13% (N=120) of single units in these cats were OB and even less (2%) were OS, while 5% were DS. A considerable degree of recovery was observed in cats after 2 weeks out of the dark. Fifty-four percent (N=46) of neurons were OB, 11% OS, and 20% DS. There was no clear indication of different degree of specificity between simple and complex cells. The recovery process seemed to have asymptoted by 8 weeks and in the 3-6 month group 75% (N=84) of neurons were OB, 26% OS and 20% DS. The peak firing rates were intermediate in value between recently-deprived cats and normal cats.

The results of this study demonstrate that neurons in area 18 are very susceptible to dark rearing as are cells in area 17. The effects of dark rearing on single unit physiology are quite profound affecting not only orientation and direction selectivity but peak firing and latency of response as well. All types of cortical cells seem susceptible to dark rearing and recover to essentially the same degree. The rapid recovery of single unit trigger feature specificity with clear changes observable after only two weeks of recovery indicates that marked plasticity is still present in cortical circuitry of cats several months old.

2045 BLINDNESS IN MONKEYS AFTER LESIONS OF NONVISUAL CORTEX: NOT A VISUAL-MOTOR DISCONNECTION EFFECT. Richard K. Nakamura and Mortimer Mishkin. Lab. Neuropsychol., NIMH, Bethesda, MD 20014.

We previously reported that a large cortical lesion in the monkey outside the areas known to be necessary for visual discrimination learning resulted in behavioral blindness (Neurosci. Abs. 3: 571, 1977). The lesion, placed in one hemisphere, completely spared both the visual cortex (striate, prestriate, and inferior temporal) and the limbic cortex (medial temporal, ventral frontal, and cingulate) but included all other cortical areas: the other hemisphere was visually deafferented by optic tract section and forebrain commissurotomy. Histology and evoked potentials in several such preparations indicated that, in the hemisphere with the ablation, the geniculostriate pathway was intact as intended. Nevertheless, these animals behaved as though blind for periods ranging from 25 to over 400 days, showing partial recovery thereafter.

The present study asked whether the blindness was the result of a disconnection of the visual system in one hemisphere from the motor system in the other hemisphere. To test this, three animals were prepared as above but with the forebrain commissures left partly or completely intact. These animals thus had connections preserved between an intact visual system on one side and an intact output system on the other. Yet, they too were blind, in this case for a median period of 76 days. Subsequent division of the forebrain commissures did not result in a second period of blindness. The results demonstrate that the blindness is not a disconnection effect and suggest further that during blindness the visual signal is not processed beyond striate cortex, for otherwise the signal should have been transmitted across the preserved commissural channels (which start at the striate-prestriate border) to the motor system. This analysis implies that the territory ablated normally provides some activating input, direct or indirect, that is necessary for cortical visual processing.

In a further study, the original lesion was subdivided in an effort to localize the area supplying the activating input. It was found that a sensorimotor lesion (Brodmann's areas 1-6) failed to produce blindness in any of three monkeys. The complementary lesion, however, consisting of dorsal prefrontal, inferior parietal, insula, and superior temporal areas, did produce blindness in three monkeys for a median period of 27 days. These localization results provide additional evidence that the blindness is not a visual-motor disconnection effect, and suggest instead that it is a profound form of visual neglect, since parts of the lesion that produced blindness are known to produce neglect. Accordingly, the basis of neglect too could be impaired processing within the cortical visual system due to loss of activating input, though a less complete loss than that producing blindness.

Supported by NIMH fellowship #1F32-MH05273

2046 SELECTIVE UPTAKE OF [^{3}H]-γ-AMINOBUTYRIC ACID (GABA) AND [^{3}H]-GLYCINE BY NEURONS OF THE AMACRINE LAYER OF CAT RETINA. Y. Nakamura, B.A. McGuire* and P. Sterling. Dept. Anat., Sch. of Med., Univ. Pa., Philadelphia, PA 19104.

In reconstructing microcircuitry it is of considerable help to label neurons by means that may relate their morphology to their function. We injected eyes intravitreously with radioactive putative transmitters, GABA or glycine (100 μCi in 10 μl.) and perfused the animals one hour later. Series of 70-200 consecutive sections from regions near the area centralis were prepared as electron microscope autoradiograms. In the GABA experiments 2% of the cells in the amacrine cell layer were heavily labeled (34x background) and proved to be "interplexiform" cells (IPCs) whose reconstructed processes could be followed into both the outer plexiform layer and inner plexiform layer (IPL), where they made conventional synapses. There were about 90 IPCs/mm^2, but they were unevenly distributed, being closely spaced in some areas (20-45 μm apart) and absent in others (eliptical regions averaging 320x160 μm). An additional 20% of the amacrine layer cells were also labeled but less heavily (9x background). These were true amacrines, slightly larger and darker than the IPCs, with fine processes reconstructed through the outer 1/3 of the IPL. That GABA-labeled amacrine processes were also found diffusely through the IPL and particularly in the innermost region (near the ganglion cells) suggests that the GABA amacrines are of a "diffuse" or multistratified variety. In the glycine experiment, 15% of the amacrine layer cells were heavily labeled (16x background) and sent fine processes radially through at least the outer 1/3 of the IPL. An additional 33% of the amacrine layer cells were labeled, but less heavily (6.4x background). These sent one or more stout processes vertically through at least 3/4 the depth of the IPL. Large (2-3 μm), vesicle-filled varicosities arose from both cell bodies and the vertical processes and were also labeled. We conclude that amacrines showing selective uptake of GABA or glycine belong to distinct morphological classes and speculate that, when their synaptic connections are reconstructed, knowledge of their biochemical characteristics will provide clues to their physiological role. (supported by NIH EY00828)

2047 THE ROLE OF MÜLLER CELLS IN THE GENERATION OF THE RETINAL B-WAVE RESPONSE: A SOURCE DENSITY ANALYSIS. Eric A. Newman* (SPON: Daniel Kurtz). Research Lab of Electronics, Massachusetts Institute of Technology, Cambridge, MA. 02139

The origin of the electroretinogram b-wave response was investigated by determining its current source density distribution in the dark adapted eyecup of the frog, Rana pipiens. Local b-wave amplitude was measured as a function of depth within the choroid, retina and vitreous. B-wave current flow was determined by dividing incremental b-wave amplitude by the radial component of incremental tissue resistance. Current source density was calculated from the spatial derivative of the current distribution. These measurements show that the b-wave is generated by a proximal current source which is restricted to a narrow (∿10 μm) region bordering the retinal surface and a diffuse current sink, extending from approximately 10% to 80% retinal depth. The sink of b-wave current has two prominent peaks: near the outer nuclear layer and near the border of the inner plexiform and inner nuclear layers. This source density pattern suggests that the distribution of b-wave currents is determined by Müller cells, which are the only retinal elements extending from the retinal surface into the distal retina.

The two peaks of the b-wave current sink have similar retinal locations as the sources of the distal and proximal light-evoked increases in extracellular K^+ concentration ($[K^+]_o$) recently observed by Dick and Miller (Neuroscience Abstracts, 1977). The currents associated with the two portions of the b-wave sink have time courses similar to those of the two sources of K^+_o as well: the proximal current sink develops more slowly than the distal one. These observations support the model of b-wave generation based on passive Müller cell depolarization driven by increases in $[K^+]_o$. (Supported by NIH grant 5 T01 EY00090 and by a grant from the Bell Telephone Laboratories, Inc.)

2048 DESCRIPTIVE AND QUANTITATIVE EM STUDIES OF THE OPTIC TECTUM OF XENOPUS FOLLOWING ENUCLEATION. J. J. Norden, A.-J. C. Ostberg* and J. A. Freeman. Vanderbilt Univ., Nashville, TN and University College, London, England.

Using quantitative EM, 40,000 synapses have been counted in the superficial layers of control and denervated tecta in Xenopus from 12 hrs.-4 mos. following unilateral enucleation in an attempt to determine if sprouting of nearby fibers, which also innervate the superficial tectum, occurs after deafferentation. By 5 days, the total number of synapses on the denervated side drops to less than 50% of the synapses on the control side. This decrease in the number of synapses is still apparent 4 mos. following enucleation. Few vacated post-synaptic sites are seen at any time following enucleation. Most pre- and post-synaptic profiles are removed by gliosis while still in synaptic contact. Thus, we found no evidence for sprouting in the tectum of Xenopus at any time after deafferentation.

These results are in contrast to a report of collateral sprouting in the superior colliculus of the rat following unilateral enucleation (Lund and Lund, '71). In the latter study, there was a significant change in the number of flattened vesicle containing profiles making asymmetric contacts following enucleation. This indicated that remaining fibers had sprouted to occupy post-synaptic sites vacated by degenerating optic nerve terminals. In order to compare the results of the two studies, an additional 800 synapses in normal and denervated tecta in Xenopus were characterized according to a number of parameters with particular attention paid to vesicle morphology and type of synaptic contact. In Xenopus, however, the change in the number of flattened and spherical vesicle containing profiles and types of synaptic contacts could be accounted for in terms of the loss of retinal afferents alone.

We conclude that sprouting and reoccupation of synaptic sites does not occur after deafferentation in the tectum of Xenopus. And because post-synaptic sites are removed during degeneration, neither do vacated sites remain uninnervated. These findings have important implications for how synaptogenesis must occur during regeneration of the optic nerve in Xenopus. Optic nerve fibers must "induce", de novo, synthesis of post-synaptic densities rather than displace sprouted fibers or innervate vacated sites.

2049 SPATIO-TEMPORAL PROPERTIES OF SIMPLE AND COMPLEX CELLS IN THE CAT VISUAL CORTEX (AREA 17). H.C. Nothdurft* (SPON: K. M. Gottschaldt). Max Planck Institute for biophysical chemistry, Dept. Neurobiology, D 3400 Göttingen-Nikolausberg, West-Germany.

The temporal properties of spatial interaction were measured with pairs of visual stimuli (dots or lines) presented separately or in combination with various onset-intervals.

When stimulated with parallel lines, those complex neurons which were direction sensitive to a moving line responded also better to the corresponding sequence of line onset. The interaction was either facilitatory or suppressive (or both) and could last up to 600 ms. This sequence sensitivity was a uniform property within the receptive field and did not depend on stimulus position.

In contrast, the responses of simple cells did not show strong sequential sensitivity but revealed the spatial properties of excitatory and inhibitory regions in the receptive field. Commonly, the inhibition was more dominant and seemed to be longer lasting than in neurons of the lateral geniculate nucleus.

In relation to orientation specificity there was no clear example of facilitation along the optimal axis when tested with combinations of dots. The time course of suppression in the non-optimal orientation was not uniform though in some complex cells it was quite short.

The different spatial and dynamic properties of simple and complex cells are also revealed in their transfer of various complex pictures.

2050 PROPERTIES OF CORTICAL NEURONES ACROSS THE 17/18 BORDER: VELOCITY CHARACTERISTICS. Guy A. Orban, Henry Kennedy+ and Hugo Maes+. Laboratorium voor Neuro- en Psychofysiologie, K.U.L. Leuven, B-3000 Belgium.

Velocity influence on area 18 neurones with eccentric fields was described by Orban & Callens (EXBRAP 30: 125, 1977). The aim of the present experiments was to extent this study to the whole of area 18 and to examine whether or not the velocity response curves allow to distinguish between area 17 and area 18 neurones and to trace the 17/18 border. Oblique penetrations in a frontal plane (from medial to lateral) were made from area 17 towards area 18 in anesthetized (N_2O/O_2) and paralyzed cats. Neurones were studied both qualitatively on a plotting table and quantitatively with computer (PDP 11/40) controlled stimuli rear projected on a polacoat screen. The hand plotting allowed classification of units according to the criteria of Kato et al. (J. Neurophys., in press) and determination of receptive field (RF) location and of optimal stimulus parameters. The influence of stimulus velocity was investigated quantitatively with the multihistogram method. Velocity response (VR), velocity latency and velocity directionality curves were plotted. The two types of neurones : velocity sensitive (which have their latency changing with velocity) and velocity specific (or velocity tuned, which have a constant latency) are found in central and in peripheral regions of area 18. However the shape of the VR-curves of velocity sensitive neurones changes from a broad band curve to a high pass filter curve with increasing distance from the vertical meridian (VM). The velocity specific units are more numerous in central projections. There is an excellent correlation (within 100 μ) between changes in VR-curves and the retinotopy indicating the 17/18 border. The correlation between the electrophysiologic indication of the 17/18 border and the histology is much poorer. Indeed on Nissl sections the 17/18 border can only be indicated with a precision of the order of 1 mm. Three conclusions can be drawn from our study. (1) Velocity is a much more important parameter for area 18 than area 17. (2) Velocity specific neurones which belong to the movement analysing system (Orban, Perception 6: 501, 1977) are more common in central regions. (3) The current histological techniques are too coarse for an exact localization of the 17/18 border.

2051 STROBE REARING REDUCES SENSITIVITY TO SLOW MOTION. T. Pasternak, W.H. Merigan, J.L. Brown. Center for Visual Science, University of Rochester.

Cats were reared from birth to at least one year in a room illuminated by a short duration strobe flash (40/min). This illumination provided pattern visual stimulation but precluded the perception of motion. Earlier we reported (Pasternak, Merigan and Brown, Society for Neuroscience Annual Meeting, Anaheim, 1977) that cats reared in such conditions have greatly reduced spatial contrast sensitivity, especially to high spatial frequencies, and show a reduction in high frequency cutoff of over two octaves.

We report here data on the sensitivity of strobe reared animals to motion. Low velocity thresholds were measured using moving random dot patterns. In a two-alternative forced-choice procedure the cats were presented with moving vs stationary patterns. A correct nose pressing response toward the moving stimulus was rewarded with pureed beef. A modified method of constant stimuli was used and a threshold measured in each session.

All three strobe reared cats tested could discriminate moving from stationary dot patterns. However, the low velocity thresholds of these cats were greatly elevated compared to normal cats. While thresholds for normal animals were in the range of 1.1-2.7 deg/sec, thresholds for strobe reared cats were between 4.6-12.2 deg/sec.

The loss of sensitivity to low velocities of movement and the earlier finding of extreme loss of sensitivity to high spatial frequencies suggest that the sustained system is primarily affected by strobe rearing.

2052 VISUAL-MOTOR PROPERTIES OF UNITS IN THE SUPERIOR COLLICULUS OF THE ALERT CAT. Carol K. Peck and Madeleine Schlag-Rey, Dept. of Psych., Pomona College, Claremont, CA 91711, and Dept. of Anat., UCLA, Los Angeles, CA 90024.

Although the visual properties of cells in the superficial layers of the cat's superior colliculus (SC) have been studied in many laboratories, there is much less information on the intermediate and deep layers of the SC. In the monkey, these layers contain cells related to eye movements (EMs). We have studied the relationships between visual and eye movement properties of cells in the SC of the cat.

Single unit activity was recorded in both the SC of alert cats, with the head fixed during recording sessions. The cats had been trained to make voluntary saccades in anticipation of visual targets but were not trained to fixate the test stimulus (Schlag-Rey & Schlag, J. Neurophysiol., 1977). Receptive fields were mapped by computing the difference between the position of a visual target and the position of the eyes on trials when the eyes were stationary. In addition, presaccadic activity could be clearly separated from visually-evoked responses since the cats would often fixate a target with considerable delay.

Although movement was often the most effective stimulus for these units, all of them responded to stationary stimuli and many showed maintained activity, in contrast to previous reports in the paralyzed cat. Most units had some directional preference and some were very sensitive to changes in the direction of motion (e.g., the number of spikes in bursts decreased to 50% of maximum as the direction changed by 30^{o}-40^{o}).

One type of unit, frequently encountered, responded phasically to the onset of a visual stimulus and also discharged prior to a targeting EM, even if that EM occurred much later or after the stimulus was turned off, yet the same units would not discharge before EMs in complete darkness. Among tonically active units, two types could be contrasted. One type discharged from the onset of a stimulus until the cat made a targeting EM in the direction of the stimulus, while the other type became tonically active only if the cat fixated the stimulus. The absolute location of the stimulus (i.e., with respect to the head-body axis) was important for some units. This was observed both in units with phasic "on" responses to a stimulus distant from the point of fixation and also in units which responded during fixation.

The properties described above are consistent with the hypothesis that the SC is involved in coding appropriate EMs to visual stimuli.

Supported by NS-04955.

2053 REGIONAL SPECIALIZATIONS IN THE OPTIC TECTUM OF AN IGUANID LIZARD, DIPSOSAURUS DORSALIS. Ellengene H. Peterson, Department of Anatomy, University of Chicago, Chicago, Illinois.

In Dipsosaurus dorsalis, the optic tectum consists of: 1) the superficial strata, composed of seven alternating cell and fiber layers; 2) the middle strata; and 3) the deep strata, consisting of three monolayers of somata alternating with cell free zones. In addition to this pattern of lamination, the tectum in Dipsosaurus exhibits a series of regional specializations. Specifically, the tectum can be divided into at least three morphologically distinct regions on the basis of normal cytoarchitecture and the pattern of ganglion cell afferents as visualized in silver and autoradiographic material. These three areas are arranged as strips oriented approximately parallel to the rostrocaudal axis of the tectum. Seen in cross section they are: the mid-dorsal tectum, the lateral tectum, and the ventral rim. 1) In the mid-dorsal tectum, the laminae are relatively thick in the rostral half tectum. This expansion is most pronounced in the middle strata and reaches its maximum extent approximately midway along the rostrocaudal axis. In silver preparations, the mid-dorsal tectum contains a discrete patch of degeneration at the shortest survival times (5 days). In autoradiographic material, grains are concentrated over the three most superficial fiber layers and, at the shortest survival times, grain density is relatively high over the mid-dorsal expansion. 2) In the lateral tectum, the pattern of degeneration is different from that seen dorsally. At 5 and 10 days survival, little degeneration is evident, but at 15 days, three distinct bands of silver particles can be seen. At 21 days, degeneration in lateral and dorsal tectum are equally dense. In autoradiographic material, the laminar pattern of grain densities is similar to that seen dorsally but, at short survival times, it is less dense. 3) The ventral rim of the tectum can be distinguished in silver material by the presence, at all survival times, of two bands of large caliber fibers which enter the tectal layers parallel to the surface and run approximately 1/3 the way up the lateral wall of the tectum. They are accompanied by coarse argyrophilic debris, particularly over the deeper fiber layer. The three dense bands of silver particles seen in the lateral tectum do not extend into this ventral rim. In autoradiographic material, relatively few grains are seen in this region at the shortest survival times.

The optic tectum of Dipsosaurus thus exhibits a number of regional specializations. If the retina projects topographically onto the tectal surface, then these subdivisions of the tectum may reflect regional specializations in the ganglion cell layer of the retina. (Supported by A.P. Sloan Postdoctoral Fellowship and PHS Grant NS 12518)

2054 RETINAL MECHANISMS OF LIGHT ADAPTATION IN THE ALBINO RAT. Maureen K. Powers and Daniel G. Green. Vision Research Laboratory, University of Michigan, Ann Arbor, Michigan 48109.

In the isolated retina of the skate, sensitivity to light is regulated by two separate mechanisms, one located in the distal retina and the other in the proximal retina[1]. Light adaptation affects the two mechanisms differently. For example, dim background lights may depress sensitivity of proximal elements (e. g., ganglion cells) without influencing sensitivity of distal elements (e. g., receptors) at all. We have examined the relations between proximal and distal retina during light adaptation in the intact retina of the albino rat.

Using conventional techniques and diffuse illumination, we recorded the ERG b-wave, the isolated receptor potential (obtained by injecting aspartate into the vitreous) and single ganglion cell fibers in the optic tract. We measured two aspects of these responses: threshold (defined as 1 extra spike per stimulus presentation for ganglion cells, and as a 50 μV change of potential for the receptor potential and the b-wave) and the intensity-response function (number of spikes above nonstimulated levels elicited from ganglion cells, and μV change above noise for the receptor potential and the b-wave).

With no background present, the intensity needed to evoke a threshold response was about 100 times greater for the receptor potential than for either the b-wave or the ganglion cells. However, the intensity-response functions of all three measures were similar in shape when response was expressed as percent of maximum. Backgrounds that decreased b-wave threshold by 1 or 2 log units had the same or greater effect on ganglion cell threshold. The same background lights decreased the maximum amplitude of the b-wave's intensity-response function, but not of the ganglion cells'. The presence of background lights that increased ganglion cell and b-wave thresholds more than 50-fold had no effect on receptor potential threshold or intensity-response function.

We have shown that the receptor potential, b-wave and ganglion cells in the rat respond similarly in the absence of background lights, but differently in their presence. Our results are in good agreement with those of Green et al.[1] and with earlier work by Dowling[2] in the rat. We conclude that retinal mechanisms of light adaptation in the rat resemble those in the skate, and we suggest that they may be similar in other vertebrates as well. (Supported by EY00379 to D. G. G.)

[1]Green, Dowling, Siegel and Ripps (1975) J. Gen. Physiol. 65:483.
[2]Dowling (1967) Science 155:273.

2055 INVOLVEMENT OF SECONDARY COMMISSURES IN INTERHEMISPHERIC TRANSFER M. Ptito, G. Page, F. Lepore and M.C. Lassonde. Lab. de Neuropsychologie, Univ. du Quebec, Trois-Rivieres and Univ. de Montreal, Montreal, Canada.

Brain lesion studies have shown that interhemispheric transfer is present in animals bearing a transection of the corpus callosum and the optic chiasm. Such transfer is possible only if one postulates that visual information is conveyed across the midline via the secondary of subcortical commissures. The present experiment was undertaken to evaluate this hypothesis. Adult cats underwent a section of the left optic tract (LOT) and a destruction of the cortical visual areas (VA) in the contralateral hemisphere (17, 18, 19 and suprasylvian area). The intact directly deafferented hemisphere is thus only innervated via indirect subcortical commissures, such as the intratectal. After the LOT lesions, the animals learned a series of visual pattern discriminations in a Thompson box. The animals easily learned with either or both eyes the different pattern discriminations in a time comparable to controls or split-chiasm subjects. After undergoing the cortical resection (VA), they were retested in the previously learned tasks and taught new ones. Results showed that all subjects were able to achieve criterion on the previously learned discriminations. However, they were very slow at the beginning of the relearning period and required approximately twice as many trials to relearn the discrimination as after the LOT lesions. Initial performance on the new pattern discrimination was similar to that at the end of the relearning phase. However to achieve criterion the animals required about four to five times as many trials as after the LOT lesions. An additional lesion was then made at the level of the intratectal commissure (IC) to evaluate the importance of this commissure in supporting the residual vision demonstrated in the cats. The animals were tested on both the previous and on the new discrimination tasks. Preliminary results seem to indicate that IC cats have difficulty performing the previously learned discrimination problems. Data on new learning is still too sketchy to be evaluated. However, based on their ability to learn pattern discriminations following VA lesions and on the partially demonstrated relearning difficulties after IC sections, it may be tentatively concluded that the intratectal commissure is involved in the interhemispheric transfer of visual information.

2056 STRONG AND WEAK TRIADIC INPUT TO RELAY CELLS OF THE LATERAL GENICULATE. Salvatore C. Rapisardi* (Spon: J. H. LaVail) Dept. of Anatomy, Howard University, Washington, D. C. 20059

Nineteen terminal boutons of retinal fibers in the dorsal lateral geniculate of the cat have been examined throughout their extent in long series of uninterrupted, consecutive thin sections. The profiles postsynaptic to the retinal terminal (RT) fall into two categories. Both types show an electron lucent cytoplasm, but one (F profiles) contains loosely packed pleomorphic vesicles and no ribosomes. The second type (D profile) exhibits numerous ribosomal rosettes, but no vesicles. Triadic arrangements occur frequently. In such cases an F profile postsynaptic to an RT is presynaptic to a D profile which is postsynaptic to the same RT.

The RTs fall into two groups according to the extent to which they are involved in triads. In one group (9 RTs) over 95% of the total synaptic contacts made by the retinal afferent took part in triads. In the second group (10 RTs) less than 50% of the synapses made by the retinal afferent took part in triads. There are also differences between the triads found in each group. There is a great variability in the number of RT and F synapses received by any particular D element. Individual D processes which are part of triads in the high triad group of RTs receive about equal numbers of RT and F synapses, but D processes from triads in the low triad group receive about five times as many RT synapses relative to their F input. This suggests that a triad in the low triad group of RTs is less effective than a triad in the high triad group.

Reconstructions of D profiles postsynaptic to the RTs with high triadic involvement have grape-like appendages similar in appearance to those found on the dendrites of Guillery Class II cells. Models of D processes postsynaptic to RTs of low triadic involvement are relatively smooth, but show small appendages and resemble the dendrites of Guillery Class I cells.

The hypothesis that the function of the triad is a feed-forward inhibition upon relay cells is supported by Sterling and Davis'* finding that the F profiles accumulate DABA. Since the physiologically defined X and Y cells have been associated with Class II and Class I cells, respectively (Levay and Ferster, JCN, 172, 77; Kalil and Worden, JCN, 178, 78), the heavy triadic involvement of Class II cells suggested here is consistent with the Stevens and Gerstein* report of a retinally generated feed-forward inhibition (following an initial excitation) that is almost exclusively associated with X cells. *See abstracts this volume (supported by Grant #NS-11614)

2057 CELLS OF ORIGIN OF DESCENDING TECTOFUGAL PATHWAYS IN THE BIRD. A. Reiner and H.J. Karten. Dept. Psychiat. and Behav. Sci., S.U.N.Y. at Stony Brook, N.Y., 11794.

In birds, the tectum gives rise to two major descending systems, the ipsilateral tectopontine-tectoreticular pathway (ITP) and the crossed tectobulbar pathway (CTB). The ITP terminates within the lateral reticular formation (FRL) of the brainstem and within the lateral pontine nucleus (PL). The CTB decussates ventral to the oculomotor complex and terminates throughout the paramedian brainstem. These two major descending tectofugal pathways have been described in all vertebrates studied. The horseradish peroxidase technique was used in the present study to determine the laminar distribution of the cells of origin of these descending tectal systems in the pigeon.

Unilateral injections into the terminal field of the CTB at a variety of caudal pontine and rostral medullary sites resulted in labeled cells exclusivily in layer 13 of the contralateral ventral tectum. Unilateral injections into rostral pontine portions of the CTB yielded labeled cells in layers 13-15 of the contralateral dorsal tectum, as well as in layer 13 of the contralateral ventral tectum. These results suggest that rostrally terminating portions of the CTB arise from the deepest lamina of the dorsal tectum, while the more caudally terminating portions of the CTB arise from layer 13 of the ventral tectum. Unilateral injections into the ITP that included both PL and overlying FRL resulted in labeled cells in the ipsilateral tectum in layers 8 and 10-15. Injections confined to PL resulted in labeled cells in these same seven layers. All injections, both paramedian and lateral, resulted in labeled cells at two subventricular sites within the optic lobe, nucleus intercollicularis (ICo) and FRL. Label in ICO was predominantly ipsilateral to the injections, while label in FRL was predominantly ipsilateral to ITP injections but bilateral to CTB injections. These results suggest that both tectal and tegmental cell groups give rise to projections to paramedian and lateral zones of the brainstem.

The tectofugal projection upon nucleus rotundus of the diencephalon arises from layer 13. The present results indicate that lower brainstem regions may receive input similar to that received by nucleus rotundus since they also receive layer 13 input. Of particular interest is the observation that PL and possibly FRL are in receipt of input from tectal cells in layers 8 and 10. Cells of these layers characteristically extend their dendrites into tectal retino-recipient layers and have small visual receptive fields (1-10 degrees).

Supported by USPHS grant 1 F32 NS 05682-01 to A.R.

2058 SOME OBSERVATIONS ON THE ORGANIZATION OF THE CAUDAL POLE OF THE THALAMUS (LATERAL PULVINAR OR PLγ) IN THE MACAQUE MONKEY. Michael Rezak. Department of Anatomy, University of Illinois Medical Center, Chicago, Illinois 60612.

The organization of the caudal pole of the thalamus is poorly understood. Cytoarchitecturally it is comprised of extensions of the medial pulvinar and the lateral pulvinar. The lateral pulvinar has been shown to have three subdivisions and it is its most caudal subdivision or PLγ which forms the lateral part of the caudal pole of the thalamus. The connectional organization of this region was studied using retrograde (HRP) and anterograde (autoradiographic) tracing methods. Microinjections of tritiated amino acids into prestriate cortex (areas 18 and 19) revealed a highly convergent input to PLγ. Interestingly, discrete microinjections (0.1 µl) of tritiated amino acids in PLγ revealed no reciprocal projections to prestriate cortex. Instead PLγ was found to project to the temporal lobe. More specifically, PLγ has as its cortical target layers III, IV and I of inferotemporal cortex (areas 20 and 21). This projection to cortex follows a loose topographic organization such that ventral regions of PLγ project to posterior portions of inferotemporal cortex while dorsal PLγ projects to more anterior and ventral regions of inferotemporal cortex. No evidence for visuotopically organized connections of PLγ was found. It should be noted that recent physiological studies also have shown that inferotemporal cortex does not contain a detailed visuotopic map. The lack of reciprocity between PLγ and prestriate cortex was further underscored in our HRP studies where no peroxidase positive cells were found in PLγ after HRP injections in prestriate. Peroxidase positive cells were found in PLγ after HRP injections in areas 20 and 21. Thus, PLγ plays a heavy role in associating occipital cortex with inferotemporal cortex and thereby establishes a cortico-thalamocortical route which must operate in parallel with the well known cortico-cortical routes in the progressive relay of visual information.

(Supported by University of Illinois Campus Research Board and Biomedical Research Support Grants)

2059 RETINAL INPUT TO THE HAMSTER'S SUPERIOR COLLICULUS: CONDUCTION VELOCITY DISTRIBUTION AND CORRELATION WITH RECEPTIVE FIELD PROPERTIES. Robert W. Rhoades and Leo M. Chalupa. Dept. of Psych. University of California, Davis, CA 95616.

In view of the current interest in the tripartate division of ganglion cell types in the mammalian retina and the role of these neurons in the organization of the receptive fields of cells in central visual structures, we sought, in the present study, to (1) determine the conduction velocity (CV) distribution of the retinal input to the hamster's superior colliculus (SC), and (2) to correlate afferent conduction velocities with the response properties of tectal cells. The experiments were accomplished by employing standard extracellular recording techniques in the SC and electrical shocks delivered to the contralateral optic nerve (ON) and optic chiasm (OX). In an additional series of animals we also attempted to delineate the nature of the retinal input to the ipsilateral SC. Here the ON electrode was positioned behind the eyeball ipsilateral to the SC from which we recorded.

Cells driven reliably by shocks delivered to the ON or OX were encountered throughout the depth of the tectum. However, as might be expected from the anatomical distribution of the retinal input to the SC (Chalupa and Rhoades, in preparation), the incidence of driven cells decreased markedly in the tectal laminae ventral to the stratum opticum. The distribution of CV's for the retinal afferents to the hamster's colliculus was quite broad (ranging from 1.7 to 25.5 m/sec) and clearly bimodal with a broad peak centered at 6 m/sec and another narrower peak at 12 m/sec. A few tectal cells were innervated by axons having conduction velocities in excess of 20 m/sec. These data, when combined with the distribution of ganglion cell sizes in the hamster's retina (Tiao and Blakemore, 1976, JCN, 168, 439-458), suggest that all retinal ganglion cell types in this species project to the colliculus.

Afferent conduction velocity was clearly correlated with directional selectivity. Ninety percent of the tectal neurons receiving inputs from axons having CV's of less than 5 m/sec were directionally selective while only 41% of those neurons having inputs from more rapidly conducting fibres (>5 m/sec) exhibited selectivity.

We tested 116 cells in the anterior portion of the colliculus for responsivity to photic stimulation delivered to the ipsilateral eye and electrical shocks applied to the ipsilateral ON. Of these 11% exhibited some degree of binocularity while only 6% were responsive to ON shocks. These electrophysiological findings correspond well with the limited nature of the retinal input to the ipsilateral SC (Chalupa and Rhoades, in preparation).

2060 VISUAL MASKING BY REMOTE STIMULI IN MONKEY SUPERIOR COLLICULUS NEURONS. Barry J. Richmond and Robert H.Wurtz, NIMH, Bethesda, Md. 20014

In the monkey superior colliculus some cells show a suppression of activity following saccadic eye movements due to a corollary discharge (Richmond and Wurtz, Neuroscience Abstracts, 1977) in contrast to a visual masking effect seen in the striate cortex (Judge and Wurtz, Neuroscience Abstracts, 1977). We have now studied visual responses of cells in the superficial layers of the superior colliculus to see if visual masking is also prominent in this structure.

Single cell responses were recorded in awake rhesus monkeys (Macaca mulatta) while the monkey fixated a spot of light on a tangent screen. All cells with either para-foveal or peripheral visual fields showed masking of 20 msec test spots of light by a preceding stationary "masking" spot (usually by 50 msec) when both stimuli were in the excitatory receptive field center. In addition, when a small spot placed far out of the excitatory visual field was used as a stationary masking stimulus, the response of the cell to the flashed test stimulus in the excitatory receptive field was attenuated, although not usually eliminated. The distant stimulus did not itself modify the low background rate of discharge of the cell. The effect was a visual interaction and did not require a saccade to the target as did the visual enhancement effect reported previously. The masking seems to be similar to previously reported distant interactions in cells of cat superior colliculus (Rizzolatti et al., J. Neurophysiol., 1974).

A visual interaction also modified the response to moving visual stimuli. Many cells in the superior colliculus have only a weak response to rapid stimulus movement such as occur during saccadic eye movements (900°/sec for the rhesus monkey). However, if the sweep was arranged to cross only the excitatory receptive field of the cell, the response to the sweep increased markedly. We have not determined whether the effect on the sweep response is due simply to the adjacent surround or to a more remote area such as described above.

Both the effects on stationary and on moving stimuli by stimuli outside the excitatory central receptive field area are visual interactions resulting from extended surround effects. These masking effects are likely to act in addition to the suppression resulting from a corollary discharge to reduce the response of a colliculus cell during eye movements.

2061 THE EFFECT OF ADAPTING TARGET LOCATION UPON THE GAIN OF THE SURROUND RESPONSE MECHANISM OF X CELLS IN CAT RETINA.
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The spatial distribution of the surround response mechanism (SM) of X cells was assessed with unmodulated adapting targets placed in different regions in the receptive field (RF). The adapting targets were a spot placed in the middle of the RF center and annuli concentric with this spot. The annuli had mean diameters varying from 0.57° to 2.0°; all adapting stimuli had the same flux. Two measures of the gain of the SM were used: the ability of a flashing annulus to reduce the on-response to a flashing spot in the RF center - the on-inhibition measure - and the magnitude of the off-discharge to an annulus flashed alone in the RF periphery - the off-excitation measure. The relationship between adapting target location and the gain of the SM was curvilinear for the on-inhibition measure but linear for the off-excitation measure. The greatest reduction in the gain of the SM was observed for the adapting annulus with a mean diameter of 0.96° when the on-inhibition measure was used and 1.75° when the off-excitation measure was used. The adapting spot (0.28°, 0.5°) was the least effective of the adapting targets in both experiments.

The results of the experiment using the on-inhibition measure support a RF model where the SM is very weak or non-existent in the middle of the RF center in X cells. The results from the experiment using the off-excitation measure do not support this model.

2062 COMPLEX PATTERN DISCRIMINATION IN THE ALBINO RAT: ROLE OF STRIATE CORTEX AND THE IPSILATERAL RETINO-CORTICAL PATHWAY.
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Monocular deprivation in the rat, as in the cat and monkey, produces a variety of morphologic and physiologic abnormalities which alter the development of normal behavior. A chief advantage of the rat as a model system is the fact that the optic nerve fibers, unlike those of the higher species, are almost totally crossed. Thus, the relationship between the neurologic alterations and behavior may be easier to assess. We were curious about the role of the small ipsilateral projection in visual performance; specifically, the ability of this pathway to mediate complex pattern discrimination. Earlier studies investigating this question have produced ambiguous results (Chang, Psychol. Abstr. 3491, 1937; Lashley, Psychol. Rev. 31:369,1924; Muntz and Sutherland, J. Comp. Neur. 122:69,1964).

Adult albino rats (N=10) were first trained to discriminate columns and rows of 5 mm squares in a fully automated discrimination apparatus. On completion of training, unilateral (left hemisphere) lesions were performed on all animals. Lesions were made in striate cortex (area 17 of Krieg) by thermocoagulation. The contralateral (right) eyelid was sutured in 4 of the rats, while in the remaining 6 animals the ipsilateral (left) eye was closed. Following a post-operative recovery period all animals were tested for retention.

Animals relearning the discrimination with the crossed fibers (left eye-right hemisphere) required a mean of 173 trials to regain criterion performance. The mean number of trials for the animals using the uncrossed projections (right eye-right hemisphere) reached 1520. In fact, 4 of the 6 animals using the ipsilateral projections failed to reach criterion within 1800 trials.

These results indicate that the uncrossed projections from retina to striate cortex are grossly deficient in mediating a complex pattern discrimination. Secondly, they show the importance of area 17 for successful performance on this task. Finally, they strengthen the assumption that subtle alterations in the morphology of striate cortex may be related to the decrements in behavior produced by visual deprivation.
(Supported by NIMH Grant MH RO1-27424)

2063 DIFFERENCES BETWEEN NASAL AND TEMPORAL RETINA IN SIAMESE AND NORMALLY PIGMENTED CATS.
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It has been previously reported (Stone, Rowe & Campion, J. Comp. Neurol., in press) that there is commonly a reduction in ganglion cell density in siamese retinas which is particularly apparent in the area centralis and least apparent in the visual streak. This pattern of ganglion cell loss was interpreted as a selective loss of X- type ganglion cells, since it is well established that X-cells are the most frequent cell type in the area centralis, but that W-cells are most frequent in the visual streak (Rowe & Stone, J. Comp. Neurol., 169, 99-126.). These observations are here extended to a comparison between more peripheral regions of nasal and temporal retina. In normally pigmented cats the mean soma diameter of ganglion cells in temporal periphery is consistently larger than in nasal periphery (e.g. 15.6 μm vs 13.7 μm) and this is associated with a reduction, in nasal retina, of the number of ganglion cells in the 14-22 μm range. These cells typically constitute about 61% of the ganglion cell population in temporal periphery, but only about 46% in nasal periphery. In siamese retinas mean soma diameter is also greater in temporal than in nasal periphery (e.g. 14.3 μm vs 13.5 μm), but the difference is consistently less than seen in common cats. The number of cells in the 14-22 μm range is also consistently higher in temporal than in nasal retina in the siamese (40% vs 30%), but the increase is not as marked as that observed between corresponding regions of common cat retinas. Mean soma diameter is also consistently higher in temporal retina of common cats than in temporal retina of siamese (15.6 μm vs 14.3 μm), but such differences were not consistently seen in nasal regions. These data suggest that in normally pigmented cats, X-cells are relatively more frequent in temporal than in nasal retina. The differences between siamese and normally pigmented cats are consistent with a selective loss of X-cells in siamese retinas, since the differences between normally pigmented and siamese retinas are more pronounced in temporal than in nasal retina. Preliminary analysis of the retinas of newborn kittens indicates that these patterns of naso-temporal differences in ganglion cell composition are present at birth in both common and siamese cats.

2064 WHY DOES MONOCULAR PARALYSIS OF ADULTS YIELD X-CELL LOSSES WHILE VISUAL DEPRIVATION OF INFANTS YIELDS Y-CELL LOSSES?
W.L. Salinger, M.G. MacAvoy* and P.E. Garraghty. Dept. Psych., UNC-G, Greensboro, NC 27412.

In the adult cat, both binocular lid suture and monocular paralysis have been shown to reduce the encounter rate for X-cells (cells are characterized solely on the basis of response to optic chiasm shock-OX latency) in the lateral geniculate nucleus (LGN). In contrast, kitten onset eyelid suture produces a pattern of Y-cell losses. These contrasting results raise the possibility that the nature of the effects produced by environmental modification may in part be age dependent rather than simply a function of the type of visual deprivation. To evaluate this possibility the effects of monocular paralysis in infancy were examined.

The left eyes were paralyzed in kittens with no prior visual experience at 24 days of age. Following surgery the animals were reared to maturity in a normally illuminated colony. OX latencies for each of 395 cells encountered in the right LGN of these subjects were measured, and compared with those obtained from subjects with adult onset monocular paralysis.

Infant onset monocular paralysis resulted in a reduced encounter rate for both X- and Y-cells. These losses were evident in both the layer innervated by the mobile "nondeprived" eye (A_1) and in laminae subserved by the paralyzed eye (A and C). In contrast, infant onset lid suture produces no reported effects on the nondeprived laminae.

A parallel between the effects of monocular paralysis of infants and adults is seen in that the A_1 layer, innervated by the mobile eye, suffers a partial X-cell loss in both instances. In monocularly paralyzed kittens, however, the additional loss of Y-cells suggests that here, monocular paralysis has features typical of other infant onset perturbations.

Taken together, these results suggest that the age of onset of environmental modification constitutes an important determinant of the extent to which X- or Y-cells are affected. These data further indicate that the impact of sensory disruption is partially dependent on the nature of that disruption. To the extent that the age of onset of the disruption is itself a critical factor, one should be cautious in attempting to explain the effects of a particular type of environmental modification on the basis of its stimulus properties alone.

2065 TRANSIENT VARIABILITY CHANGES IN CAT RETINAL GANGLION CELLS DURING ADAPTATION TO DIFFUSE ILLUMINATION. Arthur C. Sanderson, Dan Schweitzer-Tong, and Wlodzimierz M. Kozak. Biomedical Engineering Program, Carnegie-Mellon University, Pittsburgh, PA 15213.

The spike discharge of cat retinal ganglion cells was recorded extracellularly from single optic tract axons, and the variability of the interspike intervals was studied during the transient period following a sudden step change in diffuse retinal illumination. Step changes of one log unit in the range 10^{-4} to 10^{+1} cd/m^2 with a fully dilated pupil (equivalent to a retinal luminance of about .026 to 2600 scotopic trolands) were utilized. 30 sec to 5 min adaptation time was allowed between steps.

Variability of the maintained discharge was described using the index of the best-fit gamma distribution of interspike intervals. The gamma index varied from 1 to 8. ON-cells tended to fire more regularly (higher index) at higher illumination levels, while OFF-cells tended to fire more regularly at lower illumination levels.

A nonstationary estimator of the gamma index was developed to characterize the variability during the transient adaptation period. This estimate is based on a moving window procedure which corrects for the inherent dispersion of interval lengths due to the changing spike rate. The corrected moving window estimate is smoothed in time, then ensemble averaged over a set of responses. The transient was analyzed for a period of 30 sec following the step change. During the transient, the gamma index started at a maximum of 1-3 times the steady-state value, then decayed with a time constant similar to that of the rate change.

Analysis of the transient variability data showed a power law relation between coefficient of variation and mean interval with a power between 0 and 1. This relation did not hold for steady-state values at different light intensities. Based on considerations of neuronal firing models, these results suggest that the principal source of variability occurs prior to the ganglion cell, and that fluctuations are scaled by the adaptation mechanism in the manner of a multiplicative gain control, for example, through cellular or local circuit mechanisms, rather than adjusted additively by network inhibition.

(Supported by NSF grant ENG75-17536 and NIH Training Grant 5-T32-GM07477.)

2066 A BIOCHEMICAL STUDY OF ISOLATED GLIAL (MÜLLER) CELLS FROM THE TURTLE RETINA. P. Vijay Sarthy* and Dominic Man-Kit Lam*. (Spon: D. D. Louie). Baylor College of Medicine, Houston, Texas 77030.

A method has been developed for the preparation of large numbers of glial (Müller) cells from the turtle retina. After proteolytic dissociation of the retina, Müller cells were separated from retinal neurons by velocity sedimentation at unit gravity. Fractions containing over 90% morphologically identifiable Müller cells were prepared by this procedure. Fractions containing only Müller cells were obtained by drawing selected cells individually into a micropipette under visual observation. Biochemical analyses of isolated Müller cells showed that 1) these cells did not synthesize and accumulate ACh, GABA, or catecholamines when incubated with appropriate radioactive precursors; 2) the specific activities of choline acetyltransferase (EC 2.3.1.6), glutamate decarboxylase (EC 4.1.1.15) and tyrosine hydroxylase (EC 1.14.16.2) in these cells were less than 2% of those found in the retina; 3) Müller cells, however, contained high activities of transmitter degrading enzymes - acetylcholinesterase (EC 3.1.1.7) and γ-aminobutyrate-transaminase (EC 2.6.1.19); and 4) the cells also possessed high levels of two presumably glial-specific enzymes - glutamine synthetase (EC 6.3.1.2) and carbonic anhydrase (EC 4.2.1.1). These results, together with other findings, suggest that Müller cells are not capable of neurotransmitter syntheses but possess the enzymes necessary for two important roles in the retina: 1) the inactivation of certain transmitters after synaptic transmission by uptake and degradation, and 2) the maintenance of acid-base balance and the provision of a stable micro-environment in the retina by removal of metabolic products such as carbon-dioxide and ammonia.

Supported by NIH grant EY 02423.

2067 CHANGES IN LGN CELL SIZE DO NOT NECESSARILY CORRELATE WITH CHANGES IN CORTICAL BINOCULARITY, IN CATS WITH MONOCULAR VISION. P.B. Schechter. U. of Chicago, Dept. of Ophthalmology, Chicago, Ill. 60637.

Cats raised with monocular vision have few visual cortical cells driven by the deprived eye. Also, cells in the LGN laminae receiving input from the deprived eye are smaller than those in the laminae receiving input from the experienced eye. Early work by Wiesel & Hubel (J. Neurophysiol. 26, 1963) suggested that the cortical ocular dominance change is caused by pattern deprivation, while the LGN cell size change is caused by light deprivation. Some recent studies have suggested, however, that changes in cortical binocularity and LGN cell size "...are produced by a common causal factor" (Movshon & Dursteler, J. Neurophysiol. 40, 1977), or that LGN changes are secondary to cortical changes (Cragg, Anker & Wan, J. Comp. Neur. 168, 1976). The present results address this controversey.

Four kittens were dark reared until their 30th day of life. One was given 3 hrs of monocular vision on day 30 (3 hr kitten), 2 were given 4 hrs per day on days 30, 31 and 32 (12 hr kittens), and one was given 5 hrs per day on days 30-33 (20 hr kitten). All kittens were then dark reared until day 42, when recordings were taken from their visual cortices. A fifth kitten was normally reared, with one eye sutured, until day 42 (MD). After the recordings, 100 cells were measured in the right and left laminae A of each kitten's LGN.

In the 3 hr kitten and one 12 hr kitten, about equal numbers of cells were monocularly driven by each eye. In the other 12 hr kitten, the 20 hr kitten, and the MD kitten, nearly all cells were monocularly responsive to the experienced eye. However, only in the MD kitten was there a significant difference between mean cell size of the two laminae A. These results do not support the speculations of Movshon & Dursteler (1977) or of Cragg et al. (1976), but are cnosistent with the suggestion of Wiesel & Hubel (1963), concerning the relationship between cortical and LGN changes resulting from monocular vision.

2068 PERMANENCE OF BEHAVIORAL AND DENDRITIC SPINE DEFICITS IN THE MONOCULARLY DEPRIVED ALBINO RAT. Michael L. Schwartz and Lawrence A. Rothblat. Dept. Psychol., George Washington University, Washington, D.C. 20052

Monocular deprivation in the rat significantly reduces the density of dendritic spines in visual cortex contralateral to the deprived eye (Fifkova, J. Comp. Neur. 140:431,1970). In addition, the ability of these animals to discriminate complex visual pattern is severely impaired (Schwartz and Rothblat, Neurosci. Abstr., 1976). We have recently found that the period during which spine density and behavior can be altered begins around the time of eye opening and extends approximately 30 days. Deprivation in adults leaves both measures unchanged. In the present study we attempted to determine whether these morphological and behavioral changes can be reversed by a period of normal experience.

All animals (N=34) were monocularly deprived for 30 days by suturing one of the lids just prior to normal eye opening. At 45 days of age, 20 of the animals were reverse-sutured. Ten of these rats (deprived group) began behavioral testing on the following day. The remaining 10 (recovery group) were allowed 30 days of normal experience prior to training. A third group was not reverse-sutured and performed behavioral tasks using the non-deprived eye (non-deprived group).

All animals were trained to discriminate columns and rows of 5 mm squares in a fully automated discrimination apparatus. Thirty trials were given each day until the rat met a criterion of 90% correct responses in a daily session or until 1800 trials were completed. The results revealed that both the deprived ($\bar{X}$=1524) and recovery ($\bar{X}$=1191) groups were significantly impaired ($p < .01$) relative to animals learning with the non-deprived eye ($\bar{X}$=721). Further analysis revealed no significant difference between the deprived and recovery groups on this task. Dendritic spine density was assessed by processing the brains according to the Rapid Golgi technique. Preliminary analysis of this material shows evidence of little, if any, recovery in spine density. The number of spines in the deprived hemisphere of recovery animals was reduced by 13% in comparison to a 16% reduction in the deprived hemisphere of rats examined immediately following 30 days of deprivation.

It can be concluded from these results that there is little evidence for recovery from either the behavioral or dendritic spine deficits that follow monocular deprivation in the rat. These results further support our previous finding of a sensitive period for visual system development in the rat.

(Supported by NIMH Grant MH R01-27424)

2069 PRENATAL DEVELOPMENT OF EFFERENT PROJECTIONS FROM THE VISUAL CORTEX OF THE RHESUS MONKEY. C.J. Shatz & P. Rakic. Dept. of Neuroscience, Children's Hosp. Med. Center, Boston, MA 02115 & Sect. of Neuroanatomy, Yale Univ. Sch. of Medicine, New Haven, CT 06510.

The time of neuron origin and the development of afferent connections in the rhesus monkey's visual system have been studied previously (Rakic,'77) but little is known about the formation of efferent projections from the visual cortex. We investigated the development of the cortical projections to the lateral geniculate nucleus (LGN), superior colliculus (SC) and pulvinar (Pul) by means of the orthograde axonal transport of radioactive tracers. Seven fetal monkeys, aged from embryonic day 63 (E63) to E95 were exteriorized by Cesarian section and 0.1 µl ^{3}H-proline (20-40 µCi) was injected into the occipital cortex. Each fetus was restored to the uterus and 24 hours later was removed, fixed by perfusion, and its brain was processed for autoradiography.

In the autoradiographs of the E63 fetus no radioactive label was seen in the corticothalamic radiations or within the LGN, SC, or Pul. In fetuses injected at E69, E71 and E78, label was present in the cell-poor zones surrounding the LGN. Within the LGN, label was confined to the lateral-most margin: the prospective magnocellular layers (Rakic, '77). The remainder of the nucleus was free of label. The large injection sites made it impossible to determine whether visuotopic order is present at these early ages. In fetuses injected at E83 and E85, the portion of the prospective parvocellular region of the LGN adjacent to the white matter also contained label. In the oldest monkey (E95), label extended throughout both the parvo- and magnocellular layers, as in the adult. By E83 the labelling pattern in the LGN appeared characteristically wedge-shaped and appropriately located with respect to the cortical injection, indicating that topographic order in the corticogeniculate projection is established.

The development of the cortical projection to the SC is similar in time course to that to the LGN. Between E69 and E78, label was confined to the stratum opticum; by E85 it extended into the superficial gray. In the Pul, substantial amounts of label were present as early as E69, indicating that the development of this projection may even precede those to the LGN and SC. Topographic order was present by E95 in SC and E83 in Pul.

The efferent pathways from the visual cortex in primates are therefore present by the middle of gestation. Their development is in rough synchrony with that of the afferent pathway: axons from the LGN are present within the occipital lobe by E78, but do not enter the cortical plate until about E90 (Rakic,'77). Moreover, the cortical efferent pathway is topographically ordered prior to the lamination of the LGN and before the segregation of afferents from the two eyes. Supported by N.I.H. grants NS 11233 and EY 05172.

2070 LIGHTING CONDITIONS DURING DISSECTION AFFECT PROPERTIES OF GANGLION CELLS. Jeremy M. Shefner and Michael W. Levine*. Psychology Dept., Univ. of Illinois at Chicago Circle.

The response properties of goldfish retinal ganglion cells in isolated retinae maintained with pure O_2 were compared for retinae dissected in dim red light and retinae dissected in normal room lights. Chromatic opponency was a regular feature of photopic ganglion cells in retinae dissected in dim red light, but ganglion cells from retinae dissected in room lights responded as though their only input was from the long wavelength sensitive ("red") chromatic mechanism. This deleterious effect of room lights did not have to be exerted during the dissection; cells from retinae dissected in dim red light, but then exposed to room lights, were also devoid of any input other than from the red mechanism. Thus, this effect is apparently due to the presence of the high luminance light itself, and not to photomechanical changes occurring during dissection.

In many other respects, ganglion cells observed in retinae dissected under the two lighting conditions display similar properties. Both spatially linear ("X-like") and non-linear ("$\bar{X}$") cells may be found in goldfish retinae independent of lighting conditions during dissection. Also, neither the mean nor the variance of the maintained discharge of ganglion cells in the dark differed as a function of which dissection was performed.

2071 A WIRING DIAGRAM FOR THE RECEPTIVE FIELDS OF BIPOLAR CELLS IN THE GENERALIZED VERTEBRATE RETINA. Robert Siminoff, BRI, UCLA, Los Angeles, CA 90024.

A model of the generalized vertebrate retina was developed based on a synthesis of the literature. The bipolar cells are organized into concentric receptive fields- the center excitatory area formed by direct inputs from photoreceptors and the surround inhibitory area formed by indirect inputs from photoreceptors via horizontal cells. A wiring diagram for the C-type bipolar cell is presented. The cones are organized into 2 alternating rows-- row A is formed by a red cone alternating with a green cone and row B is formed by a blue cone alternating with either a red or green cone. The direct cone input to the central region comes from a hexagon of 9 like-cones from 5 rows of cones in the mosaic. The surround region is formed by inputs from 6 C-type horizontal cells- each of which receives inputs from a hexagon of 9 opponent-color cones. Thus, if the central region is red-sensitive the surround region is green-sensitive. Electrical coupling of like-photoreceptors via high resistance " gap " junctions and stray light produce the added feature of the geometric center having the highest sensitivity (or weighted input) and the sensitivity decreases radially from the center. Additionaly, overlapping of the central and surround regions is produced. The horizontal cells act as inverters of variable gain. A L-type horizontal cell receives inputs from all cones within a given hexagon and produces a form of negative feedback to the cones. This results in a decrease in the effects of stray light and produces modifications in the receptive field organization of the bipolar cell. The L-type bipolar cell is similarly organized as the C-type except that the central region of each of the 6 horizontal cells receives inputs from a hexagon of 19 cones of the 3 spectral types of cones. Electrical coupling between like-horizontal cells produces spatial summation so that as the light intensity increases negative feedback via the L-type horizontal cell spreads laterally to minimize the lateral spread of light stimulus due to increased stray light. The ganglion cell reflects the organization of the bipolar cell. Two types of bipolar cells are present in parallel- the HPBC where direct inputs produce hyperpolarization and the DPBC where direct inputs produce depolarization. Thus each wiring diagram produces two types of bipolar cell - hyper/ dep and dep/ hyper.

2072 THE ACCESSORY OPTIC SYSTEM: A VISUAL SYSTEM IN VESTIBULAR COORDINATES. John I. Simpson and Robert E. Soodak*. Dept. Physiol. & Biophys., N.Y. Univ. Med. Ctr., 550 First Ave., New York, NY 10016

The accessory optic system in mammals is, in general, composed of three terminal nuclei (dorsal, lateral and medial) innervated by primary visual fibers. Response properties of cells of the medial terminal nucleus (MTN) were investigated using extracellular recording in anesthetized, paralyzed rabbits. MTN cells typically have a high background activity (25-50 spikes/sec.) and respond to chiasm stimulation with a latency of 2-2.5 msec. All cells influenced by moving patterns show both direction and speed selectivity. Effective stimuli are large (20-30° square), slowly moving textured patterns. Preferred directions are vertical with a posterior component; cells preferring upward movement are twice as numerous as those preferring downward movement. The preferred and null directions of MTN cells are not 180° apart. For example, if the preferred direction is up with a posterior component, the null direction is down with a posterior component. Best modulation occurs at about 0.5-1°/sec. Activity increases in a sustained manner 2-3 times over background for preferred direction movement and can be silenced for null direction movement. MTN cells respond primarily only at onset of illumination. The marked similarities between properties of MTN cells and of on, direction selective retinal ganglion cells in rabbit suggest that this ganglion cell class provides the primary visual input to MTN and is the major contributor to the accessory optic tract. Collectively, the direction preferences of on, direction selective ganglion cells define three directions in visual space: anterior, up and posterior, and down and posterior. Some cells in the nucleus of the optic tract (NOT) have response properties identical to those of MTN cells, except that the preferred direction is from posterior to anterior (Collewijn, Brain Res., 1975). These pretectal cells may, in fact, be part of the accessory optic system. Studies using HRP techniques show that MTN projects to the NOT region. This projection may act to synthesize an inhibitory signal for posterior movement. We propose that the three directions in visual space represented in the accessory optic system are derived from the orientation of the three semicircular canals of the vestibular system and that this organization allows for interaction of visual and vestibular signals in a single coordinate system. (Supported by PHS Grant NS-13742).

2073 VISUAL ACUITY IN VISUALLY DEPRIVED CATS - BEHAVIORAL CORRELATES OF SINGLE-CELL ELECTROPHYSIOLOGY? Douglas C. Smith*, Randy Lorber*, Ann Kirk* and Michael S. Loop. Dept. Physiol., Univ. of Illinois, Urbana, Ill. 61801.

Visual deprivation through the first 3-4 mo. of a kittens life is known to produce profound changes in the responsivity and stimulus specificity of single cells in striate cortex. For example, following rearing with monocular deprivation (MD) only about 5% of area 17 cells respond to visual stimulation of the deprived eye (Wiesel & Hubel, '63; '65; Kratz et al., '76). In addition, other manipulations are associated with differences in the percentage of responsive cells. For instance, around 18% of striate cells are responsive to the intially-deprived eye following postcritical-period reverse suture (RS) (Smith et al., '78). Further, an average of 30% of the cells can be driven following postcritical-period enucleation of the nondeprived eye in a MD cat (MD-DE) (Kratz et al., '76; Smith et al., '78). Following binocular deprivation (BD) about 50% of the cells in striate cortex are responsive (Kratz & Spear, '76; Pettigrew, '74; Wiesel & Hubel, '65). Finally, in MD's with the other eye removed shortly after birth (MDE), about 68% of the cells are responsive which is less than the 78% found to be responsive to a single eye in normal cats (Kratz & Spear, '76). Moreover, while the percentages of cells displaying direction and/or orientation selectivity do not vary systematically across all of these groups, the actual numbers of selective cells do. This is due to differences in the percent responsive in each condition.

We have been measuring visual acuity in several of these deprivation conditions. Using the Mitchell et al., ('76) jumping stand we measured behavioral visual acuity for high contrast square wave gratings (luminance 13 cd/m^2). Thus far, we have established the visual acuity for 2 RS, 3 MD-DE, 2 BD and the nondeprived eye in 5 MD cats. The mean acuity for RS cats is 1.6 c/d, for MD-DE cats is 2.7 c/d, for BD cats is 3.5 c/d, and is 5.1 c/d for a nondeprived eye. Additional animals are currently being tested in each group as well as MD cats with the deprived eye opened and MDE cats. The results of these additional cats will be presented. Thus far, visual acuity appears to be highly correlated with the percentage of responsive cells and the number of cells displaying stimulus specificity.

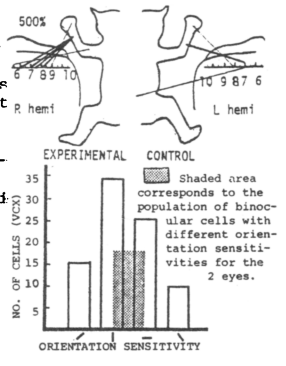
Supported by grants EYO 7005 and MH 30936.

2074 RESPONSE OF FLY GIANT OPTIC NEURONS TO INTENSITY CHANGES AND MOVING PATTERNS. Spencer L. SooHoo and Lewis G. Bishop. University of Southern California, Los Angeles, CA 90007.

When a tethered flying insect sees relative angular motion, it will move so as to null out this relative motion. Goetz measured flying torque produced by a tethered fly and found that thrust response was maximum when a horizontally oriented striped pattern was moved vertically; the turning torque was maximum when a vertically oriented striped pattern was moved horizontally. Two functionally independent neural systems were suggested for the control of flight. Intracellular recording and staining showed that in the lobula plate there are anatomically and functionally distinct horizontal and vertical movement detectors. This report deals with the electrical responses of the large vertical cells. These cells respond to changes in light intensity with a graded response. The response consists of a depolarization upon which noise-like fluctuations are superimposed. These fluctuations are reduced by signal averaging; their power spectra density resembles that of random, band-limited noise. Relative to the monopolar neurons of the lamina or to the retinula cells, the vertical cells show a small dynamic range in their response to changes in light intensity. A striped pattern moving in the downward direction evokes a depolarization and increased fluctuations in the membrane potential; upward motion evokes a hyperpolarization and increased fluctuations in the membrane potential. A moving stimulus evokes a response whose magnitude is equal to that evoked by a stationary stimulus several orders of magnitude brighter. At low contrast frequencies the contrast frequency of a striped moving pattern appears in the response.

2075 EARLY EXPERIENCE AFFECTS BRAIN DEVELOPMENT IN NORMALLY REARED KITTENS. D.N. Spinelli and F. Jensen. Depts. of COINS and Psychology, University of Massachusetts, Amherst, MA 01003.

To elucidate the impact that early experience has on the developing brain in normally reared kittens, we experimented as follows. Kittens raised in a normal environment were trained for 8 min. a day to a simple task: vertical bars presented to one eye signalled an unsafe condition and the kitten had to lift the appropriate forearm or be shocked on it; on lifting, the unsafe stimulus was turned off and the safe one on in the other eye. Training began at 4 weeks of age, 5, and 11 in 3 groups. The kittens learned the task uneventfully. At 13 weeks of age, single cells were recorded from postcruciate gyrus (PC), primary visual cortex (VCX) and visual association cortex (VASCX). PC shows a greatly enlarged forearm locus for the trained forearm, up to 3 mm in diameter; whereas the locus for the untrained forearm has a diameter of .5 to 1 mm. Further, 70% of cells in the trained side respond to visual stimuli with orientation identical to the one used during training. In the untrained forearm locus, 30% of cells respond to visual stimuli and orientation sensitivity is randomly distributed. VASCX shows a preponderance of cells prefer orientations used during training and often respond to forearm stimuli. Surprisingly large effects (considering the brevity of visual training compared with total visual experience) are present in VCX: 1) a large shift toward monocularity; 2) orientations used during training are significantly more represented than others; 3) most important, the presence of cells sensitive to vertical bars for one eye and horizontal bars for the other. This shows, for the first time, that early experience can enlarge the cortical locus of a body part in PC and tune VCX cells to unusual stimuli in normally reared kittens. No atrophy from disuse can exist in these animals because they are not deprived and cells with these properties are not present in normal adults. We conclude that early experience in developing organisms powerfully affects the developing brain and impacts on concurrent experiences. These results are significant not only because of what they tell us concerning how experience is stored in the brain, but (as we believe that these changes once produced are permanent) because of the implications they carry concerning early experiences in human children.



2076 EFFECTS OF SELECTED LESIONS IN VISUAL CORTEX ON INTERHEMISPHERIC TRANSFER OF FORM DISCRIMINATIONS IN CATS. James M. Sprague, Giovanni Berlucchi*, Antonella Antonini* and Alfredo Simone*. Dept. Anat., Sch. Med., Univ. Pennsylvania, Phila., PA 19104 and Inst. Physiol., Univ. Pisa, 56100, Pisa Italy.

Suprasylvian lesions removing cortical areas 7 and 21, and portions of area 19 and of the lateral suprasylvian area (LSA) were placed unilaterally in split-chiasm cats. By comparison with the non-lesioned side and with cortically intact split-chiasm and split-brain cats, form discrimination learning with the eye on the lesioned side was severely retarded. This deficit cannot be attributed to an unintentional undercutting or damage of areas 17 and 18, since the laminae of the lateral geniculate nucleus (LGN) showed minimal retrograde atrophy; degeneration was found in NIM and in the inferior and lateral pulvinar nuclei. In addition, interhemispheric transfer of these discriminations to the lesioned side was absent or poor, while transfer in the opposite direction was normal. A cat with a suprasylvian lesion undercutting areas 17 and 18 (and severe atrophy of LGN as well as inferior and lateral pulvinar) was unable to learn from discriminations with the eye on the injured side, despite prolonged training with that eye and normal learning with the other eye. Another cat with a suprasylvian lesion selectively removing the anteromedial and posteromedial portions of LSA (Clare-Bishop area) showed no learning deficit using the eye on the injured side, but poor transfer to that side.

In contrast, split-chiasm cats with unilateral or bilateral lesions largely removing the commissurally connected portions of visual cortical areas 17, 18 and 19 showed good interhemispheric transfer of monocularly learned pattern discriminations. The capacity for interocular transfer in these cats was in fact little or no different from that of split-chiasm cats with an intact cortex.

The results support the hypothesis of a major involvement of cortical areas other than 17 and 18 in learning and interhemispheric transfer of form discriminations in the cat.

Supported by research grants from NIH (EY00577) and CNR, Rome, (70.0168/18).

2077 LATERAL GENICULATE INTERNEURONS ACCUMULATE EXOGENOUS [^{3}H]-2,4- DIAMINOBUTYRIC ACID (DABA). Peter Sterling and Thomas L. Davis.* Dept. Anat., Sch. Med., Univ. Pa., Philadelphia, PA 19174.

Optic axons in the A-laminae of the cat lateral geniculate nucleus (LGN) contact directly the dendrites of relay cells (classes I, II) and also synaptic terminals containing flattened vesicles (F-terminals). The F-terminals in turn contact relay cell dendrites (Guillery, Z. Zellforsch. 96:1, 1969) and appear to be inhibitory. They arise as axons or presynaptic dendrites from interneurons (class III) which are the smallest neurons in the A-laminae and form about 25% of the population. We wanted to see whether the class III neurons or their processes had the ability to selectively accumulate gamma-aminobutyric acid (GABA) or its analog, DABA, since many neurons which appear to use GABA as a transmitter can accumulate these compounds. [^{3}H]-DABA was injected (50μCi in 2μl) into the A-laminae. One hour later the cat was perfused with buffered glutaraldehyde-paraformaldehyde and the geniculate prepared for light and electron microscope (EM) autoradiography. Neurons intensely labeled with silver grains were observed interspersed with unlabeled neurons for several millimeters around the injection site. We reconstructed from serial, one-micron autoradiograms 134 neurons in a patch of lamina A and found that the labeled cells formed about 25% of the population. They were the smallest cells, (12-18μm) and lacked the multilaminar body which is the hallmark of the somewhat larger (16-24μm) class II relay cell (LeVay and Ferster, J.C.N. 172:563, 1977). In EM autoradiograms the F-terminals were intensely labeled while optic terminals (RLP) and presumed cortico-geniculate terminals (RSD) were not. Astrocytes and oligodendrocytes were heavily labeled but not as intensely as the cell bodies and processes of the interneurons. These observations, plus the finding that the GABA synthetic enzyme, glutamic acid *decarboxylase, exists in certain neurons of the rat* LGN, suggest that GABA may be the transmitter for the inhibitory intrageniculate interneuron. (See also abstracts of Rapisardi and Stevens and Gerstein.) (Supported by NEI grant RO1EY00828)

2078 A SYNAPTIC TRIAD AS THE INPUT TO "X" LATERAL GENICULATE CELLS. John K. Stevens, George L. Gerstein, Dept. Physiology, Univ. of Penn., Phil, Pa.

Using single electrodes we have recorded from 26 unit-pairs in cat lateral geniculate nucleus (LGN). In each case these pairs consisted of a small monophasic positive action potential (AP), followed by a larger biphasic AP. The small AP has been identified via electrical stimulation as an optic tract (OT) fiber and, by exclusion we assume the large AP represents an LGN cell. Crosscorrelograms between these OT fibers and LGN cells revealed a very consistent relationship. After a single optic tract AP the probability of finding a LGN AP was enhanced for 1.0 to 1.5 msec (1st peak). This 1st peak in the correlogram was followed by a decrement in the firing probabiliy of the LGN cell for a 1.0 to 2.0 msec period (F period). The F period was followed in turn by a second increment in the firing probability of the LGN cell (2nd peak).

Special pattern crosscorrelograms demonstrate that these three components are contingent only upon a single AP from the OT fiber and not on the firing of the LGN cell. Thus, the F period and 2nd peak do not represent an intrinsic properity of the LGN cell itself (absolute refractory period), but rather must represent an external neural circuit. The simplest possible circuit consistent with these data would be a synaptic triad, where an OT fiber would excite both a LGN cell and an interneuron. The interneuron would in turn inhibit the LGN cell to produce the F period. The 2nd excitatory peak would represent a rebound effect.

We obtained response planes on 18 pairs. In 17 of these both the OT fiber and LGN cell had heterogeneous response planes ("X" cells). The remaining OT-LGN pair had homogeneous planes ("Y" cells). On six occasions cells recorded immediately preceeding or following an OT-LGN pair had homogeneous response planes. Thus, it is, unlikely that this "X" cell bias can be explained solely by electrode sampling errors.

The suggested circuit and its almost exclusive *association with "X" cells is attractively consistent* with the anatomical finding of Rapisardi that the LGN synaptic triad is associated with Guillery class II cells and supports the Sterling and Davis suggestion that the F terminals in the triad which accumulate DABA are derived from inhibitory interneurons. (See abstracts in this volume. Supported by NIH grants EY01832 and NS05606.)

2079 CODING OF TEMPORAL CONTRAST BY CAT RETINAL GANGLION CELLS. Vesna G. Sutija. Dept. Physiol. & Biophys., Univ. Miami Sch. of Med., Miami, FL 33152.

Responses from single ganglion cell fibers in the optic tract of cats were recorded extracellularly with laquer coated tungsten electrodes, and separated into X and Y using the linearity of spatial summation criteria. Subsequently a spot of light, frequency and amplitude modulated, was positioned concentrically with the receptive field center, while the periphery received steady background light. The background luminance level, the diameter of the spot and the waveform (sinusoidal or square) was also varied. The on-center X showed saturation with higher levels of background luminance and larger spot diameters. The waveform did not significantly influence their following rates at higher frequencies or the peak frequency response. The off-center X acquired properties similar to the off-center Y at lower background luminances (in terms of their high frequency following rates and the peak frequency response). Both on-center and off-center Y improved with higher background levels, larger spot diameters, and with squarewave stimulation (i.e. increased their bandwidths and their peak response shifted). For on-center X, the response to squarewave modulated stimulus could be predicted from the response to sinusoidally modulated light; all other subgroups showed marked nonlinearities. These findings are inconsistent with models of separate spatial and temporal information processing in the visual system. (Supported by N.I.H. post-doctoral fellowship Grant #5F32EY05021-02).

2080 THALAMIC PROJECTIONS TO ELECTROPHYSIOLOGICALLY DEFINED VISUAL AREAS IN THE CAT. Laura Symonds*, Alan Rosenquist, Stephen Edwards, Larry Palmer. Dept. Anat., Sch. Med., Univ., of Penna., Phila., PA 19104; and Dept. Anat., Sch. Med., Univ. of Virginia, Charlottesville, VA 22901.

Evidence from a number of different sources suggests that the posterior "visual" thalamus of the cat may be divided into at least three zones extending from medial to lateral in the dorso-medial to ventrolateral plane. Using the scheme and nomenclature of Updyke (J. Comp. Neurol., 173:81-122, 1977), we have made injections of tritiated amino acid into three extrageniculate thalamic subdivisions: lateral posterior interjacent (LPi), lateral posterior lateral (LPl), and pulvinar (P). LPi corresponds to the tecto-recipient zone, LPl to the cortico-recipient zone, and P to the pretecto-recipient zone. The patterns of cortical connectivity of these thalamic subdivisions were related to electrophysiologically and anatomically-defined visual areas of the cat cortex. In the suprasylvian cortex (Palmer, et. al., J. Comp. Neurol., 177:237-256, 1978), areas PMLS and VLS are labeled after LPl but not LPi or P injections. Areas DLS, PLLS, and PS (Heath and Jones, Ergebn. Anat. Entwickl.-Gesch., 45:1-64, 1971) are labeled after both LPi and LPl but not after P injections. Of the two rostral suprasylvian areas, ALLS appears to receive no thalamic input from the regions covered by our injections, and AMLS receives projections from the ventral but not the dorsal portions of LPl. Other data suggest that this latter finding may be explained by the existence of an extensive representation of the lower visual field in AMLS and the probability that lower fields are represented ventrally (and rostrally) in LPl. The "splenial visual area" of Kalia and Whitteridge (J. Physiol., 232:275-283, 1973) was labeled after injections into P and lateral portions of LPl but not after injections into LPi or medial portions of LPl. Area 7 is labeled after injections into P but not into LPl or LPi. Area 19 is labeled following injections into LPl and P, but not LPi. Area 20 appears on the basis of both electrophysiological recording and thalamic connectivity to consist of two subdivisions, and both of these are labeled after injections into LPi, LPl and P. We conclude that Updyke's division of the posterior thalamus into zones is largely supported by the uniqueness of their projections to identified cortical areas. (Supported by Grants BNS 02453 from NSF, and T32 EY 07035-02 from NIH).

2081 ACTIVITY OF NEURONS IN VISUAL CORTEX OF THE ALERT MACAQUE EVOKED BY STATIONARY AND MOVING STIMULI IN THREE-DIMENSIONAL SPACE. William H. Talbot* and Gian F. Poggio, Dept. of Physiology Johns Hopkins School of Medicine, Baltimore, MD 21205

A previous study from this laboratory demonstrated sensitivity to depth of neurons in striate and prestriate cortex responding to stimuli moved in frontoparallel planes in front of and behind the plane of fixation. Two major groups of depth sensitive neurons were found: neurons that responded over a narrow range of depths centered near or at the fixation plane (*tuned excitatory, tuned inhibitory*) and neurons that responded over an extended range of depths on one or the other side of the fixation plane (*near, far*). We have now added to that study by separately analyzing binocular interaction during static and dynamic stereoscopic stimulation. Bar stimuli were presented against a background of dynamic random noise using a dichoptic dot matrix display. Trained monkeys fixated a small target during each stimulus presentation. The fixation target was shifted on the screens of the stimulator to a position that ensured that the response field for each neuron was centered on the display. Size and orientation of bar stimuli were usually adjusted for maximal response. Single cortical neurons were routinely tested with bars moving in frontoparallel planes (a dichoptic simulation of our earlier experiments), stationary bars at various apparent depths, and bars moving with constantly changing disparity that appeared to move toward and away from the monkey in various directions. Our results indicate that activity of most foveal cortical neurons is affected by stimuli moving in depth. For many cells classified as *tuned* from responses to moving bars of fixed disparity, tests with stationary bars and bars moving in depth confirm that these cells are primarily sensitive to positional disparity of retinal images. Neurons showing extended depth sensitivity when tested with stimuli of fixed disparity can be subdivided by dynamic disparity testing: some respond to stimuli that appear to move in depth with activity that is predictable from their responses to fixed disparity stimulation; others respond with activity that is opposite to prediction. Finally, a relatively large number of neurons that have little or no depth sensitivity when tested with fixed disparities are quite sensitive to the direction of apparent stimulus movement in depth. The characteristics of these neurons revealed when retinal image velocity and direction differ in the two eyes seems in some cases to be due to an ocular directional dominance that is not apparent in responses to monocular stimuli or to binocular stimuli that cause identical image movements in both eyes. (USPHS 5 PO1 6828)

2082 SEROTONIN-A NEUROTRANSMITTER OF THE BOVINE RETINA, Thomas N. Thomas, John W. Zemp and Dianna A. Redburn+, Department of Biochemistry, Medical University of S.C., Charleston, S.C. 29403 and +Department of Neurobiology and Anatomy, Univ. Texas Med. School, Houston, Tex. 77025

Serotonin (5-hydroxytryptamine, 5-HT) is an established neurotransmitter of the brain but very little information is available regarding its role as a retinal neurotransmitter. Some recent reports have demonstrated the presence of 5-HT and high affinity uptake systems for 5-HT in chick and rabbit retina. Our investigations indicate the possibility of 5-HT being a neurotransmitter of the bovine retina.

Slices of fresh bovine retina were incubated at 37°C in oxygenated Krebs-Ringer medium containing 50 µM pargyline and [^{3}H] 5-HT (10^{-8}M to 10^{-6}M). Low concentrations of [^{3}H] 5-HT were used so as to measure the high affinity uptake. These conditions favour uptake into serotonergic neurons. The uptake of [^{3}H] 5-HT by bovine retina is sodium and temperature dependent, ouabain sensitive, and it is saturable. A Michaelis-Menten plot of the uptake data gave an apparent Km of 4.3 x 10^{-7}M and a Vmax of 294.3 pmol/g/10 min. The uptake system also demonstrated a high degree of pharmacologic specificity. Reserpine (2 x 10^{-4}M) completely eliminated the uptake whereas chlorimipramine (10^{-4}M) produced 85% reduction in uptake. By using a technique that has been successfully used to study the release of neurotransmitters from brain and retina, we were also able to demonstrate K^+ stimulated, Ca^{2+} dependent release of [^{3}H] 5-HT from bovine retina. This is the first demonstration of evoked release of 5-HT from retina of any species. Spectrophotofluorimetric analysis of bovine retina demonstrated the presence of 5-HT and its major metabolite 5-HIAA.

This report demonstrates the presence of appreciable amounts of 5-HT and 5-HIAA in the retina, a high affinity uptake system, and K^+ stimulated Ca^{2+} dependent release. Evidence presented here supports the possibility of serotonin being a neurotransmitter of the bovine retina. Psychoactive and hallucinogenic drugs have been shown to alter serotonergic systems in the brain. The demonstration of serotonergic system in the retina would have profound pharmacological implications and would provide further insight into the mode of action of these drugs.

2083 EFFECTS OF MONOCULAR DEPRIVATION ON CELLS IN THE CAT'S LATERAL SUPRASYLVIAN VISUAL CORTEX. Lillian Tong* and Peter D. Spear. Dept. Psychol., Univ. of Wisconsin, Madison, WI 53706.

In experiment I, effects of rearing with monocular lid-suture (MD) on neurons in the lateral suprasylvian visual cortex (LS) were investigated. Nine MD cats were studied, and 239 cells were recorded in both the binocular and monocular segments of LS cortex. In normal cats, 65% of the binocular segment cells are driven by both eyes (Spear & Baumann, 1975). Following MD, however, nearly all cells (83%-90%) were driven exclusively by the experienced eye in the binocular segment of both hemispheres. The receptive field properties of these cells appeared normal: 75% were direction selective, 6% were movement sensitive, 10% responded best to stationary flashing stimuli, and 9% had indefinite receptive fields. In the monocular segment contralateral to the deprived eye, the deprived eye was able to drive many LS cortex cells. However, the receptive field properties of these cells were very abnormal; e.g., none of them were direction selective. Thus, both binocular competition and deprivation per se play a role in the effects of monocular lid-suture on LS cortex neurons.

Experiment II investigated whether these effects were due to abnormal visual cortex inputs (from areas 17, 18, and 19) or to changes in the thalamic-LS cortex pathways independent of the visual cortex. This was tested by recording from 159 cells in 9 MD cats which had areas 17, 18, and 19 removed bilaterally as adults. In the binocular segment contralateral to the deprived eye, the deprived eye drove 67% of the cells after visual cortex removal, compared to only 17% when visual cortex was intact. Thus, the visual cortex appears to suppress thalamic-LS cortex inputs from the contralateral deprived eye in MD cats. In addition, the ipsilateral experienced eye drove 69% of the LS cortex cells after visual cortex removal (36% were binocularly driven). This contrasts with normally reared cats, in which the ipsilateral eye drives only 14% of the LS cortex cells after visual cortex removal (Spear & Baumann, 1978). Thus, during rearing with MD, the ipsilateral experienced eye develops thalamic-LS cortex inputs which are not present in normally reared cats. The receptive field properties of cells driven by both the deprived and experienced eyes were abnormal following visual cortex removal in the MD cats; e.g., only 10% were direction selective. Thus, the visual cortex inputs provide direction selectivity to LS cortex cells in MD cats, just as in normally reared cats (Spear & Baumann, 1978). Taken together, the results of experiment II indicate that the effects of MD on LS cortex cells are produced both by an abnormal input from areas 17, 18, and 19 (which suppresses thalamic inputs from the deprived eye) and by changes in the thalamic-LS cortex pathway independent of the visual cortex (new inputs from the experienced eye).

2084 EFFECTS OF UNILATERAL AND BILATERAL LESIONS OF THE SUPRASYLVIAN AREA ON INTEROCULAR TRANSFER IN THE CAT. Michel Turcotte*, Maurice Ptito, Maryse C. Lassonde and Franco Lepore. Lab. de Neuropsychologie. Univ. du Québec, Trois-Rivières and Univ. de Montréal, Montréal, Canada.

The suprasylvian area (SSA) has been shown both anatomically and physiologically to be involved in visual processing. Recent studies (Berlucchi et al., Exp. Brain Res. 31, 1978) using visual cortical lesions excluding the suprasylvian area failed to abolish interhemispheric transfer. The authors concluded that transfer must depend on other cortical areas, such as the suprasylvian area. In the present experiment, eight adults cats underwent a section of the optic chiasm, followed by a unilateral destruction of the SSA. They were then tested monocularly on several pattern discrimination tasks both in a Thompson box and a Lashley type jumping stand. One group started with the eye ipsilateral (EI) in the cortical lesion the other with the eye contralateral (EC) to the SSA lesion. Animals in both groups learned the pattern discriminations equally well in an equivalent member of trials. The degree of interocular transfer was then assessed, that is, they were retested on the same patterns using the untrained eye. Partial results showed that interocular transfer was present in all subjects from both groups. However, more precise statistical analyses showed that transfer in the EI group was much better (index of interocular transfer derived from Murdock's formula: 0.93) than in the EC group (index of interocular transfer: 0.23). To further evaluate the importance of SSA in interocular transfer the SSA in the unlesioned hemisphere was next destroyed. The cats were first retested on the previously learned discriminations. No apparent deficits were noticed when testing was carried out using the hemisphere lesioned first. However performance decreased when testing with the eye connected to the newly lesioned hemisphere. Once criterion was achieved on all previously learned patterns, new pattern discriminations were given to one or the other eye. Again learning was comparable for both sides. However, when interocular transfer for the newly learned patterns was measured, it was found to be very poor in either direction (index of transfer: 0.20). These results support previous findings on the importance of the suprasylvian area in interhemispheric transfer of visual discriminations, although they also indicate that it is probably not the exclusive region for such a function.

2085 INTERACTIONS OF STRIATE AND POSTERIOR PARIETAL CORTEX IN SPATIAL VISION. Leslie G. Ungerleider and Mortimer Mishkin. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20014.

This study investigated the contribution made by inputs from striate cortex to the visuospatial functions of posterior parietal cortex. In Exp. 1, monkeys received a three-stage operation intended to serially disconnect posterior parietal from striate cortex. At each stage they were tested for retention of the landmark task, a visual spatial discrimination sensitive to the effects of bilateral posterior parietal damage (Pohl, JCPP 82: 227, 1973). The results showed: no deficit after the initial unilateral posterior parietal lesion; severe deficit after the addition of a total contralateral striate lesion; and moderate deficit after disconnection of the intact posterior parietal and striate areas by section of the corpus callosum. Subsequent removal of the remaining posterior parietal cortex yielded no effect, indicating that tectofugal inputs to this cortex had played no significant role in the recovery following disconnection. Taken together, the results demonstrate that the visuospatial functions of posterior parietal cortex depend on corticocortical inputs from striate cortex. This parallels an earlier finding that corticocortical inputs from striate cortex are crucial for the pattern recognition functions of inferior temporal cortex. However, the relative effects of the striate and callosal lesions in the two studies were reversed, suggesting that posterior parietal cortex is more dependent on ipsilateral striate inputs than is inferior temporal cortex. In Exp. 2, monkeys received bilateral lesions of the striate areas representing either central vision (lateral striate) or peripheral vision (medial striate). They were tested both on a pattern discrimination task, to assess residual inferior temporal function, and on the landmark task, to assess residual posterior parietal function. On the former task, only the lateral striate lesion produced a deficit, whereas on the latter task, the two lesions produced equally severe deficits. Apparently, inputs from central vision are especially important for the pattern recognition functions of inferior temporal cortex, but inputs from central and peripheral vision are equally important for the visuospatial functions of posterior parietal cortex. Thus, although interactions with striate cortex are critical for both the parietal and temporal areas, the striate inputs to the two are organized differently: relative to inferior temporal cortex, posterior parietal cortex receives a greater contribution from inputs representing both the contralateral and the peripheral visual fields. These differences, which are seen in the receptive field properties of posterior parietal vs. inferior temporal neurons, may reflect differences in the cortical processing required for spatial vs. object vision.

(Supported by NIH Fellowship EY05009)

2086 THE CLASS V CELL IN THE LATERAL GENICULATE COMPLEX AND LATERAL POSTERIOR COMPLEX OF THE CAT THALAMUS. B. V. Updyke. Dept. of Anat., L.S.U. Med. Ctr., New Orleans, LA 70112.

In 1970 Famiglietti (1) described an unusual cell type (class V cell) in Golgi impregnations of the cat lateral geniculate nucleus, and presented evidence suggesting that these cells possess both a myelinated axon and presynaptic dendrites. I have reexamined the morphological features of Golgi stained class V cells in the dorsal lateral geniculate complex (LGNd & MIN) and in the lateral posterior complex. Golgi staining was combined with Nissl counterstaining (2) in order to assess the distribution of class V dendrites relative to cytoarchitectural boundaries.

Class V cells are found within all layers of the LGNd, and within MIN and the lateral posterior complex. These cells have medium sized perikarya and smooth varicose or moniliform dendrites which branch dichotomously and usually taper to fine beaded terminal processes. Dendritic appendages are relatively sparse and typically occur as either single or serial swellings on threadlike processes.

Many of these cells have dendrites which are exceptionally long (500 - 700μm), resulting in large but sparse dendritic arbors. A striking feature of these arbors is the tendency for the dendrites to span both laminar and nuclear borders. Dendrites were seen to lie within and to cross the intralaminar zones of the LGNd; to extend medially beyond lamina A_1 into MIN, and dorsally beyond lamina A into the perigeniculate nucleus. Class V cells within the pulvinar send dendrites laterally into the external medullary lamina.

It was possible to recognize only the axon initial segments. Given the otherwise satisfactory quality of the impregnations, this consistent failure of the distal portions of the axons to stain is indicative of myelinated axons.

The anatomical evidence thus suggests that class V cells constitute a special class of thalamo-cortical relay cell. Work is now underway to reexamine Famiglietti's (1) suggestion that these cells also possess presynaptic dendrites; a feature which would imply additional "interneuron-like" functions.

Although one might speculate that cells with such striking morphology should respond differently from conventional relay cells, there seems to be no physiological confirmation for this notion. One is tempted to conclude that they have been overlooked in physiological studies of the cat LGNd.

Supported by EY 01925.

(1) Famiglietti, E.V. Jr. 1970 Brain Res., 20: 181-191.
(2) Geisert, E.E. Jr. & Updyke, B.V. 1977 Stain Technol., 52: 137-141.

2087 THE DISTRIBUTION OF CELLS PROJECTING INTERHEMISPHERICALLY IN EXTRASTRIATE VISUAL CORTEX OF THE MACAQUE. D.C. Van Essen* and J. L. Bixby* (SPON: J. Myerson). Division of Biology, California Institute of Technology, Pasadena CA 91125.

Axons in the corpus callosum that project to extrastriate visual cortex terminate preferentially in regions representing the vertical midline of the visual field. This characteristic pattern of connections provides a valuable anatomical method for assessing visual topography over large regions of cortex. We have extended this approach by comparing the distributions of the cells of origin and the terminal projections of the callosal fibers. The splenium of the corpus callosum was transected and a Gelfoam pad soaked with horseradish peroxidase (HRP) was applied to the cut surfaces of the callosum. After survival times of 3 or 6 days the brain was fixed, sectioned and reacted for HRP activity; in the 6-day survival animal additional sections were stained for degenerating terminals. The positions of HRP-labelled neurons were marked on drawings of individual sections and on two-dimensional, "unfolded" reconstructions of extrastriate occipital cortex (cf. Van Essen and Zeki, J.Physiol., 1978). The regional distributions of degeneration and HRP-labelled neurons were very similar throughout the occipital lobe. Thus, callosal-projection cells in one hemisphere terminate in a mirror-symmetric pattern in the opposite hemisphere. These experiments do not, however, reveal the degree to which the projections from any one locus are restricted to the exactly corresponding contralateral locus within the overall zone of terminations.

In most cortical regions all of the HRP-labelled cells were situated in layers II and III, and most labelled cells were obviously pyramidal in shape. An additional population of pyramidal cells in layers V and VI was labelled in several restricted portions of the anterior bank of the lunate sulcus, the prelunate gyrus, the posterior bank of the superior temporal sulcus, and the intraparietal sulcus. Whether these regions lie within topographically-defined visual areas V2, V3, V3A, or V4 is not yet known, but the existence of regional variations in the laminar distribution of callosal-projection neurons may provide a useful anatomical basis for distinguishing functionally significant visual cortical subdivisions. Supported by NIH grant 1 RO1 EY02091-01

2088 THE PROJECTION OF THE PRETECTUM UPON THE INFERIOR OLIVARY COMPLEX: AN AUTORADIOGRAPHIC AND HORSERADISH PEROXIDASE ANALYSIS IN THE TREE SHREW, THE RAT AND THE CAT. J.T. Weber, M.F. Huerta, M. Behan*, G.J. Royce and J.K. Harting. Department of Anatomy, University of Wisconsin, Madison, Wisconsin, 53706.

We have been studying the organization of visual inputs to the inferior olivary complex. Thus far, in several mammals, we have identified a substantial projection to the medial accessory olive which arises from the intermediate and deep layers of the superior colliculus. In the present communication, we present data which show that a second primary retinal recipient zone, i.e., the pretectum, also sends a substantial projection to the inferior olive.

We placed injections of ^{3}H-proline into the pretectal complex of tree shrews, rats and cats. In each of the three mammals, transported protein can be seen overlying the ipsilateral dorsal cap of Kooy. This projection is especially dense in the tree shrew and the rat. Two additional ipsilateral pretectal olivary projections were observed in rats and cats: one to the rostral portion of the dorsal accessory olive, and one to the beta nucleus. In the cat, sparse label overlies a portion of the ipsilateral medial accessory olive, which is outside the region we previously identified as receiving input from the superior colliculus.

In an attempt to identify the specific cells of origin of the pretecto-olivary pathway, we injected horseradish peroxidase (HRP) into the inferior olive of several cats and one rat. Following large olivary injections in the cat, backfilled neurons can be identified within the nucleus of the optic tract (NTO), the anterior (APN) and the posterior (PPN) pretectal nuclei. In contrast, subsequent to HRP injections confined to the caudal portions of the inferior olive, backfilled neurons are present only within the NTO and the PPN.

Injections of HRP into the inferior olive of the rat result in backfilled neurons being present only within the NTO and the APN. However, since the injection did not involve all olivary targets of the pretectum, it follows that additional pretectal cell groups in the rat might project to the inferior olive.

In summary, our findings reveal that there are species differences in the pattern of terminations of the pretecto-olivary pathway. Furthermore, it appears, at least in the cat, that the pretecto-olivary pathway arises from several pretectal cell groups.

Supported by Grants EY01277, BMS76-81882 and NS013453.
J.T. Weber is supported by NIMH Fellowship MH05601.

2089 CONNECTIONS OF STRIATE CORTEX WITH THE POSTERIOR BANK OF THE SUPERIOR TEMPORAL SULCUS IN MACAQUE MONKEYS. R. E. Weller* and J. H. Kaas (Spon: R. A. Bombardieri). Depts. of Psychology and Anatomy, Vanderbilt University, Nashville, TN 37240.

In New World monkeys and prosimians, electrophysiological mapping methods have been used to reveal a topological representation of the visual hemifield in the temporal lobe that has been called the Middle Temporal Visual Area (MT). In these primates, MT is also characterized by having reciprocal connections with striate cortex (Area 17). MT has not been defined by mapping experiments in Old World monkeys, but a number of investigators have shown that a region of cortex on the posterior bank of the superior temporal sulcus (STS) in the temporal lobe receives projections from striate cortex. Furthermore, Ungerleider and Mishkin (Anat. Rec., '78) have recently reported that the patterns of projections from striate cortex to STS in macaque monkeys corresponds to the known retinotopic organization of MT, and they suggested that the receiving zone in the STS is the homologue of MT. The present results support that conclusion.

Connections of cortex on the banks of STS were determined by injecting ^{3}H-proline, horseradish peroxidase (HRP), or ^{3}H-proline plus HRP into various locations in striate cortex of 10 cerebral hemispheres in 7 macaque monkeys (Macaca fascicularis). The ^{3}H-proline experiments defined a region along the posterior bank of STS with input from striate cortex. Most of the label was concentrated in layer IV and the inner part of layer III. The location of the label in STS varied with the injection site, and multiple injection sites resulted in multiple zones of label in STS. HRP injections in striate cortex resulted in HRP-positive cells in the same region of STS that received projections from striate cortex. A large injection zone in striate cortex resulted in a large region of labeled cells in STS of about 10mm in width. Most of the labeled cells were in layer VI, and many of these cells could be identified as pyramidal neurons. Occasionally, HRP-positive neurons were also found in layer V, and a few labeled cells were encountered in layer III. In addition, a few scattered labeled neurons were sometimes seen outside of the STS. Labeled neurons were also found in Area 18 following the striate injections, and these neurons were largely in layer V. For comparison, large HRP injections were made in Area 17 (and extending partly into Area 18) in both hemispheres of one squirrel monkey, a New World monkey. As expected, a large number of cells in layer VI of MT were labeled.

The results show that a region of STS in macaque monkeys is reciprocally connected with striate cortex in a pattern very similar to that of MT in New World monkeys and prosimians. We conclude that a region of STS in Old World monkeys contains MT.

Supported by NIH Grant EY-02686.

2090 ALBINISM & OPTIC NERVE DEFORMITIES: ALBINO VS. BLACK C57BL/6J-c^{2J} MICE. I.S. Westenberg. V.A. Hosp., Phoenix, AZ 85012.

The incidence of deformed optic nerves (ON) in albino rats of the Sprague Dawley strain has been reported as 70-97%, vs. 2% in pigmented rats of the Long-Evans strain. This is relevant to recent studies of abnormal retinal projections linked to albinism in rats, because most such reports have involved this albino strain. In the original report there were two independent variables, albinism (albino vs. pigmented) and strain (Sprague Dawley vs. Long-Evans). As in previous "between-strains" comparisons of Sprague Dawley rats vs. pigmented rats of other strains, there was no way to factor out the effects of albinism (genetic differences at the C locus) from the effects of strain (genetic differences at other loci); i.e., the variables of albinism and strain were confounded. The goal of the present experiment was to determine the role of one of these variables, albinism, in ON deformities.

In the present experiment albinism was the independent variable and strain was held constant. Specifically, the independent variable was 1 gene at the C locus, while genes at all other loci were held constant across all subjects; i.e., subjects were albino or pigmented but otherwise genetically identical (coisogenic). Such a "within-strain" comparison is possible with inbred pigmented and albino mice of the C57BL/6J-c^{2J} strain. Albino C57BL/6J-c^{2J} mice have 2 mutant c^{2J} genes at the C locus (c^{2J}/c^{2J}), while pigmented C57BL/6J-c^{2J} mice have 1 normal "+" gene and 1 mutant c^{2J} gene at the C locus ($+/c^{2J}$). Otherwise, pigmented and albino C57BL/6J-c^{2J} mice are genetically identical. Thus albinism can be varied while strain is held constant.

The ON of 109 albino C57BL/6J-c^{2J} males were compared to the ON of 113 black C57BL/6J-c^{2J} males; each of 63 litters provided at least 1 albino and 1 black male. All mice were bred in the Phoenix V.A. mouse colony from Jackson Laboratory stock. As part of another experiment the right eye of each mouse was removed at 100 days of age; later each was perfused with saline and fixative. Each left eye was removed, and each brain was removed with ON intact. Grossly abnormal ON were observed in 1 albino and 3 black males; a large kink occurred in the right ON of 1 albino and 2 black mice and in the left ON of 1 pigmented mouse. Each of the 4 mice was from a different litter and had albino and pigmented male littermates with normal ON. The deformity was not related to maternal genotype or pedigree. The incidence of deformed ON in C57BL/6J-c^{2J} mice corresponds to that of pigmented Long-Evans rats rather that that of Sprague Dawley albinos. It was lower for albino mice than for pigmented mice of the same inbred strain. Thus, when strain is held constant, albinism is not related to increased ON deformities, at least not in C57BL/6J-c^{2J} mice.

Supported by NIH Grant No. 1 R01 EY 01888-01 and Fight For Sight, Inc., (NY), Grant No. G-599.

2091 QUANTITATIVE EM ANALYSIS OF THE DORSAL LATERAL GENICULATE NUCLEUS OF MACACA MONKEYS. James R. Wilson and Anita E. Hendrickson. Dept. of Ophthalmology, Univ. of Wash., Seattle, WA., 98195.

The number of vesicle-containing profiles and synaptic densities in the parvo- and magnocellular laminae and interlaminar zones of the dorsal lateral geniculate nucleus (dLGN) were counted and their relative frequencies determined in normal adolescent monkeys. Comparable counts were also made in the dLGN of adolescent monkeys deprived from 2 weeks postnatally by monocular lid-suture.

In the parvocellular laminae and in all of the interlaminar zones of the dLGN from normal Macaca, approximately 50% of the synaptic densities seen were from small profiles with closely-packed round vesicles (RSD; the majority most likely originating in cortex) ending on dendrites. About 20% of the synaptic densities were made by larger profiles with loosely-packed round vesicles (RLP; of retinal origin) ending on dendrites, and a similar number (~20%) were from flattened vesicle-containing profiles (F; probably intrageniculate origin) which also ended on dendrites. A small percentage (~10%) of the total synaptic densities were presynaptic to profiles containing flattened vesicles; these densities were formed by all three terminal types. Within the magnocellular laminae the relative number of F profiles and their synaptic densities was found to be higher than in the parvocellular or interlaminar zones. The profile number per unit area in the laminar and interlaminar zones was comparable; the lack of RLP terminals in the interlaminar zones was made up by increased numbers of RSD, F, and unclassified profiles. A Golgi analysis of the dLGN neurons indicated that the dendrites within the interlaminar zones were from a variety of neurons with cell bodies located both in the laminar and interlaminar zones. The only difference noted at the EM level for the counts made in the dLGN of deprived monkeys was an increase in the ratio of RSD to F profiles in the interlaminar zones - no quantitative differences were found between the deprived, non-deprived, or normal laminae of the dLGN in these counts.

We infer from the greater relative frequency of F terminals in the magnocellular laminae that there is probably more inhibition upon the proximal dendrites of these cells, which might relate to several electrophysiological aspects of Y-cell characteristics in the magnocellular laminae. These counts also demonstrate the importance of the interlaminar zones since these zones contain large numbers of synaptic densities and dendrites. The change noted in the deprived monkeys further emphasizes the possibility of significant interactions occurring in these interlaminar zones.

This research supported by PHS grants EY01208, EY07013, EY07130, RR00166, and RCDA EY39039 to A.H.

2092 CIRCLING FOLLOWING UNILATERAL LESIONS OF THE SUPERIOR COLLICULUS CORRELATED WITH CORTICAL NOREPINEPHRINE LEVELS. Jacqueline M.S. Winterkorn, Robert A. Ross and Thomas H. Meikle, Jr. Cornell Univ. Medical Coll., New York, N.Y. 10021

Unilateral lesions of the superior colliculus in cats have been reported to produce compulsive ipsiversive circling, which has been attributed to destruction of neurons in deeper layers of the colliculus which project through the tectospinal tract to the reticular formation and cervical spinal cord. Other reports have stressed that lesions interrupting ascending catecholamine pathways in the brainstem also are associated with post-operative circling. Because these catecholamine pathways ascend in the midbrain tegmentum subjacent to collicular neurons from which the tectospinal tract arises, and because lesions of the superior colliculus frequently encroach upon the tegmentum, the present study was designed to test the hypothesis that ipsiversive circling following unilateral ablation of the superior colliculus is due, not to interruption of tectospinal efferents from the colliculus, but rather to interruption of pathways ascending in the tegmentum ventral to the superior colliculus.

In each of 28 adult cats, one of the following unilateral brain lesions was made: superficial superior colliculus; deep superior colliculus; tectospinal tract; locus coeruleus; midbrain tegmental locus of dorsal adrenergic bundle. On each cat, behavioral tests, histological confirmation and biochemical assays of levels of activity in forebrain of the biosynthetic enzymes for dopamine and for norepinephrine were performed independently.

The results indicate that (1) superficial lesions of the superior colliculus do not produce circling and do not produce any change in the biochemical parameters studied; (2) lesions of the superior colliculus which include deep layers and encroach to varying degrees upon midbrain tegmentum produce varying degrees of ipsiversive circling highly correlated with decreased norepinephrine in ipsilateral cortex; (3) unilateral lesions of the tectospinal tract do not produce circling and do not produce any change in biochemical parameters; (4) lesions of the locus coeruleus produce significant decreases in cortical norepinephrine but do not produce ipsiversive circling; (5) discrete lesions of a small region of midbrain tegmentum can produce ipsiversive circling together with decreased cortical norepinephrine.

These results strongly suggest that circling after unilateral lesions of the superior colliculus is due not to interruption of the tectospinal tract but to damage of a critical region in the midbrain tegmentum, ventral to the superior colliculus. Although the dorsal adrenergic bundle passes through this critical region, ablation of the adrenergic cell bodies in the locus coeruleus does not produce ipsiversive circling.